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Research Article

POST-HARVEST STUDY OF OKRA (*ABELMOSCHUS ESCULENTUS* (L.) MOENCH) FRUITS AND PHYTOPATHOLOGICAL EFFECT OF ASSOCIATED MICROFLORA

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ABSTRACT
Okra (*Abelmoschus esculentus* (L.) Moench of family malvaceae) is an important vegetable crop attacked by several pathogens during storage. Seventy eight fruit samples of okra from different sights i.e. market, farmers field and farm storage (houses) were collected (during 2011; 2012 and 2013) from districts of Rajasthan. Eleven major fungal (*Aspergillus flavus , A. niger, A. nidulens, A. fumigates, Alternaria alternate, Curvularia lunata, Rhizopus nigricans, Cladosporium oxysporum, Penicillium chrysogenum, P. citrinum, Stachybotrus atra, Chaetomium globosum, C. murorum, Rhizoctonia bataticola*), and four bacterial genera (*Actinomycetes sp., Erwinia caratovora, Xanthomonas campestiris* pv. *campestris*, *Xanthomonas campestiris* pv. *malvacearum, Pseudomonas syringae* pv. *syringae*) were found associated with post-harvested diseases or spoilage of okra fruits in the study. It was concluded that fungal pathogens cause the damage at high temperature, low relative humidity with poor aeration and bacterial damage at high humidity and low aeration. These pathogens showed 04–72 % loss due to said pathogens.

Key Words: *Okra, Post-Harvest Diseases, Microflora, Artificial Inoculation, Fungal Species, Bacterial Species, Post- Harvest Losses*

INTRODUCTION
Okra (*Abelmoschus esculentus* (L.) Moench) as lady's finger, is an important vegetable crop under tropical and subtropical conditions including India of malvaceae. In India, it is grown over an average of 4.52 lakh in mts. with a production of 43 Lakh tones (Anonymous, 2011).

The plant is an erect, coarse, robust annual herb in which flowers are borne singly in the leaf axile on peduncles. It has 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 approximately contains; 86.1% moisture, 9.7% carbohydrates, 2.2% protein, 1.0% fibers, 0.2% fats, and 0.9 % ash. The ripen 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; Sharma *et al.*, 2013).

The diseases of okra caused by microorganisms i.e. fungi, bacteria, viruses either due to contaminations and infections occur during growing season or due to infection incidental during harvesting, processing and packing or transportation process.

The amount of damage and loss associated with the diseases varies greatly with commodity, processing, growing conditions and the way of handling. The post-harvest diseases spooned during transit and storage throughout the country and sources of continuous drain on our already acute food situation and lead to a

waste of labor, time, land and money. In India, no exact estimates of such losses are available for post-harvest or storage diseases of vegetable.

Since the associated microflora reduces the quality, price rate or market value of this vegetable therefore aim of present investigation is to study the major fruit diseases in storage and their post-harvest impact on stored fruits.

MATERIALS AND METHODS

The study was classified in two parts related to collection of fruit samples and secondly study of micro flora associated with stored fruit samples. The study has been classified in following headings as: (a) Pathogens affecting seed and fruits storage (b) Potential losses due to different microorganism and (C) Storage of fruits.

(a) Pathogens Affecting Seed and Fruits Storage

Naturally infected seeds and fruits of okra were collected at different stages of growth from farmer's fields, houses and market. To avoid rot or disease study the fruits collected to be free from mechanical injury, surface of fruits sterilized and stored at cool, proper ventilated space.

The symptoms during and after storage of selected to infected fruits were recorded. The infected tissue of these fruits were surface sterilized and plated on Petriplates containing Potato Dextrose Agar (PDA) medium under aseptic conditions.

The microflora and causal organisms were transferred to PDA and cultures were again plated and reisolated (Anonymous, 1996). The pathogenicity of microorganism was confirmed by inoculating healthy fruits for identical symptoms. All the bacterial cultures were isolated cultured and maintained on Nutrient Agar (NA) medium.

(b) Potential Losses due to Different Microorganism

For the study, 78 fruit samples ok okra from different sights i.e. market, farmers field and farm storage (houses) were collected in growing seasons 2011; 2012 and 2013 from major growing districts of Rajasthan. Incidence of various post-harvest diseases caused by different microorganisms on fruit and seeds were recorded immediately after sample collection.

The causal organism and symptoms of particular diseases was attributed after isolation, identification and pathogenicity test. The rotting of fruits by various fungal and bacterial pathogens in storage was induced by artificial inoculation to assess the potential losses.

