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RESEARCH ARTICLE

Rot Causing Capacity of Fungi Associated with Sweet Potato [*Ipomoea batatas* (L.) Lam.] Tubers

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ABSTRACT

An extensive survey was conducted in order to assess the fungi with post-harvest decay of sweet potato tubers in market and field from different localities like- Agra, Firozabad, Etah. In all 30 samples from market and 15 samples from field were collected. The collected tubers were separated into healthy and diseased. The average length of tubers was also measured from the samples in each locality. The minimum average length of tuber was 11.4 mm in the samples Firozabad locality and maximum tuber length was 15.9 mm in the samples of Etah locality was recorded. The internal mycoflora showed 5 species i.e. Aspergillus nidulans var. acristatus, A. terreus, Cladosporium cladosporioides, Fusarium equiseti and Penicillijm melegrinum. The most abundant internal fungi were Cladosporium cladosporioides, Fusarium equiseti, Penicillium meleagrinum, which showed 27.0 to 32.4% abundance. All 5 species isolated from tubers were screened from tubers were screened for pathogenicity test. Only 3 species i.e., Aspergillus nidulans var. acristatus, Cladosporium cladosporiodes and Penicillium meleagrinum were found to cause rot by disc inoculation, wound inoculation and knife injury and spore spray inoculation technique. The maximum 15.0% rotting was shown by Aspergillus nidulans var. acristatus while Cladosporium cladosporioides showed 13.0% rot and Penicillium meleagrinum showed 14.2% rot after 20 days at 28 ± 1ºC temperature. The use of improved sweet potato variety, good storage facilities and adequate control measures need to be encouraged in order to reduce rot disease in sweet potato tubers.

Key words: *Sweet Potato, Fungi, Pathogenicity, Inoculation, Post-Harvest*

INTRODUCTION

Ipomoea batatas Lamk. (Fam. convulvulaceae) commonly known as 'sweet potato' is one of the important cultivated crops of India. It is widely cultivated throughout the tropical and in some parts of temperature regions. Sweet potato is grown as annual herbaceous vine producing succulent tuberous roots. The area under sweet potato cultivation in India is about 4 lakh acres, the major areas are Bihar, Uttar Pradesh, Mysore, Kerala, Mumbai, Orissa, Madras and Madhya Pradesh.

The sweet potato is largely attacked in the fields by several fungal, bacterial and viral diseases which reduce the yield of underground tubers. Some of the important fungal diseases are leaf spot caused by *Cercospora batatae*, white blister caused by *Cystopus ipomoeae Panduratae* (Anonymous, 1950), Black rot caused by *Ceratocystis fimbriata* (Lu and Feng, 1988), Rhizopus rot caused by *Rhizopus stolonifer* (Sinha and Prasad, 1986) and collar rod caused by Sclerotium rolfsii (Sivaprakash and Kandaswamy, 1983 and Kareppe 1991) and scap disease caused by *Sphaceloma batatas* (Bagit and Gapasin, 1987 and Paningbaton, 1987).

A number of other pathogenic form are also known which cause high damage to the tubers in fields, market and storage in post-harvest conditions. Oyewale (2006) reported that a number of fungi viz. *Motierella ramanniana, Rhizopus stolonifer, Mucor pusiluss, Botrytis cinerea, Erysiphe polygoni* and *Aspergillus flavus* were associated with post-harvest fungal rot of sweet potato. According to a report of Charles Tortae *et al.* (2010), *Aspergillus flavus* was the most dominant fungal species during post-harvest storage condition of sweet potato followed by *Aspergillus niger, Rhizopus stolonifer, Trichoderma viride, Fusarium oxysporum, Penicillim digitatum, Cladosporium herbarum* and *Aspergillus ochraceus*. Washington (2013) reported the soft rot

disease of sweet potato storage roots and post-harvest storage rot by the fungi *Fusarium solani* and *Macrophomina phaseolina*.

The present study was carried out to isolate and identify fungi caused rot disease in sweet potato tubers. The significance of the present work lies with the rapid incidence of decay of the vegetable and its management.

MATERIALS AND METHODS

COLLECTION OF SAMPLES:

The samples were collected in sterilized glossy bamboo paper bags to avoid external contamination. The bags were wraped in packets and sterilized in autoclave for 1 hrs at 15 lbs pressure and 121ºC temperature before sample collection. After sterilization the packets were kept in hot air oven at 60ºC for 3 hrs to remove the moisture. The samples were collected from different localities of Agra, Firozabad and Etah (Table- 1) from both fields and markets.

