International Journal for Research in Applied Sciences and Biotechnology

ISSN: 2349-8889
Volume-8, Issue-1 (January 2021)

www.ijrasb.com

https://doi.org/10.31033/ijrasb.8.1.2

Screening of Microflora Associated with Brinjal Seeds (*Solanum melongena* L.) in Western Part of India

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ABSTRACT

Total 110 seed samples of brinjal (*Solanum melongena* L.) were collected from 12 major growing districts of Rajasthan and subjected to dry seed examination (DSE). DSE revealed asymptomatic (07.75-97.5%), moderately discolored (04.50-67.50%) and shriveled discolored (03.25-38.75%) seeds. Symptomatic seeds in DSE showed various types of discolorations, deformation like white crust on seed surface. The seeds with water soaked symptoms and spots on seed surface were also observed such seeds on incubation yielded bacterial species. The incubation of symptomatic seeds about 21 fungal species of 14 genera and 3 bacterial species were observed *viz. Alternaria alternata, A. solani, Aspergillus flavus, A. niger, Cladosporium oxysporium, Curvularia lunata, Fusarium oxysporium, F. solani, Rhizoctonia bataticola* and *Rhizopus nigricans* etc and bacterial species like *Xanthomonas axonopodis* var. *vesicatoria, Ralstonia solanacearum* and *Pseudomonas aeruginosa*. The microflora severely affects seed germination (failure or delayed germination), wilting and rotting of seedlings, bacterial oozing, collapse of hypocotyls and cotyledonary leaves which resulting seedling mortality. High yield of microflora was obtained on standard blotter method (SBM) and agar plate method (APM).

Keywords- Brinjal Diseaes, Phytopathological Effects, Seed-Borne Microflora, Incidence of Microflora, Seed Sample Survey.

I. INTRODUCTION

Brinjal (*Solanum melongena* L.) is origin of Indo- Burma region belongs to family Solanaceae and commonly known as egg plant, aubergine, or guinea squash. It is a warm-season, non-tuberous, summer vegetable grown in tropical and temperate parts of the world [1]. Now the plant is widely cultivated in all over India, particularly in West Bengal, Orissa, Andhra Pradesh, Gujarat, Bihar, Madhya Pradesh, Maharashtra, Chhattisgarh, Karnataka and Haryana as major brinjal growing states. Rajasthan accounts brinjal for about 21803 MTs with an area of 4633 Ha [2]. Several diseases pose a great threat in cultivation of brinjal. These diseases not only reduce the yield but also deteriorate the quality of fruits. Fungal diseases are very much hazardous to brinjal plants in Rajasthan region because of temperature flux. Various types of diseases as leaf spot, leaf blight, root rot, fruit rot and post-harvest diseases found to be associated with brinjal. The seed to seedling transmission of seed-borne pathogens create alarming situation. The seed-borne fungi infect brinjal seedlings and cause severe losses due to damping-off, collor rot, stem canker, leaf blight, fruit rot that resulting in premature defoliation, less in number and size and quality of fruits up to 20-30% [3]. Screening of literature reveals that no planned and specific survey has been made as yet on the parasitic and storage diseases of plant in Rajasthan. During this investigation a number of fungi and bacterial species were found to be associated with the various plant parts. These infected fruits are short lived and greater loss is caused during transit and storage. The seeds are planting material for new generation but affected to various plant pathogens reduced to yield and spread of pathogens to next generation. The aim of present study is to investigate the major seed-borne disease, their effect on germination, yield loss and their incidence in context of Rajasthan.

II. MATERIALS AND METHODS

I.Study area and collection

The experiment was conducted in PG Department of Botany, Agarwal P.G. College, Jaipur (Rajasthan) in 2012 to 2016. The survey was conducted in 12 major brinjal seed growing district of Rajasthan. A total of 110 seed samples of brinjal were collected from different farmer field, storage houses and open market to know the diversity and incidence of microflora associated with them.

II.Detection of seed-borne pathogen

The seeds samples were subjected to Dry Seed Examination (DSE), Standard Blotter Method (SBM) and Agar Plate Method (APM) for detection of seed-borne pathogen. In dry seed examination, 100 seeds were taken randomly and examined by naked eye as well as by stereo binocular microscope (Nikon). In SBM, 100 untreated; 100 pretreated seeds (treated with available 2% aqueous sodium hypochlorite) were placed on water soaked blotter papers in Petri dishes. The Petri dishes containing seeds were incubated at 22± 2 ºC under alternating cycles of 12 hours near ultraviolet (NUV) light and darkness for 7 days. Seed germination percentage and seedling symptoms were recorded on 8th day of incubation. The

external and internal seed-borne fungi are identified or detected by two important commonly methods viz. Blotter method and Agar plate Method. In APM, the seeds were incubated on Potato Dextrose Agar (PDA) and Nutrient Agar (NA) for isolation of fungi and bacteria, respectively [4,5]. Percentage of incidence and germination of mycoflora was calculated by following formula:

$$\text{Incidence } \% = \frac{\text{No. of seeds infected to pathogens}}{\text{Total number of seeds observed}} \text{X100}$$

(iii) Identification of Pathogen:

Growth of different type of fungal pathogen was examined under the steriobinocular microscope and identified by various characteristics, staining techniques etc.

