

Studies on fungi associated with storage rot of Sweet potato [*Ipomoea batatas* (L.) Lam.] root tubers in Odisha, India

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Abstract

An extensive survey was conducted in order to assess the fungi associated with post-harvest decay of sweet potato root tubers in different market places of Odisha, India, during 2014-15. Rotten sweet potato samples were collected from six different market places such as Bhubaneswar, Cuttack, Jajpur, Puri, Balasore and Bhadrak. The five fungal species namely *Aspergillus flavus*, *Aspergillus niger*, *Fusarium oxysporum*, *Geotrichum candidum* and *Rhizopus oryzae* were isolated from the rotten samples. Of these, *Rhizopus oryzae* showed the highest percentage of frequency of occurrence followed by *Fusarium oxysporum*, *Aspergillus niger*, *Aspergillus flavus* while *Geotrichum candidum* had the least percentage of frequency. Pathogenicity tests revealed that all the isolated fungi were pathogenic to sweet potato root tubers. However, *Rhizopus oryzae* was found to be most pathogenic leading to rapid disintegration of the infected tubers within 15 days of inoculation, while *Geotrichum candidum* was the least pathogenic. The study on the effect of three solid nutrient media on growth of these test fungi revealed that Sabouraud Dextrose Agar supported highest growth followed by Czapek Dox Agar and Potato Dextrose Agar. The use of improved sweet potato varieties, good storage facilities and adequate control measures need to be encouraged in order to reduce storage rot of sweet potato root tubers in Odisha

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Introduction

Food scarcity is the world's third most pressing problem after poverty (FAO, 1997). Several countries faced food scarcity which is one of the important major problems through the globe. According to a report around 1 billion people are being faced by severe hunger in these nations of which 10% actually die from hunger-related complications. This problem arises due to inadequate agricultural storage and produce preservation from microbes-induced spoilages (Salami and Popoola, 2007, Kana *et al.*, 2012). Root crops are the energy-rich edible underground plant structures developed from modified roots (Okigbo, 1989). Sweet potato contains a wealth of orange-hued carotenoid pigments. In countries throughout Africa, in India and in the Caribbean, sweet potatoes have been shown to be a highly effective way of providing school age children with sizable amounts of their daily vitamin A. It has been shown to be a better source of bioavailable β -carotene than green leafy vegetables. It yields high amount of energy per unit area per unit time and is expected to bridge the food storages and malnutrition. It is among the world's most important, versatile, and underutilized food crops that rank fourth among the food crops after rice, potato, and wheat and seventh in terms of total production (Low *et al.*, 2009, Hu *et al.*, 2004). These crops are drought tolerant and provide a wide harvesting window which makes it act as a famine reserve.

Post-harvest deteriorations caused by microbial invasion of the tubers are the most important causes of loss in its production and contribute hugely to the unsuccessful long term storage of the root tubers (Okigbo *et al.*, 2009).

Several fungi has been reported to cause post-harvest decay of sweet potato include, *Fusarium oxysporum*, *Ceratocystis fimbriata*, *Fusarium solani*, *Monilochaetes infuscans*, *Macrophomina phaseolina* and *Botrydiodiplodia theobromae* (Clark and Hoy, 1994). Onuegbu (2002) reported that *Penicillium* sp. *Ceratocystis fimbriata*, *Diaporthe batatalis*, *Aspergillus flavus* and *Aspergillus niger*

were responsible for post-harvest decay of sweet potato tuber. Oyewale (2006) reported that a number of fungi viz. *Motierella ramanniana*, *Rhizopus stolonifer*, *Mucor pusillus*, *Botrytis cinerea*, *Erysiphe polygoni* and *Aspergillus flavus* were associated with post-harvest fungal rot of sweet potato. According to a report of Charles Tortoe *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*, *Penicillium digitatum*, *Cladosporium herbarum*, and *Aspergillus ochraceus*. Lewthwaite *et al.* (2011) reported the black rot disease of the sweet potato caused by *Ceratocystis fimbriata*. 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*. Holmes and Clark (2002) reported the *Geotrichum candidum* storage rot of sweet potato. However, few works has been carried out on the post-harvest fungal storage loss of sweet potato root tubers throughout the world but very less work as conducted in Odisha.

