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Studies on fungi associated with storage rot of onion (*Allium cepa* L.) and garlic (*Allium sativum* L.) bulbs in Odisha, India

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Abstract

A survey of fungi associated with post-harvest deterioration of onion and garlic bulbs was conducted in different market places of Odisha, India, in 2014-15. Rotten samples were collected from five different markets of Bhubaneswar, Cuttack, Jajpur, Puri, Balasore and Bhadrak. The fungal species such as; Aspergillus flavus and Aspergillus niger were isolated from rotten bulbs of onion; Aspergillus niger, Penicillium sp. and Rhizopus oryzae were isolated from rotten samples of garlic. Of these, Aspergillus niger has highest percentage frequency of occurrence in onion while in garlic the frequency of Penicillium sp. was more. The percentage of their frequency is 50.3 % and 86.16 % respectively. The result of Pathogenicity test indicated that all the isolated fungi were pathogenic to their respective host and it was found that Aspergillus niger and Penicillium sp. were more pathogenic to onion and garlic respectively than that of other isolated fungi. They cause 69 % and 74 % of rotting of onion and garlic respectively. In order to reduce storage rot of onion and garlic bulbs, there is need to encourage the use of improved varieties, good storage facilities and adequate control measures.

Keywords: Identification, nutritional study, pathogenicity, post-harvest disease.

Introduction

For India, onion is one of the potential foreign exchange earners as it stood second largest producer of onions after China, producing 1.6 million MT annually[1]. Since long garlic has been known as a valuable spice for foods and a popular remedy for various ailments and physiological disorder all over the World. It is used for the preparation of chutney, pickles, curry powder, curried vegetables, meat and tomato ketchup.

During storage bulb rots cause huge onion loss. Raju and Naik elaborated that onion suffers from many post-harvest fungal diseases, like the soft rot, brown rot and smudge[2]. Several fungi such as *Fusarium* sp. *Botrytis* spp. and *Aspergilus niger*, were found on diseased onion bulbs during storage conditions[3]. Storage decay resulted in the reduction in the quantity and quality of onion that affects the market value[4]. Other important consequence may be mycotoxin contamination of the affected material[5]. Post-harvest rotting of fruits and vegetables during the supply chain has been identified as the greatest cause of post-harvest losses, which results in significant economic losses [6]. Various fungi were reported to be associated with storage rots of garlic such as *Alternaria alternarta*, *Aspergillus repens*, *Aspergillus sclerotigenum*, *Fusarium* spp.[7], *Fusarium oxysporum*, *Fusarium solani*, *Macrophomina phaseolina*[8]; *Aspergillus niger*[9], *Aspergillus* spp.[10], *Aspergillus tamari*. *Cephalosporium curtipes*, *Fusarium camptoceras*, *Penicillium paxilli*[11], *Curvularia pallescens*, *Macrophomina phaseolina*, *Penicillium rugulosum*[12], *Drechslera tetramera*, *Penicillium nigricans*[13]; *Rhizopus oryzae*[14,15], *Rhizopus* sp.[16], *Sclerotium cepivorum*[17].

The present study was carried out to isolate and identify fungi associated with storage rot of onion and garlic storage bulbs in Odisha, India. The significance of the present work lies with the rapid incidence of postharvest decay of the vegetable and its management with special reference to Odisha.

Materials and methods

The present investigation was carried out in the Post Graduate Department of Botany, Utkal University, Bhubaneswar, Odisha (India). Odisha lies between the latitudes 17.78°N and 22.73°N, and between longitude as 81.37°E and 87.53ºE. The state has an area of 155,707 km^2, which is 4.87% of total area of India, and a coastline of 450 km. During summer, maximum temperature ranges between 35-40°C and in winter the temperatures are usually between 12-14°C. The average rainfall is 150 cm experienced during July-September as the result of south west monsoon.