III. RESULTS AND DISCUSSION

(i) Dry Seed Examination

The collected seed samples revealed asymptomatic (07.75-97.5%), moderately discolored (04.50-67.50%) and shriveled discolored (03.25-38.75%) seeds (Table 1). The seed samples have dark brown or black discolored seeds 57 (03.50-32.75%), seeds with fruiting bodies 18 (03.25-17.75%), white mycelial crusted seeds 49 (01.25-25.75%), brown spotted discolored seeds 51 (03.25-31.25%), distorted or shrivelled seeds with white crust 42 (01.25-22.75%) and insect damaged or cracked seeds 37 (02.25-31.25%). The seeds having oozing, water soaked symptoms, shrivelled and discolored seeds 110 (07.50-39.25%) (Table 2). The infected seeds with symptoms act as primary inoculums [6]. Gupta et al. (1989) [7] observed discolorations on the surface of okra seeds at micropylar end caused by *Alternaria alternata, Drechslera* sp., *Cuvularia lunata* and *Aspergillus* spp. Brinjal seeds in storage infested by *Aspergillus flavus, A. niger, Fusarium moniliforme* and *Helminthosporium tetramera* [8].

(ii) Incubation Test

A total of 21 fungal species of 14 genera were yielded on SBM. Among them *Alternaria alternata* (06-33%), *A. solani* (05-31%), *Aspergillus flavus* (03-40%), *A. niger* (02-36%) *Curvularia lunata* (03-21%), *Fusarium oxysporum* (03-27%), *F. solani* (03-31%), *Rhizoctonia bataticola* (05-19%) and *Rhizopus nigricans* (05-27%) were found dominant fungi. Chlorine pretreated seeds reduces the incidence of saprophytic fungus and enhances seed germination (Table-2 and Table-3).

(iii) Incidence on agar plate method (APM)

Fusarium oxysporum, F. solani, Aspergillus flavus, A. niger, Curvularia lunata, Alternaria solani and *Rhizctonia bataicola* had high per cent incidence on PDA media. Except the fungi reported on SBM there are a few fungi named as *Aspergillus terreus, Rhizctonia solani, F. pallidoroseum, Trichoderma roseum, T. harzianum, Memnoniella echinata* and *Chaetomium murorum* were also found on PDA. The bacterial species *Pseudomonas syringae* pv. *syringae, Xanthomonas campestris* pv. *campestris* and *Erwinia caratovora* were also reported on nutrient agar media (NA) with low incidence.

(iv) Germination percentage

Poor germination was noticed in stored seeds depends on period of storage. The seedlings yield from infected seeds was found stunted and died prematurely infected with *F. moniliforme* and *F. solani*. Seeds infected with various species of *Aspergillus* showed decline in germination to an extent of 45%. Whereas seeds infected with *Alternaria alternata, A. solani* and *Curvularia lunata* reduce germination up to 39%. Similar results have been reported on brinjal seeds infected by *Alternaria solani* [9,10] and *A. porri.*

(v) Phytopathological Effects

The microflora associated with brinjal seeds caused adverse effect to seed germination and produce various symptoms on seedlings. Seeds germination was found improved after treatment in SBM. The shriveled or ungerminated seeds have oozing and rotting on SBM or such seeds showed poor and delayed germination. In this study it was observed that the germination was hampered by the species of *Alternaria, Aspergillus, Chaetomium, Cladosporium, Curvularia, Fusarium, Penicillium, Rhizopus* and *Rhizoctonia*. *Alternaria alternata, A. solani* and *Rhizoctonia bataticola* produced brown black streaks on hypocotyls, browning of radicle which later on at severe infection the whole seedling collapses. Various species of *Aspergillus* caused brown to black lesions on hypocotyls and browning of radicle. Infection of species of *Fusarium* showed yellowing, dying and wilting of seedlings.

Alternaria alternata causes shriveling, discolourations in seeds, reduced seed germination and seedling blight in other crops [11, 12]. Fruit rot in brinjal is caused by *Alternaria alternata* [13] and *Rhizopus nodosus* [14]. Wilt and fruit rot caused by various species of Fusarium [15], *F. oxysporum* f.sp. *melongenae* [16], *Verticillium dahliae* [17], *Phomopsis, Alternaria, Colletotrichum, Fusarium, Rhizopus and Cercospora* [18] and *Phoma* spp, *Botryodiplodia theobromae, Rhizopus* spp., *Absidia sp*. and *Fusarium* spp. [19].