The present study was carried out to isolate and identify fungi associated with storage rot of sweet potato root tubers in Odisha, India. The significance of the present work lies with the rapid incidence of post-harvest decay of the vegetable and its management with special reference to Odisha.

Materials and methods

Collection of samples

Sweet potato root tubers showing symptoms of rotting were randomly selected from different market places of Odisha like Bhubaneswar, Puri, Cuttack, Balasore, Bhadrak and Jajpur. The tubers were collected and kept separately in sterile polythene bags and brought to the Laboratory of Microbiology, Department of Botany of Utkal University, Bhubaneswar, Odisha (India) for phytopathological analysis.

Isolation and Identification of associated Fungi

The diseased tubers were washed with tap water and surface sterilized with 0.1% mercuric chloride solution for 2-3 minutes. The healthy samples were cut through by means of sterile knife. Slicing was done starting from the healthy portions. Pieces of 5 × 5 mm were cut and placed on potato dextrose agar (PDA) medium and incubated at room temperature for 24 to 35 hours.

Representative colony types were purified by sub-culturing on fresh PDA plates. Pure cultures were transferred to slants of PDA. Pure cultures of the isolates were grown singly on PDA for identification. The isolated fungi were identified based on the isolates colonial characteristics on culture plates and microscopic features in slide cultures. Using a sterile inoculating needle portion of each mycelial colony was aseptically taken and placed on a clean microscopic slide and teased in a drop of lacto-phenol cotton blue. The isolates were identified by the help of the available literature and further authentication was made in the Department of Mycology and Plant Pathology, Orissa University of Agriculture and Technology, Bhubaneswar (Khatoon *et al.*, 2016).

Pathogenicity test

Fresh and healthy tubers were washed with tap water and surface sterilized with 0.1% mercuric chloride solution for 2-3 minutes. Cylindrical cores were removed from the tubers with the help of 5 mm cork borer. Four millimetre (4 mm) agar discs containing 7 days old cultures of the isolates were introduced into the holes and sealed with the sterile Vaseline. Controls were set up as described except that the inocula consist of uninoculated potato dextrose agar blocks. All the treated tubers were put singly into sterile polythene bags and incubated at 28 ± 2 °C for 20 days. The roots were cut through and examined for the extent of rotting frequently till the end of the incubation period (Khatoon *et al.*, 2016).

Nutritional study

A comparative nutritional study was conducted to know the effect of three different solid nutritional media on the mycelia growth of six fungal species causing diseases in sweet potato root tubers. The test solid media were: Sabouraud Dextrose Agar, Czapek Dox Agar and Potato Dextrose Agar. The incubation period of *Aspergillus flavus*, *Aspergillus niger*, *Fusarium oxysporum*, *Geotrichum candidum* and *Rhizopus oryzae* was 7, 8, 11, 5 and 2 days respectively.

Results

Isolation of fungi from rotten sweet potato root tubers

In this present investigation, 4 genera comprising of five species of fungi were found to be associated with post-harvest storage rots of sweet potato root tubers in Odisha, India. The data presented in Table 1 revealed that 4 genera comprising of 5 species of fungi were isolated from 175 samples of rotten sweet potatoes in varying frequencies. These are *Aspergillus flavus* (Ray and Mishra, 1995; Charles Tortoe *et al.*, 2010; Onuegbu, 2002), *Aspergillus niger* (Mandal and Dasgupta, 1980; Mandal, 1981; Sharma and Sumbali, 1993), *Fusarium oxysporum*, *Geotrichum candidum* (Mandal and Dasgupta, 1980; Holmes and Clark, 2002) and *Rhizopus oryzae* (Jenkins, 1981; Ray *et al.*, 1994; Ray and Mishra, 1995). The frequency of occurrence of these five species of fungi varied from 9.1 to 38.2 %. Among the fungal species isolated from rotten samples of sweet potatoes, all the five species were isolated from samples collected from markets of Bhubaneswar, Balasore, Cuttack and Jajpur while four species were found to be responsible for rotting in samples collected from Bhadrak and Puri. The percentage of incidence was maximum in case of *Rhizopus oryzae* followed by *Fusarium oxyporum*, *Aspergillus niger*, *Aspergillus flavus* and *Geotrichum candidum*. The percentage of their incidence was 38.2, 21.2, 16, 15.5 and 9.1 % respectively (Table 1 & Fig. 1).