Collection of samples: Onion and garlic bulbs showing symptoms of rotting were randomly selected from different market places of Odisha like Bhubaneswar, Puri, Cuttack, Balasore, Bhadrak and Jajpur. The corms were collected and kept separately in sterile polythene bags and brought to the Laboratory of Microbiology, Post Graduate Department of Botany of Utkal University, Bhubaneswar, and Odisha, India for phytopathological analysis.

Isolation and Identification of associated Fungi: The diseased onion and garlic bulbs were washed with running 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 isolate's colony 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 with the help of the available literature and further authentication was made in the Department of Microbiology, Odisha University of Agriculture and Technology, Bhubaneswar.

Pathogenicity test: Fresh and healthy storage bulbs of onion and garlic were washed with running 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 bulbs were put separately into sterile polythene bags and incubated at 28 ± 2°C for 20 days. The bulbs were cut through and examined for the extent of rotting frequently till the end of the incubation period[18].

Results and discussion

Isolation of fungi from rotten bulbs of onion and garlic: The data revealed that fungi of two species of genus *Aspergillus* namely *Aspergillus flavus*[19-28] were isolated from 130 samples of rotten bulbs of onion in varying frequencies. The frequency of occurrence this genus of fungi varied from 13.84 to 86.16 %. Both the species were isolated from markets of Bhubaneswar, Jajpur, Puri and Bhadrak while only one species was from Cuttack and Balasore. The percentage of incidence is maximum in *Aspergillus niger* and comparatively low in *Aspergillus flavus*. Their percentage of their incidence was 86.16 and 13.84 respectively.

Table-1: Incidence of fungi in rotten onion bulb collected from six localities of Odisha.

Fungi	*Localities							
	I	II	III	IV	V	VI	Total	%
Aspergillus flavus	4	-	2	9	-	3	18	13.84
Aspergillus niger	37	26	11	17	8	13	112	86.16
Total	41	26	13	26	8	16	130	100

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

Table-2: Incidence of fungi from rotten garlic bulb collected from six localities of Odisha.

Fungi	*Localities							
	I	II	III	IV	V	VI	Total	%
Aspergillus niger	11	10	10	10	12	3	56	32.75
Penicillium sp.	17	20	16	15	6	12	86	50.3
Rhizopus oryzae	7	2	6	10	3	1	29	16.95
Total	35	32	32	35	21	16	171	100

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

The data revealed that 3 genera comprising of 3 species of fungi were isolated from 171 samples of rotten bulbs of garlic in varying frequencies. These are *Aspergillus niger*[29,30], *Penicillium* sp.[12,13,31] and *Rhizopus oryzae*[14,15]. The frequency of occurrence of different genera of fungi varied from 16.95 to 50.3 %. All the three species were isolated from markets of Bhubaneswar, Cuttack, Jajpur, Puri, Balasore and Bhadrak. The percentage of incidence is maximum in *Penicillium* sp. followed by *Aspergillus niger* and *Aspergillus flavus*. Their percentage of their incidence was 50.3, 32.75 and 16.95 respectively.

Pathogenisity test: The pathogenicity test revealed that both the inoculated fungal isolates were found to be pathogenic on onion bulbs. The percentage of pathogenicity was varied from 46 % to 69 %. Among both the pathogenic fungi, *Aspergillus niger*[3,31] was found to be comparatively most virulent than *Aspergillus flavus*. The inoculated pathogens on pathogenicity test cause the rotting of bulbs. Their percentage of rotting was 69% and 46 % respectively (Table-3 and Figure-2, 3).

