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INDIRA DEVIMOIRANGTHEM, P.S. NATH AND R.MONDAL

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Department of Botany,
University of Calcutta,
Kolkata 700 019, India

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Efficacy of different seed health testing methods for detection of seed borne fungal infection of Okra (*Abelmoschus esculentus*)

INDIRA DEVIMOIRANGTHEM[1*], P.S. NATH[2] AND R.MONDAL[2]
[1]Department of Plant Pathology, School of Agricultural Sciences, Nagaland University, Medziphema- 797106, Nagaland
[2]Department of Plant Pathology, Bidhan Chandra Krishi Vishwavidyalaya, Mohanpur, Nadia-7412522,West Bengal

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Seed samples of Okra collected from different small holder farmers were studied for the occurrence of seed borne fungi *in vitro*. Prevalence of seed borne fungi was analysed by five different incubation methods viz. Standard blotter method (SBM), Deep freezing method (DFM), Malt extract method (MEM), Potato dextrose method (PDA) and Water agar method (WAM). Five fungal pathogens belong to *Aspergillus flavus*, *A.niger, Fusarium* sp., *Helminthosporium* sp. and *Rhizopus stolonifer* were identified. The study on Okra seed confirmed that unsterilized (untreated) seeds show dominant occurrence of saprophytic fungi *Rhizopus* and *Aspergillus* as compared to sterilised (treated) seeds. Among the different incubation methods, Potato dextrose method is the best method for isolation and detection of different fungal pathogens followed by Standard blotter method. Dominance of saprophytic fungi such as *A.flavus, A.niger* and *R.stolonifer* were found in PDA as compared to SBM. The average percent seed infection was highest in PDA followed by SBM, ME and least in DFM. Both Potato dextrose method and Standard blotter method were statistically at par as pathogens were concerned. Untreated seeds collected from farmers showhigher percentage of incidence of fungal micro flora population than the treated seeds. Untreated seeds in majority of cases deteriorated by fungi on the other hand treated seeds showed reduction in fungal population on the seeds and thus it may be beneficial.

Keywords: Incubation methods,Okra, seed borne fungi, treated, nntreated

INTRODUCTION

Okra (*Abelmoschus esculentus*) is an important vegetable crop widely grown in tropical, subtropical and warm temperate regions of the world. It belongs to the genus Abelmoschus and family Malvaceae. It is good source of minerals, vitamins, antioxidants and fibre. In India, major okra growing states are basically Uttar Pradesh, Bihar and West Bengal.Seed is the most important input for crop production. It is estimated quality os seed accounts for 20-25% of productivity (Koundinya and Kumar, 2014).Almost 90% of the world's food crops are grown from seeds (Abdulsalam and Shenge, 2011).

Therefore, use of clean seed is an important disease control measure because, seeds transmit plant pathogens and seed is exchanged worldwide.Seed borne diseases are able to spread across international borders very easily and are often difficult to identify as their typical symptoms being rare on seed surfaces except in some legumes as economic impact has increased in recent years their important has increased with regards to many kinds of crop worldwide (Nishikawa *et al*. 2006). The association of various fungi with vegetable seeds has been reported all over the world (Dumbrel *et al.* 2011; Summiaya and Dawar, 2015; Zakaria *et al*. 2014).Therefore, seed health testing to detect seed borne pathogens is vital step in management of crop diseases.

Various factors are responsible for low yield of Okra. Seed borne fungal diseases are often the main cause. There are 10 seed borne diseases of Okra which are cause by at least 14 different seed borne fungal pathogens (Fakir, 2000).Therefore, this study was conducted with the objective of finding out the prevalence of seed borne pathogens of Okra employing different incubation methods.

*Correspondence :Indiramoirangthem2358@gmail.com

MATERIALS AND METHODS

Collection of seeds

To know the extent of seed-borne diseases of Okra, seed samples were collected from different vegetable growing districts of West Bengal in the year 2015-17. Seeds were collected from farmers of most vegetable growing districts (Nadia, 24 Parganas, South 24 Parganas and Hoogly) after harvesting of the crops. Seed samples each of Okra (local variety) were mixed thoroughly to make a composite seed samples.
Seed samples of Okra (local variety) collected were drawn random. The seeds were surface sterilized with 1% Sodium hypochlorite for one minute followed by rinsing in sterile water thrice. The seeds were divided into five lots. One lot of each seeds were incubated in the following five methods by ISTA method (1996). Ten seeds of Okra were place equidistant from each other respectively. To know the efficacy of different seed health testing methods in detecting seed-borne fungi following methods were employed as described below.