Mehta and Mehta (1989) [20] found that fruit rot *Trichosanthes dioica* and *Solanum melongena* caused by *Fusarium oxysporum* and *F. moniliforme* respectively. *Macrophomina phaseolina* was found the most pathogenic in germination trials produce both sclerotia and pycnidia on seed surface that predominantly reduce the seed quality in cucurbits [21] and chilli [22]. Black-dot root rot of eggplant caused by *Colletotrichum atramentarium* [23], leaf spot disease *Colletorichum dematium* [24] produced symptoms on leaves *Erysiphe polyphaga* [25] with whitish powdery areas on both the leaf surfaces. Seeds with white crust, discolourations and water soaked symptoms yielded Actinomycetes spp., *Xanthomonas axonopodis* pv. *vesicatoria, Ralstonia solanacearum* and *Pseudomonas aeruginosa* in this

ISSN: 2349-8889
Volume-8, Issue-1 (January 2021)

https://doi.org/10.31033/ijrasb.8.1.2

study. Such symptoms were also observed in chilli, tomato [12], pea [26], okra [27] and many other crops.

IV. CONCLUSION

In this study it is evident that samples collected from different parts of Rajasthan had considerable microflora. The percent incidence of the fungi was higher in the blotter test. The seed samples had poor germination due to storage. It is general practice in some areas that ripen fruits are dried in sun light on the soil before collecting the seeds. This practice may lead to contamination of the seeds by soil containing fungi. Species of *Fusarium, Aspergillus* produce toxins which may hasten the reduction in germination. At the time of seed collection from field the seed sample contaminated with debris promote the growth of fruit rot pathogens. Proper seed cleaning and seed dressing is essential to avoid losses in storage and control of seed-borne fungi.

V. ACKNOWLEDGEMENT

Authors are grateful to administration of VMOU, Kota; Shri Agrawal Shiksha Samiti, Jaipur, Principal, Agrawal P.G. College, Jaipur, for their valuable support, guidance of faculty members of P.G. Department of Botany, Agrawal PG College, Jaipur. The authors are also thankful to all the scientists whom work is cited and could not acknowledge unknowingly and persons that directly or indirectly engaged in writing in this paper and during practical work.

Table 1: Disease incidence occurs in dry seed examination (DSE) in brinjal seed grown in different locations of Rajasthan

S. No.	Districts	Total number of seed samples	Number of seed samples infected
1.	Jaipur	51	51(03.25-38.75)
2.	Dausa	6	06(12.25-25.50)
3.	Tonk	8	08(06.75-14.50)
4.	Jhunjhunu	5	05(05.25-13.75)
5.	Kota	7	07(08.75-19.25)
6.	Alwar	2	02(08.25-10.75)
7.	Sikar	8	08(05.25-34.75)
8.	Jalor	5	05(06.25-21.75)
9.	Nagaur	3	03(07.25-18.00)
10.	Bikaner	8	08(08.75-29.75)
11.	Jodhpur	2	02(11.25,17.50)
12.	Ajmer	5	05(09.25-21.75)
	Total	**110**	**110 (03.25-38.75)**

Table 2: Changes in seed morphology, percent incidence of pathogens associated with brinjal seeds

S. No	Type of seed discolouration	Occurrence	Incidence (RPO)	Microorganism associated with seeds
1.	Brown or black discoloured	57	03.50-32.75%	*Curvularia* spp., *Drechslera* spp. and *Alternaria* spp.
2.	Seed with fruiting bodies	18	03.25-17.75%	*R. bataticola, Aspergillus* spp
3.	White mycelial crusted seeds	49	01.25-25.75%	*Fusarium oxysporum, F. moniliforme,* Actinomyeetes spp.
4.	Brown spotted discoloured	51	03.25-31.25%	*Alternaria* spp., *Curvularia* spp., *Chaetomium* and *Myrothecium* spp.
5.	Distorted or shrivelled seeds with white crust	42	01.25-22.75%	*Aspergillus* spp., *Penicillium* spp., *Fusarium* spp.
6.	Insect damaged or cracked seeds	37	02.25-31.25%	*Aspergillus* spp., *Chaetomium* spp., *Pencillium* spp. *Rhizopus* spp., insects and

				eggs.
7.	Seeds with oozing, white crust, water soaked, shrivelled and discoloured	110	07.50-39.25%	*Ralstonia solanacearum, Xanthomonas axonopodis* pv. *vesicatoria*, *Pseudomonas aeruginosa*