Table-3: Pathogenicity of the isolates on onion bulbs

Sl. No.	Fungi	Percentage of rotting
1	*Aspergillus flavus*	46
2	*Aspergillus niger*	69

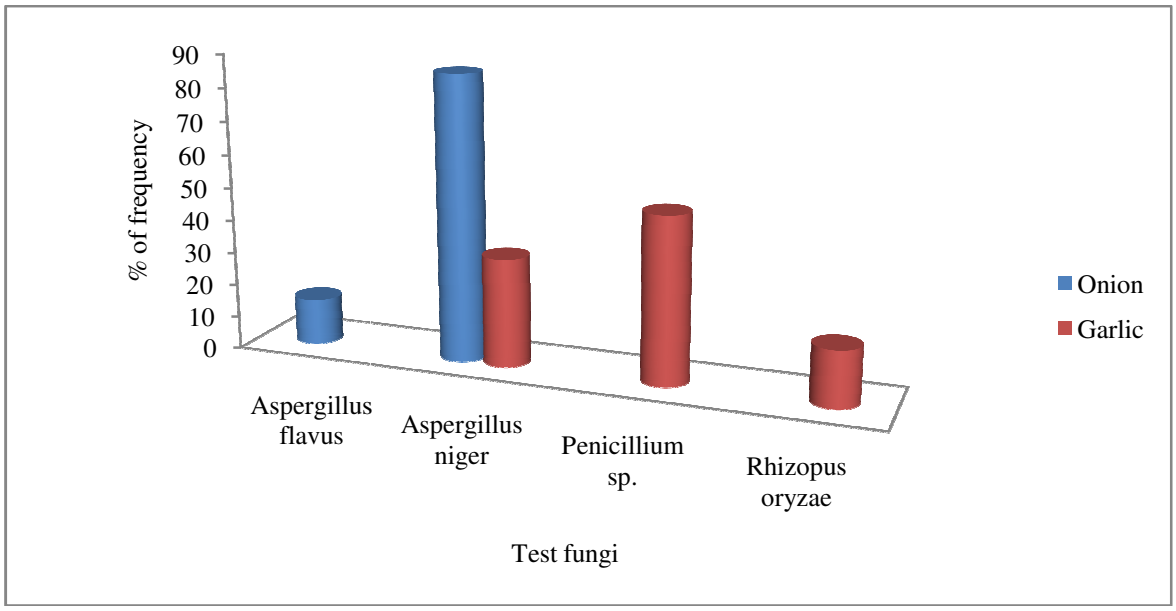

Figure-1: Incidence of fungi from rotten bulbs of onion and bulbs collected from six localities of Odisha.

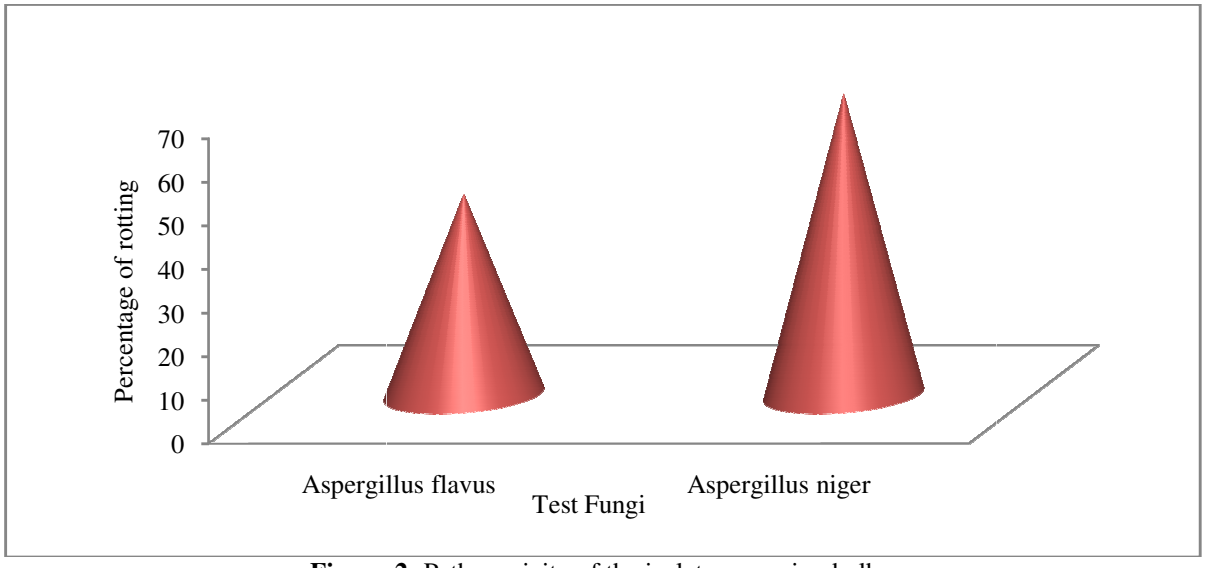

Figure-2: Pathogenicity of the isolates on onion bulbs.