(C) Storage of Fruits

Different storage methods were tried to find out the best one for storing the okra fruits. Freshly harvesting undamaged fruits were used for storage study to increase the self life. Such healthy fruits were first spread in single layer in shaded and ventilated place in layers and above the other (stack), plastic container, Iron container and air tight polyethen bags.

RESULTS AND DISCUSSION

(a) Pathogens Affecting Seed and Fruits Storage

Eleven major fungal and four bacterial genera that caused post-harvested diseases or spoilage of okra fruits were identified and isolated in different storage methods.

The fungal pathogens were identified to cause the damage at high temperature, low relative humidity with poor aeration. The symptoms caused by various pathogens have been described (table 1).

Table 1: Microorganisms associated with fruit diseases of okra under natural conditions

S. No.	Microorganisms associated with fruits	Characteristics of microorganism	Disease symptoms on fruits
		Fungal species	
1.	*Aspergillus flavus* *A. niger* *A. nidulens* *A. fumigates*	Heavy mycelia growth, mycelium bears conidiophores with conidia in basipetal order. Colour of colony was characteristic property in identification	Fruit rotting (rotten tissue become soft with browning and decay), whitish mass of fungal growth visible on infected fruits and tissue become soft and spongy (placenta of fruit also showed browning).
2.	*Alternaria alternata*	Mycelium was knotty, brown and profusely branched, conidia on conidiophores either various type of septa or oval multinucleate.	Tissue rotting and rotten tissue gives bad smell. Rotted tissue becomes spongy and black mold like or pin headed growth can be seen on the supper surface of fruit.
3.	*Curvularia lunata*		
4.	*Rhizopus nigricans*		
5.	*Fusarium moniliformae* *F. equiseti*	White cottony mycelium, highly branched, conidia semi- lunar type with transverse septa.	Rotted tissue covered with white mycelium spreaded over complete decay or rot.
6.	*Cladosporium oxysporum*	Brown to black mycelium, with erect conidiophores that bears conidia.	Brownish or blue mycelium growth on the fruit surface. The conidiophore bears conidia in basipetal order.
7.	*Penicillium chrysogenum* *P. citrinum*		
8.	*Stachybotrus atra*		
9.	*Chaetomium globosum*	Brown multicellular fruiting bodies that covered by setae or brown knotty mycelia with conidia.	Brown fruiting bodies on the fruit surface were observed. The knotty mycelia with conidia showed profuse growth.
10.	*C. murorum*		
11.	*Rhizoctonia bataticola*		
		Bacterial species	
1.	*Erwinia caratovora*	Gram's negative or positive, KOH solubility test positive or negative, cocci or rods shaped bacteria, fluorescent or non fluorescent species on KMB media.	The infected tissues turn watery show decay, rotting (fowl smell), brown spots; crust, oozing can be seen on rotting fruit surface.
2.	*Xanthomonas campestiris* pv. *campestris*		
3.	*Xanthomonas campestiris* pv. *malvacearum*		
4.	*Pseudomonas syringae* pv. *syringae*		
5.	*Actinomycetes sp.*	Gram's positive, filamentous bacteria with cottony growth.	White cottony mycelia growth on fruit surface responsible for rotting and quality if fruit.

(b) Potential Losses due to Different Micro Organism

Aspergillus species were the most common xerophyte fungal pathogen followed by species of *Alternaria, Rhizopus, Rhizoctonia, Chaetomium, Fusarium, Penicilium* and *Stachybotrytis* respectively. The bacterial pathogens *i.e. Erwinia caratovora, X. campestris* pv. *campestris, X. campestris pv. Malvacearum* caused fruit rot (fowl smell), whitish oozing and crust on fruits and seed surface.

Minor incidence on fruits was identified by the species of *Curvularia, Fusarium, Penicilluim* and *Stachybotrytis* species respectively was observed. Out of all listed bacterial pathogen. *Erwinia carotovora* (gram's positive bacteria) caused serious damaged in fruit when stored in polyethene bags without proper aeriation.

The fruits infected turned brown to black, watery (produced fowl smell).

In post-harvest studies, it was reported that *Cladosporium* sp. (Tandon and Verma, 1964) in Jammu, *Fusarium oxysporium* in Bombay (Maharastra), *Helminthosporium nodulosum* from Aurangabad (U.P.) (Tandon and Verma, 1964) and *Pythium indicum* in south India (Balakrishanan, 1948) responsible for fruit rot, root rot and seed rot in okra.