Table 3: Different percent range of microflora in untreated and pre-treated seeds and their phytopathological effects

S. No.	Fungi	Untreated			Pre-treated			Phytopathological effects
		Occurrence	RPO	Range (%)	Occurrence	RPO	Range (%)	
1	Actinomycetes	**21**	**19.09**	**01-15%**	**08**	**07.27**	**1-7%**	Delayed germination
2	*Alternaria alternate*	**37**	**33.63**	**06.63%**	**33**	**30.00**	**2-20%**	Leaf blight and leaf spot, symptoms on hypocotyls and cotyledons
3	*A. solani*	**33**	**30.00**	**05-31%**	**23**	**20.90**	**4-23%**	
4	*Arthrobotrys superb*	**17**	**15.45**	**02-13%**	**12**	**10.90**	**1-8%**	Seed rotting, discolourations
5	*Aspergillus flavus*	**43**	**39.09**	**03-40%**	**32**	**29.09**	**2-25%**	Yellowing and rotting of seedling and leaf blight
6	*Aspergillus niger*	**39**	**35.45**	**02-36%**	**33**	**30.00**	**2-26%**	
7	*Aspergillus fumigates*	**13**	**11.81**	**03-9%**	**07**	**06.36**	**1-3%**	
8	*A. candidus*	**09**	**08.18**	**03-7%**	**04**	**03.63**	**1-3%**	
9	*Chaetomium globosum*	**17**	**15.45**	**03-12%**	**09**	**08.18**	**1-7%**	Seed rotting, check the seed growth
10	*C. spinosum*	**11**	**10.00**	**01-10%**	**06**	**05.45**	**1-6%**	
11	*Cladosporium oxysporium*	**25**	**22.72**	**03-21%**	**13**	**11.81**	**2-17%**	Seedling rotting and seedling blight
12	*Colletotrichum dematium*	**15**	**13.63**	**01-12%**	**09**	**08.18**	**1-6%**	Fruit rooting and seedling rot
13	*Curvularia lunata*	**25**	**23.63**	**03-21%**	**17**	**15.45**	**1-13%**	Leaf spot and rotting
14	*C. intermedia*	**12**	**10.90**	**04-10%**	**07**	**06.36**	**1-5%**	Leaf spot and rotting
15	*Fusarium equiseti*	**11**	**10.00**	**02-8%**	**08**	**07.27**	**1-4%**	Leaf spot and rotting die back, wilting and damping off
16	*F. semitectum*	**09**	**08.18**	**01-5%**	**06**	**05.45**	**1-3%**	
17	*F. moniliforme*	**39**	**32.72**	**04-30%**	**28**	**25.45**	**1-27%**	
18	*F. oxysporum*	**31**	**28.18**	**03-27%**	**24**	**21.18**	**2-13%**	
19	*F. solani*	**37**	**33.63**	**03-31%**	**29**	**26.36**	**1-16%**	
20	*Penicillium chrysogenum*	**23**	**20.90**	**03-15%**	**13**	**11.81**	**1-6%**	Hampered the seed germination, Seedling rot
21	*Rhizoctonia bataticola*	**25**	**22.72**	**05-19%**	**18**	**16.36**	**2-13%**	Seed rot, blackening of radical and hypocotyls, stunted seedling
22	*Rhizopus nigricans*	**32**	**29.09**	**05-27%**	**13**	**11.81**	**1-11%**	Collar rot, root rot and seed rot

23	*Verticillium alboatrum*	**11**	**10.00**	**01-8%**	**06**	**05.45**	**1-3%**	Wilting, root rotting and browning, leaf blight
24	*Stachybotrys atra*	**22**	**20.00**	**02-19%**	**17**	**15.45**	**1-9%**	Rotting of seeds and seedling
S. No	**Bacteria**	**Untreated**			**Pre-treated**			**Phytopathological effects**
		Occurrence	**RPO**	**Range (%)**	**Occurrence**	**RPO**	**Range (%)**	
1.	*Ralstonia solancearum*	**75**	**68.18**	**08-70%**	**62**	**56.36**	**4-65%**	Browning on seedlings, wilting of seedlings, collapse of vascular system
2.	*Pseudomonas aeruginosa*	**46**	**44.54**	**02-56%**	**42**	**38.18**	**2-32%**	Leaf spot, seedling rot and root rot
3.	*Xanthomonas axonopodis* pv. *vesicatoria*	**62**	**56.36**	**08-82%**	**53**	**48.18**	**3-76%**	Spot and leaf blight, seedling rotting

RPO=* Relative Percent Occurrence

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ISSN: 2349-8889
Volume-8, Issue-1 (January 2021)

www.ijrasb.com https://doi.org/10.31033/ijrasb.8.1.2

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