Three pieces of blotting paper of 90 mm size were moistened with distilled water and placed in 90 mm sterilized petridishes after draining excess water. Untreated seeds placed were at equal distance in each petridishes. The plates were incubated at room temperature (25±2°C). After 7 days of incubation, the seeds were examined under stereoscopic binocular microscope for the associated fungi (Khare, 1996).

Deep Freezing blotter method

Deep freezing blotter method is similar to standard blotter method but the petridishes were incubated at 25±2°C for first 24 hrs and for next 24 hrs the plates are incubated at -20°C and then kept back under original conditions for next 6 days.

Water agar

In water agar method seeds were placed in petridishescontaing 20 ml of 2 per cent water agar. Untreated seeds placed were at equal distance in each petridishes. The plates were incubated at room temperature (25±2°C). After 7 days of incubation, the seeds were examined under stereoscopic binocular microscope for the associated fungi (Khare, 1996).

Agar plate method with malt extract

In agar plate method with malt extract agar surface sterilized seeds were placed in petridishes containing 20 ml of malt extract agar respectively and were incubated for 7 days as described under standard blotter method. After 7 days of incubation the fungal growth was examined under stereoscopic binocular microscope.

Agar plate method with potato dextrose agar

In agar plate method with PDA surface sterilized seeds were placed in petridishes containing 20 ml of PDA respectively and were incubated for 7 days as described under standard blotter method. After 7 days of incubation the fungal growth was examined under stereoscopic binocular microscope.Percentage of incidence was calculated as:

$$\% \text{ incidence} = \frac{\text{No. of seeds on which a species appeared}}{\text{Total No. of seeds observed}} \times 100$$

RESULTS AND DISCUSSION

Five different incubation methods such as Standard blotter method (SBM), Deep freezing blotter method (DFM), Malt extract method (MEM), Potato dextrose agar method (PDA) and Water agar method (WAM) were employed for detection of pathogen if any. Before placing the seeds in different methods the seeds were dipped in 1%Sodium hypochloride solution were considered as treated and those dipped in distilled water considered as untreated seeds. Efficacy of different methods for detection of fungal infection in okra seeds (untreated and treated) was presented in Table 1. It was found that PDA was the best method for isolation and detection of different fungal pathogens followed by SBM. Five fungal pathogens were isolated from PDA viz. *Aspergillus flavus, Aspergillus niger*, *Fusarium* sp., *Helminthosporium sp.* and *Rhizopus*

Table 1: Efficacy of different seed health testing methods for detection of seed-borne fungal infection of Okra

Pathogen Associated	Per cent seed infection											
	Untreated						Treated					
	A	B	C	D	E	Mean	A	B	C	D	E	Mean
Alternaria alternata	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)
Alternaria solani	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)
Aspergillus flavus	33.33 (5.81)	10.00 (3.08)	30.00 (5.56)	33.33 (5.89)	26.67 (5.13)	30.67 (5.46)	26.67 (5.15)	6.67 (2.67)	20.00 (4.64)	23.33 (4.96)	30.00 (5.56)	21.33 (4.60)
Aspergillus niger	30.00 (5.56)	6.67 (2.67)	23.33 (4.96)	30.00 (5.56)	20.00 (4.55)	22.00 (4.66)	16.67 (4.13)	3.33 (1.9)	16.66 (4.22)	20.00 (4.55)	13.33 (3.81)	14.00 3.73
Curvularialunata	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)
Colletotrichum sp	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)
Fusarium sp	6.67 (2.67)	3.33 (1.95)	0.00 (0.71)	16.67 (4.22)	0.00 (0.71)	5.33 (2.26)	3.33 (1.95)	0.00 (0.71)	0.00 (0.71)	10.00 (3.08)	0.00 (0.71)	2.67 1.74
Helminthosporiums sp.	13.33 3.50	0.00 (0.71)	3.33 (1.95)	6.67 (2.67)	0.00 (0.71)	4.67 (2.11)	6.67 (2.67)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	1.33 (1.35)
Rhizopus sp.	16.67 4.22	0.00 (0.71)	10.00 (3.24)	13.33 (3.81)	0.00 (0.71)	8.00 (2.34)	10.00 (3.24)	0.00 (0.71)	6.67 (2.67)	6.67 (2.67)	0.00 (0.71)	4.67 (2.32)
Mean	11.11 (3.33)	2.22 (1.49)	7.41 (2.72)	11.11 (3.33)	7.41 (2.72)		7.04 (2.65)	1.11 (1.05)	4.81 (2.19)	6.67 (2.58)	4.81 (2.19)	
Factors	Pathogen		Methods	Pathogen x Methods			Pathogen		Methods	Pathogen x Methods		
C.D. (P=0.05)	0.52		0.38	1.15			0.49		0.36	1.09		
S.Em. (±)	0.18		0.14	0.41			0.17		0.13	0.39		