Table 1: Shows localities of sample collection

S.No.	Localities	No. of Samples collected		Average tuber Length in cm	% Infected tubers
		Market	Field		
1.	Agra	10	5	12.3	7.3
2.	Firozabad	10	5	11.4	5.8
3.	Etah	10	5	15.9	7.6
	Total Number of Sample collected	30	15	–	–

Each sample contained 1 kg (12–15 tubers)

The samples were brought to the laboratory and separated into healthy and diseased tubers. The sizes of some tubers were measured and percentage of diseased tubers was determined in all the collected samples.

ISOLATION OF MYCOFLORA:

The collected tubers were subjected for the isolation of mycoflora to know the spectrum of various fungi present in internal tissue.

The isolation of mycoflora was done on PDA and Czapek's Dox Agar medium. Infected tubers were surface sterilized by treating with 0.2% aqueous solution of mercuric chloride ($HgCl_2$) for 2 minutes. The treated tubers were washed repeatedly with sterilized distilled water to remove even last trace of $HgCl_2$. The infected tissue was cut out and small pieces were placed on the medium surface in the petridishes. Plates were incubated for 7 days at 28 ± 1ºC and observation were taken for the appearance of various fungi.

IDENTIFICATION, PURIFICATION AND MAINTENANCE OF CULTURES:

The fungi obtained in isolation were further purified by steak method. The axinic cultures were maintained on agar slants. The cultures were identified by following Thom and Raper (1945), Gilman (1957), Smith (1960), Subramanium (1961), Raper and Fennell (1965), Sarbhoy *et al.* (1975), Ellis (1976) and Sarbhoy *et al.* (1986). The culture's identify was confirmed by matching with standard identified cultures from CMI Kew, surrey, England and further confirmed was obtained from Division of Mycology and Plant Pathology IARI New Delhi.

DETERMINATION OF ROT CAUSING CAPACITY:

All the isolates obtained from sweet potato tubers were screened to establish their disease causing capacity (Koch. postulate). The healthy tubers, approximately of similar size were selected and were surface sterilized by treating with 0.2% mercuric chloride ($HgCl_2$) solution and then thoroughly washed with sterilized distilled water. These tubers were inoculated by individual isolate by following techniques.

(a) Spray Inoculation Technique:

The surface sterilized tuber were marked with the help of sterilized scalpel to cause injuries in the tissue of the tubers and then sprayed with the spore suspension of each isolated separately.

The spore suspension was prepared in sterilized distilled water from 7 days old culture of a species which was cultivated on PDA medium. After one hour when the surface of the tubers became free from sprayed water the inoculated tubers were placed in sterilized paper bags and inocubated at 28 ± 1ºC for 20 days. The tubers inoculated without injury were kept as control under identical conditions.

(b) Wound Inoculation Technique:
This method has been described by Granger and Horne (1924). In this method the surface sterilized tuber was cut 1.5 cm deep with the help of sterilized scalpel from all sides. The cylindrical core of tissue was removed under aseptic condition and the fungal inoculum was placed inside the holes and then the core was placed back to its original position. The wound was then sealed on the surface with molten wax. The inoculated tubers were placed in sterilized paper bags and incubated at 28 ± 1ºC for 20 days. The observations were taken to see disease development at the end of incubation period dissecting the tubers vertically. The controls were also maintained simultaneously under identical conditions.

(c) Disc Inoculation Technique:
Slices of equal diameter (2.0 cm) were made by cutting the sterilized tubers and were placed in sterilized petridishes. Each disc was inoculated with spore from 7 days old cultures of individual fungus in the centre and incubated at required temperature. The observations were taken for the development of rotting (Detmer and Moore, 1909).

MEASUREMENT OF ROT:
The percentage rot caused by each fungus was determined according to Prasad Bilgrami (1969). The whole surface area and the area showing rotten tissue were measured and percentage rot was calculated by the given formula

$$\%\text{Rot} = \frac{(W - w)}{W} \times 100$$

Where,
W = Full surface area of the tuber
w = Surface area of rotten tissue.

RESULTS

ISOLATION OF FUNGI FROM ROTTEN SWEET POTATO TUBERS:
Internal mycoflora isolated from market samples showed 5 fungal species i.e., *Aspergillus nidulans* var. *acristatus, A. terreus, Cladosporium cladosporiodes, Fusarium equiseti, Penicillium meleagrinum*. The most abundant fungi were *Cladosporium cladosporioides, Fusarium equiseti* and *Penicillium meleagrinum* which showed 27.0 to 32.4% abundance (Table- 2).

Table 2: Showing internal mycoflora of sweet potato tubers

S.No.	Fungi	c.f.u.	% A
1.	*Aspergillus nidulans var. acristatus*	4	10.8
2.	*Aspergillus terreus*	3	8.1
3.	*Cladosporium cladosporioides*	10	27.0
4.	*Fusarium equiseti*	8	21.6
5.	*Penicillium meleagrinum*	12	32.4
	Total c.f.u.	**37**	

c.f.u. = Countable fungal unit
%A = Percentage Abundance

PATHOGENICITY TEST:
In all 5 species were screened for pathogenicity (Table– 3) only 3 species were found to cause rot by all three technique i.e. wound inoculation, knife injury and spore inoculation. These

species were *Aspergillus nidulans var. acristatus, Cladosporium cladosporioides, Penicillium meleagrinum*.