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 *et al.,* 1986 a, b) were studied. Similar observations were reported in chilli (Bhale *et al.,* 2000; Panwar and Vyas, 1974), moth bean and cowpea (Verma, 1990), in cluster bean (Singh, 1953; Mihali and Alcorn, 1986) and cucurbits (Maholay, 1989; Maholay and Sohi, 1985; Chandi and Maheshwari, 1992). Dwivedi and Tandon (1976) reported nine fungal species on bottle gourd viz. *Aspergillus flavus, A. niger, Alternaria alternata, Fusarium oxysporum* and *Rhizopus nigricans* associated with seed coat and cotyledons.

The seeds of infected fruits containing seeds with white crust, water soaked symptoms were observed and such seeds on incubation yielded bacteria (Sharma *et al.,* 2013).

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 *et al.,* Ekpo and Okechukwu, 2010)), chickpea (Reddy *et al.,* 1986), crucifer seeds (Schaad and Kendrick, 1975; Schaad, 1989), rape and mustard seeds (Sharma *et al.,* 1992, 2002), pigeonpea (Sharma *et al.,* 2001), cabbage (Bandyopadhyay and Chattopadhyay, 1985; Siraree *et al.,* 2008), sunflower (Ataga, and Akueshi, 1986; Godika *et al.,* 2000) and black gram (Agrawal *et al.,* 2010) and okra (Agrawal, 2000; Sharma *et al.,* 2012).

The 32 fungal species on okra seeds from Jaipur, Rajasthan viz. Actinomycetes, *Arthrobotrys supberba, Aspergillus fumigates, Cladosporium oxysporum, Drechslera* sp., *Fusarium moniliforme, Stachyobotrys* spp., *Verticillium alboatrum* and 3 bacterial species namely *Ralstonia solanacearum* (Smith) Yabuuchi *et al., Pseudomonas syringae* var. *syringae* van Hall and *Xanthomonas axonopodis* var. *malvacearum* (Smith) Vauterin were reported.

These microorganisms not only lowered the market value of fruit but also carry the hazardous toxins with them that are harmful to the human beings and the animals. In the present investigation three important mycotoxigenic fungs viz. *Aspergillus flavus*, *Fusarium moniliforme* and *Penicillium* species the main produces of alflatoxin, zearatename and citrinin respectively were commonly occurred on seeds of infected fruits of okra.

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; Pandey and Gupta, 1986). All the indentified pathogens were isolated, pure colony raised and maintained on their respective medium. For the pathogenecity test, the fruits were artificially inoculated in healthy fruits yielded the symptoms and pathogens reisolated. Percent fruit rot or diseased fruits induced by different pathogens after artificial inoculation as shown in Table 2.

Table 2: Showing the percentage fruit rot or disease symptoms due to artificial inoculation in healthy fruits

S. No.	Name of fungal species	Percentage loss in storage
1.	*Aspergillus*	35 – 72 %
2.	*Alternaria*	22 – 63 %
3.	*Curvularia*	10 – 31 %
4.	*Rhizopus*	11 – 49 %
5.	*Fusarium*	06 – 27 %
6.	*Cladosporium*	03 – 18 %
7.	*Penicillium*	09 – 19 %
8.	*Rhizoctonia*	04 – 13 %
9.	*Chaetomium*	17 – 52 %
10.	*Stachybotryus*	15 – 33 %
	NAME OF BACTERIAL SPECIES	
1.	*Erwinia*	25 – 59 %
2.	*Xanthomonas*	20 – 42 %
3.	*Pseudomonas*	10 – 48 %

**Control (Untreated check 00%)*

(C) Storage of Fruits

For the post- harvest storage a few methods were tried and result of different storage methods revealed that storage of fruits by spreading them closely in layers at low temperature avoid the pathogen spreading in the storage. In storage, the mechanically or physically injured fruits during transit were found prone to infection and such fruits should store separately to avoid fruit to fruit (post-harvest) spreading of pathogens. The removing of diseased fruits from the storage found the best traditional methods of storage. In the laboratory experiments, storage of fruits at 80–95 RH in between 5–10^{o}C temperature in properly ventilated container found best method to improve the self life of the fruits for 15 – 20 days. The storage container should properly ventilate for long storage due to high respiration rate of okra fruits shows fast spoilage. In present study, three important mycotoxigenic fungal species (secrets carcinogenic fungal toxins) were commonly occurred on seeds and fruits of okra have been occurred. *Botryodiploridia theobromae*, *Fusarium* species and *R. oryzae* were reported to cause infection in other tropical tubers also (Acholo *et al.*, 1997; Ray *et al.*, 1996). Sweet potato tubers are also prone to several post-harvest pathogens (Schaad and Brenner, 1977; Ray and Mishra, 1996).

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