* Figures in the parentheses indicate square root transformed values

A- Std. Blotter Method; B- Deep Freezing Blotter method; C- Malt Extract Method; D-PDA Method; E- Water Agar Method

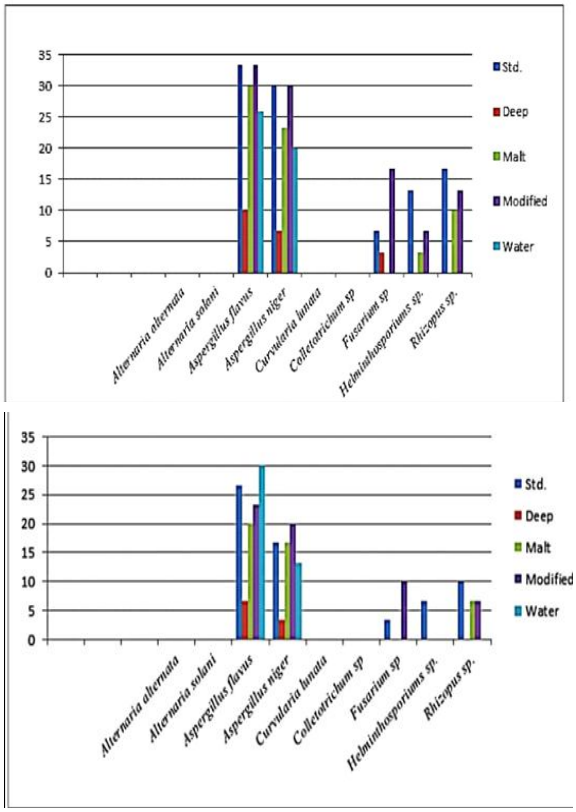

Fig.1 : Efficacy ofdifferent seed testing methods for detection of seed borne fungal infection in Okra as indicated by per cent frequency of isolated fungi in Untreated (top) and treated (bottom) figures.

stolonifer. Both PDA and SBM are statistically at par as far pathogens were concerned.

As evident from Table1 and Fig.1, the fungal species isolated from Okra seeds (untreated) in PDA method, were *Aspergillus flavus*, *Aspergillus niger*, *Fusarium sp., Helminthosporium sp* .and *Rhizopus stolonifera*; the frequency percentage rate in this method was 33.33, 30.00, 16.67, 6.67 and 13.33 respectively. The fungal species detected following SBM were *Aspergillus flavus, Aspergillus niger, Fusarium sp., Helminthosporium sp.* and *Rhizopus stolonifer*, and the frequency percentage rate in this method was 33.33, 16.67, 6.67, 13.33 and 16.67 respectively. In MEM, four fungal pathogens were isolated i.e.*Aspergillus flavus, Aspergillus niger,Helminthosporium sp*. and *Rhizopus stolonifer* and the frequency percentage in this method were 30.00, 23.33, 3.33 and 10.00 respectively. In WAM, only two fungal pathogens were isolated i.e.*Aspergillus flavus and Aspergillus niger* and the frequency percentage in this method were 26.60 and 20.00 respectively. From DFM, the following fungal pathogens were isolated i.e.*Aspergillus flavus, Aspergillus niger*

and Fusarium sp. the frequency percentage in this method were 10.00, 6.67 and 3.33 respectively.