Table-4: Pathogenicity of the isolates on garlic bulbs

Sl. No.	Fungi	Percentage of rotting
1	*Aspergillus niger*	36
2	*Penicillium* sp.	74
3	*Rhizopus oryzae*	44

The pathogenicity test of garlic bulbs revealed that all the fungal isolates were pathogenic on the host. The percentage of pathogenicity was varied from 36 to 74 %. Among all the pathogenic fungi, *Penicillium* sp. was found to be comparatively most virulent followed by *Rhizopus oryzae* and *Aspergillus niger*. The inoculated pathogens on pathogenicity test cause the rotting of bulbs. Their percentage of rotting was 74 %, 44 % and 36 % respectively (Table-4 and Figure-4, 5).

(A) (B) (C)

A= Uninoculated, B= Inoculated by *Aspergillus flavus*, C= Inoculated by *Aspergillus niger*.

Figure-3: Pathogenicity of the isolates on onion

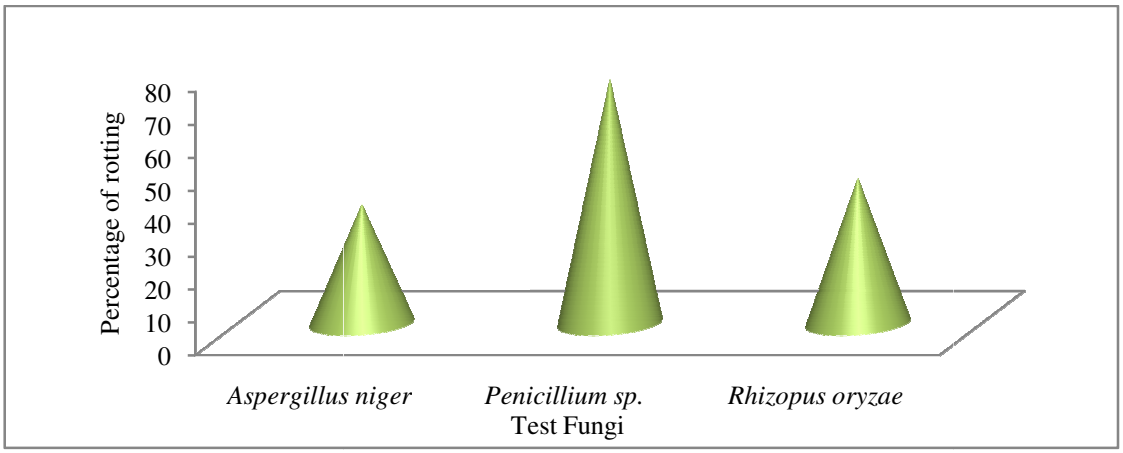

Figure-4: Pathogenicity of the isolates on garlic bulbs

(A) (B) (C) (D)

A= Uninoculated, B= Inoculated by *Aspergillus niger*, C= Inoculated by *Penicillium* sp., D= Inoculated by *Rhizopus oryzae*.

Figure-5: Pathogenicity of the isolates on garlic

Conclusion

The present study revealed that several fungi have been found to be responsible for post-harvest decay of onion and garlic bulbs during storage condition in Odisha. These pathogens are responsible for to enormous loss of these bulbs not only in terms of quantity but also reduce its economic and nutritive value. Some of these fungi are capable of producing mycotoxins which are hazardous to the health of consumers. As such urgent attention is required for the management of disease, thereby increasing the economic yield of the produce. This will ensure substantial contribution of the crop to food supply and national economy.

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