Table 1. Incidence of fungi from rotten roots of sweet potato collected from six localities of Odisha.

Fungi	Localities						Total	%
	I	II	III	IV	V	VI		
<i>Aspergillus flavus</i>	8	7	4	4	5	-	28	16
<i>Aspergillus niger</i>	5	4	6	6	9	7	37	21.2
<i>Fusarium oxysporum</i>	4	2	5	-	5	11	27	15.5
<i>Geotrichum candidum</i>	3	3	4	3	1	2	16	9.1
<i>Rhizopus oryzae</i>	22	12	8	11	4	10	67	38.2
Total	42	28	27	24	24	30	175	100

I = Bhubaneswar, II = Cuttack, III = Jajpur, IV = Puri, V = Balasore, VI = Bhadrak

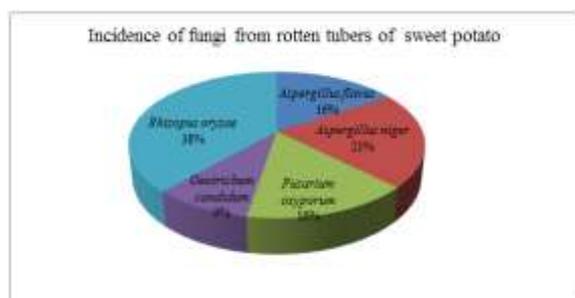


Fig. 1. Incidence of fungi from rotten tubers of sweet potato collected from six localities of Odisha.

According to Charles Tortoe *et al.* (2010), *Aspergillus flavus* was the most dominant fungal species followed by *Aspergillus niger* and *Fusarium oxysporum*. The infection of sweet potato root tubers by these isolated fungi was characterized by several symptoms. *Aspergillus flavus* infection was characterized by the development of wet mass of green colour spores over the surface. Snowdor (1991) reported that these fungi create local discoloration of the surrounding tissues of infected tubers. *Aspergillus niger* infection was characterized by softening of internal tissue, development of black spore mass over the infected area. According to a report of Mandal (1981), *Aspergillus niger* infection on sweet potato started around a wound as small water soaked dull area with white mycelial growth, followed by black sporulation of the fungus. *Geotrichum candidum* infection was characterized by softening of internal tissue with foul odour. Mishra and Rath (1986) reported that *Geotrichum candidum* storage rot is a minor

disease was occurred on injured or physiologically week roots, generally these were noticed during late winter or autumn. *Fusarium oxysporum* infection was characterized by white dry mycelial growth over the cut and buries. Walker in 1952 stated that the *Fusarium* rot of sweet potato was widely prevalent in United States since 1890. Neilsen and Moyer (1979) reported that, several species of *Fusarium* were cause minor losses in sweet potato roots at temperature below 10.6 °C. *Rhizopus oryzae* infection was characterized by the development of cottony white coloured mycelial growth over the wounded area. It was observed that more post-harvest loss of sweet potato occur during summer due to high ambient temperatures and relative humidity in Odisha. This post-harvest loss due to temperature and relative humidity was also reported by Nahunnaro, 2008. It was observed that these pathogenic organisms gain entry into tubers probably through the breaks forms during harvesting, or through other natural openings like cracks and buries on the tubers surfaces sustained during harvesting, transit or storage (Okigbo *et al.*, 2009). Normally, the fungi causing rot in sweet potato are lesion pathogens. The rot changes the consistency of flour from the roots making them no longer suitable for consumption or causing a considerable loss in market value (Rees, 2003). Unhealthy store conditions lead to the absorption of moisture by produce in storage as a result of defects in the storage facility, thus encouraging the development of hot-spots and moulds (Shukla *et al.*, 2012).