Similarly, efficacy of different methods for detection of fungal infection in okra seeds (treated) is also presented in Table 1 and Fig.1.It was revealed that PDA was the best method for isolation and detection of different fungal pathogens followed by SBM. Fungal species isolated from okra (treated) seeds in PDA method were-*Aspergillus flavus, Aspergillus niger, Fusarium sp, Helminthosporium sp*.and *Rhizopus stolonifer*, with frequency rates of 23.33, 20.00, 10.00 and 6.67 respectively. The fungal species detected following SBM were *Aspergillus flavus, Aspergillus niger, Fusarium sp., Helminthosporium sp*.and *Rhizopus stolonifer*; the frequency rate in this method was 26.67, 16.66, 3.33, 6.67 and 10.00 respectively. From MEM, the following pathogens wereisolated-*Aspergillus flavus, Aspergillus niger* and *Rhizopus stolonifera*; the frequency percentage rate were 20.00, 16.66 and 6.67 respectively. The fungal species isolated from DFM, *Aspergillus flavus* and *Aspergillus niger* and the frequency percentage rate were 6.67and 3.33 respectively. In WAM, the following fungal pathogens were isolated i.e.*Aspergillus flavus* and *Aspergillus niger* and the frequency percentage in this method were 30.00 and 13.33 respectively. It was noted that untreated seeds have occurrence of good number of fungi *Aspergillus niger, A.flavus* and *Rhizopus stolonifer* indicating that untreated seeds have more dominance of saprophytic fungi. The studies on Okra seeds showed that untreated seeds in majority of cases deteriorated by fungi on the other hand treated seeds showed reduction in fungal population on the seeds and thus it may be beneficial

The present findings clearly showed that from tested seed samples considerable number of seed borne fungal pathogens belonging to different genera *Aspergillus flavus, Aspergillus niger, Fusarium sp., Helminthosporium sp*.and *Rhizopus stolonifer* had been detected in okra seeds. A considerable number of seed borne fungal pathogens belonging to different genera *A.niger, Fusarium, Penicillium, Colletotrichum and Macrophomina* had been detected in okra seeds by many researchers (Akter, 2008; Fakir, 2000: Jamadar *et al*. 2001; Islam, 2012; Sultana, 2009). The result is in close proximity with the findings of Summiaya *et.al.* (2015) reported that agar plate method was found best for isolation of fungi followed by SBM in okra.

REFERENCES

Abdulsalam, S., Shenge, K.C.2011. Seed borne pathogens on farmer saved sorghum (*Sorghum bicolour* L.) seeds. *J. Stored Products and Postharvest Res*.**2**:244-28.

Akter, N.2008. Effect of plant extract on the management of seed borne fungal diseases of okra, MS Thesis, Department of Plant Pathology, BAU, Mymensingh. pp.36 and 74.

Christen, C.M., Lopez, F. 1964. Pathology of stored seeds. *Proc. Inter. Seed Testing Assn*. **28**:701-711.

Dumbrel S., GuldekarD. ,Potdukhe S.R. 2011. Survey of seed borne fungi of onion (*Allium cepa* L.) from various locations of Maharashtra. *J. Soils Crops*. **21**: 221-224.

Fakir, G.A.2000. An annotated list of seed-borne diseases in Bangladesh. Seed Pathology Laboratory, Department of Plant Pathology, Bangladesh Agricultural University Mymensingh pp.20.

Islam, S. 2012. Health and quality of okra seeds collected from monirampurupazilla of Jessore district and their treatment by plant extracts MS Thesis, Department of Plant Pathology Bangladesh Agricultural University, Mymensingh. pp.76.

ISTA 1996. International Rules for Seed Testing, Annexes 1996. *Seed Sci. and Technol.* **24** (Suppl.): 13-19, 93-122, 247-252.

Jamadar, M.M, Ashok S, Shamrao J, Sajjan A, Jahangidar S.2001. Studies on seed mycoflora and their effect on germination of colour graded okra [*Abelmoschus esculentus* (L.) Moench]. *J. Crop Res*.**22**: 479-484.

Khare, M. N. 1996. Methods to test seeds for associated fungi. *Ind.Phytopath*: 319-328.

Koundinya A. V. V. ,Pradeep Kumar P. 2014. Indian vegetable seeds industry: Status and Challenges. *Int. J. Plant Animal Environ. Sci.* **4**: 62-69.

Nishikawa, J., Kobayashi, T., Shitara, K., Chibana, T., Natsauki, T. 2006.Seed borne fungi detected on stored solanaceous berry and their biological activities. *J.Gen.PlantPathol.*, **72**:305-312.

Sultana, L. 2009. Assessment of health status of TLS (Truthfully Labeled Seeds) in the markets of Bangladesh. M.S. thesis, Dept. of Plant Pathol., BAU, Mymensingh.pp.39.

Summiaya, R., Shahnaz, D. 2015. Seed-borne mycoflora associated with okra [*Abelmoschus esculentus* (L.) Moench]. *Pak. J. Bot*. **47**:747-751.

Zakaria, L., Weng, K. S., Li, Y.T. 2014. Diversity of fungi isolated from vegetable seeds. *Malaysian J. Microbiol*. **10**: 155-160.