The result of percentage rotten tissue is shown in Table- 4. The result indicated that 11.3 mm tissue was rotten by *Aspergillus nidulans var. acristatus*, 11.7 mm tissue was rotten by *Cladosporium cladosporioides* and 12.0 mm tissue was rotten by *Penicillium meleagrinum*.

Percentage of rotten tissue showed by *Aspergillus nidulans var. acristatus* was 15%, *Cladosporium cladosporioides* was 13.0% and *Penicillium meleagrinum* was 14.2% in 20 days and 28 ± 1ºC temperature (Table 4 and Fig. 1).

Table 3: Screening of fungi for rot development in sweet potato tubers

S. No.	Fungi	Inoculation Technique		
		Tuber Disc	Wound	Knife Injury and spore spray
1.	*Aspergillus nidulans* var. acristatus	+	+	+
2.	*Aspergillus terreus*	–	–	–
3.	*Cladosporium cladosporiodes*	+	+	+
4.	*Fusarium equiseti*	–	–	–
5.	*Penicillium meleagrinum*	+	+	+

Table 4: Pathogenicity of the isolates on sweet potato tubers

S. No.	Fungi	Size of rotten tissue in mm	Average	% Rotting	Average of % rotting
1.	*Aspergillus nidulans* var. acristatus	10.1, 11.5, 12.3	11.3	14.6, 15.0, 15.4	15.0
2.	*Cladosporium cladosporioides*	12.0, 11.5, 11.3	11.7	12.9, 13.3, 13.0	13.0
3.	*Penicillium meleagrinum*	11.5, 12.0, 12.6	12.0	14.1, 13.9, 14.7	14.2
4.	Control	–	–	–	–

Temperature 28 ± 1ºC
Days = 20
Technique = Wound Inoculation.

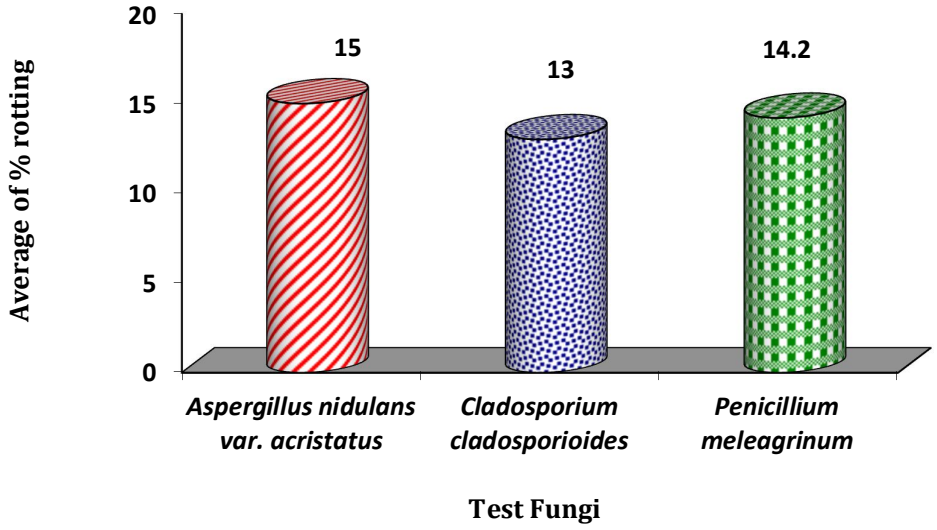

Fig. 1: Pathogenicity of the isolates on sweet potato tubers

DISEASE SYMPTOMS

The rotting was started from the point where the tuber was wounded and inoculated. At the early stage the disease progressed from the infected region. The tissue turned into a dark-brown mass still retaining the soft texture. After 15 days the region assumed a black colour

owing to loss of water and the disease tissue hardened. The knot was characterized by shrinkage and darkening of the tuber tissue, which wrinkled and dried in the advanced stage. The underlying tissue became dark brown to black.

CONCLUSION

It was observed from the result that 3 species i.e., *Aspergillus nidulans, Cladosporium cladosporioides, Penicillium melegrinum* were found to cause rot and these were considered potent pathogens which cause great losses to sweet potato tubers. The fungus entered the tuber through wounds in the form of bruises or deep cut and subsequently deeply infested the tissue. Many workers have established this fact. As the disease progressed, the hyphae of the fungus penetrated deeply and became established in the inter and intercellular spaces. The cell walls of the host tissue were broken down and the stored material was utilized by the pathogens. This act resulted in the development of rot.

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