Table 2. Pathogenicity of the isolates on sweet potato root tubers

Sl. No.	Fungi	Percentage of rotting
1	<i>Aspergillus flavus</i>	31
2	<i>Aspergillus niger</i>	56
3	<i>Fusarium oxysporum</i>	26
4	<i>Geotrichum candidum</i>	14
5	<i>Rhizopus oryzae</i>	74

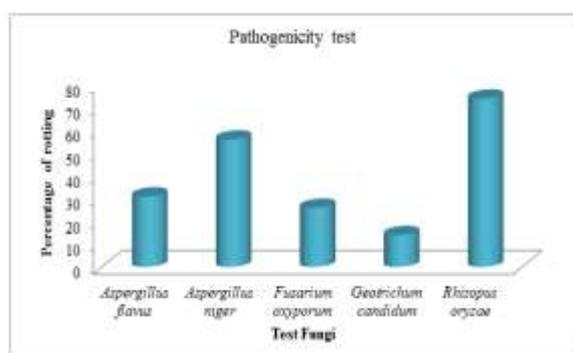


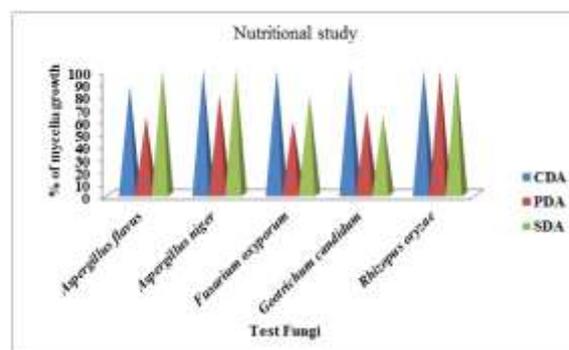
Fig. 2. Pathogenicity of the isolates on sweet potato root tubers

The use of improved sweet potato varieties, good storage facilities and adequate control measures need to be encouraged in order to reduce storage rot of sweet potato root tubers in Odisha.

Pathogenesis test

The pathogenicity test revealed that all the fungal isolates were pathogenic on sweet potato root tubers. Their percentage of rotting was varied from 14 % to 74 %. Among all the test pathogenic fungi, *Rhizopus oryzae* was found to

be comparatively more pathogenic than others followed by *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum* and *Geotrichum candidum*. The inoculated pathogens on pathogenicity test cause the rotting of sweet potato tubers under storage. The percentage of rotting was found to be 74 % by *Rhizopus oryzae*, 56 % by *Aspergillus niger*, 31 % by *Aspergillus flavus*, 26 % by *Fusarium oxysporum* and 14 % by *Geotrichum candidum* (Table 2 & Fig. 2). Upon re-isolation, the rotten tissues yielded a fungus which was identical with the original fungus inoculated. On artificial inoculation of these isolated fungi to the tuber host showed almost same kind of symptoms which was observed in natural infection condition. On pathogenicity test, *Geotrichum candidum* leads to be responsible for fast rotting of sweet potato tubers followed by *Aspergillus niger*, *Rhizopus oryzae*, *Fusarium oxysporum* and *Aspergillus flavus*.



CDA= Czapek Dox Agar, PDA= Potato Dextrose Agar, SDA= Sabouraud Dextrose Agar

Fig. 3. Effect of three solid nutrient media on the growth of five test fungi under study.

Table 3. Effect of three solid media on the growth of isolated fungi:

Test Organisms	Percentage of growth of fungal mycelia		
	CDA	PDA	SDA
<i>Aspergillus flavus</i>	87.5	62.5	100
<i>Aspergillus niger</i>	100	80	100
<i>Fusarium oxysporum</i>	100	58.75	80
<i>Geotrichum candidum</i>	100	67.5	63.75
<i>Rhizopus oryzae</i>	100	100	100

CDA= Czapek Dox Agar, PDA= Potato Dextrose Agar, SDA= Sabouraud Dextrose Agar

Nutritional study of isolated fungi

The data presented in Table 3 revealed that there was no significant difference among the media tested, on mycelial growth of five fungal species. Among the three solid nutrient media tested, Sabouraud Dextrose Agar medium supported maximum radial growth of most of the fungi except *Fusarium oxysporum* and *Geotrichum candidum*. Maximum growth of these fungi was obtained on Czapek Dox Agar. *Rhizopus oryzae* showed its 100 % growth on all three tested media. On an average, Sabouraud Dextrose Agar supported maximum growth followed by Czapek Dox Agar and Potato Dextrose Agar (Table 3 & Fig. 3).

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