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Original Research Article

Studies on Fungi Associated with Storage Rot of Carrot (*Daucus carota* L.) and Radish (*Raphanus sativas* L.) in Odisha, India

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Abstract: An extensive survey on fungi associated with post-harvest deterioration of carrot and radish storage roots was conducted during 2014-2015 in different market places of Odisha, India. Rotten samples were collected from five different localities such as Bhubaneswar, Cuttack, Jajpur, Puri, Balasore and Bhadrak. Three fungal species such as *Aspergillus niger*, *Geotrichum candidum* and *Rhizopus oryzae* were isolated from the rotten samples. Of these, *Geotrichum candidum* has highest percentage frequency of occurrence in both carrot and radish. In carrot next to *Geotrichum candidum*, *Rhizopus oryzae* showed more frequency of occurrence than *Aspergillus niger* but in case of radish the case is just opposite, here the percentage frequency of occurrence of *Aspergillus niger* was found to be more than *Rhizopus oryzae*. Pathogenicity tests revealed that all the isolated fungi were pathogenic to their respective host storage roots. However, *Rhizopus oryzae* was found to be most pathogenic on carrot leading to rapid disintegration of the infected roots while *Geotrichum candidum* was the least pathogenic. In case of radish, *Aspergillus niger* was found to be more pathogenic and *Rhizopu oryzae* was least pathogenic. The use of improved varieties, good storage facilities and adequate control measures need to be encouraged in order to reduce storage rot of carrot and radish storage roots.
Keywords: Isolation, nutritional study, pathogenicity, post-harvest disease

INTRODUCTION

Root crops are the energy-rich edible underground modified roots [1]. Radish is considered as the most ancient vegetables grown throughout the world [2]. In India, both the radish and carrot are cultivated throughout the states mostly as winter crop. They are rated as the low calorie root vegetables. Radish is a very good source of antioxidants, electrolytes, minerals, vitamins and dietary fibre. It contains isothiocyanate anti-oxidant compound called sulforaphane which has role against prostate, breast, colon and ovarian cancers by virtue of its cancer cell growth inhibition, and cyto-toxic effects on cancer cells [3]. Carrots are rich source of carotenes and vitamin-A. Studies have found that flavonoid compounds in such carrots may offer protection from skin, lung and oral cavity cancers [4]. Schuphan and Weiller evaluate the antibacterial properties of essential oils extracted from carrot roots. It increases the quantity of urine and helps in the elimination of uric acid. Carrot seeds are reported to be effective against the diseases of kidney and lungs [5].

According to a report, around 1 billion people are challenging by severe hunger in a number of 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 [6, 7]. Several fungi were found to be responsible for storage rot of carrots and radish. *Acrothecium carotae* [8], *Alternaria alternata* [9], *Alternaria radicinia* [10], *Aspergillus niger*, *Botrytis cinerea*, *Fusarium roseum* [11], *Fusarium avenaceum* [12, 13], *Fusarium culmorum*, *Fusarium pallidoroseum* [14], *Fusarium juruanum*, *Fusarium lateritium* [15], *Fusarium oxysporum* [16], *Geotrichum candidum* [10, 17-19] etc were found to be associated with post-harvest storage rots of carrot roots. Similarly a large number of fungi were reported to cause storage rots of radish. These are *Alternaria alternate*; *Colletotrichum capsici* [9], *Fusarium oxysporum* [20], *Fusarium* sp.; *Pestalotia* sp. [21], *Macrophomina phaseolina* [17] etc.

The present study was carried out to isolate and identify fungi associated with storage rot of carrot and radish roots 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 METHODOLOGY

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 87.53°E. 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 [22]. In summer, maximum temperature ranges between 35-40 °C and the low temperatures are usually between 12-14 °C. The average rainfall is 150 cm, experienced as the result of south west monsoon during July-September [23].

Collection of samples

Carrot and radish storage roots showing symptoms of rotting were randomly selected from different market places of Odisha like Bhubaneswar, Puri, Cuttack, Balasore, Bhadrak and Jajpur. The roots 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, Odisha (India) for phytopathological analysis.

Isolation and Identification of associated Fungi

The rotten carrot and radish storage roots 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 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, Orissa University of Agriculture and Technology, Bhubaneswar.

Pathogenicity test

Fresh and healthy storage roots of carrot and radish 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 separately 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 [24].

Nutritional study

A comparative nutritional study was conducted to know the effect of three different solid nutritional media on the mycelial growth of three fungal species causing diseases in carrot and radish. The test solid media were: Sabouraud Dextrose Agar (SDA), Czapek Dox Agar (CZA) and Potato Dextrose Agar (PDA).

RESULTS AND DISCUSSION

Isolation of fungi from rotten storage roots of carrot and radish

Table 1: Incidence of fungi from rotten storage roots of carrot collected from six localities of Odisha

Fungi	*Localities							
	I	II	III	IV	V	VI	Total	%
Aspergillus niger	5	7	8	-	3	1	24	**20.4**
Geotrichum candidum	23	8	7	-	8	12	58	**49.1**
Rhizopus oryzae	14	9	2	1	4	6	36	**30.5**
Total	**42**	**24**	**17**	**1**	**15**	**19**	**118**	**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 118 samples of rotten carrots in varying frequencies (Table 1). These are *Aspergillus niger* [11], *Geotrichum candidum* [10, 17-19, 25, 26] and *Rhizopus oryzae* [11]. The frequency of occurrence of these three fungi varied from 20.4 % to 49.1 %. All the three fungal species, all were isolated from samples collected from markets of all places viz. Bhubaneswar, Cuttack, Jajpur, Balasore and Bhadrak while only one test fungi was isolated from samples of Puri. The percentage of incidence is maximum in *Geotrichum candidum* followed by *Rhizopus oryzae* and *Aspergillus niger*. The percentage of their incidence was 49.1, 30.5 and 20.4 respectively.

Table 2: Incidence of fungi in rotten storage roots of radish collected from six localities of Odisha

Fungi	*Localities							
	I	II	III	IV	V	VI	Total	%
Aspergillus niger	6	7	14	-	9	-	36	**35.3**
Geotrichum candidum	12	7	6	13	2	3	43	**42.15**
Rhizopus oryzae	2	-	7	11	3	-	23	**22.55**
Total	**20**	**14**	**27**	**24**	**14**	**3**	**102**	**100**

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

The data revealed that 3 genera comprises of 3 species of fungi were isolated from 102 samples of rotten tubers of radish in varying frequency (Table 2). These are *Aspergillus* niger, *Geotrichum candidum* [10, 17, 18, 19, 25, 26] and *Rhizopus* oryzae. The frequency of occurrence of different genera of fungi varied from 22.55 to 42.15 %. Among the 3 fungal species isolated from rotten samples of radish, all test species were isolated from markets of Bhubaneswar, Balasore and Jajpur while 2 species from Cuttack and Puri. From the samples collected from Bhadrak only one species of fungi was found to be responsible. The percentage of incidence is maximum in *Geotrichum candidum* followed by *Aspergillus niger* and *Rhizopus oryzae*. The percentage of their incidence was 42.15, 35.3 and 22.55 respectively (Figure 1).

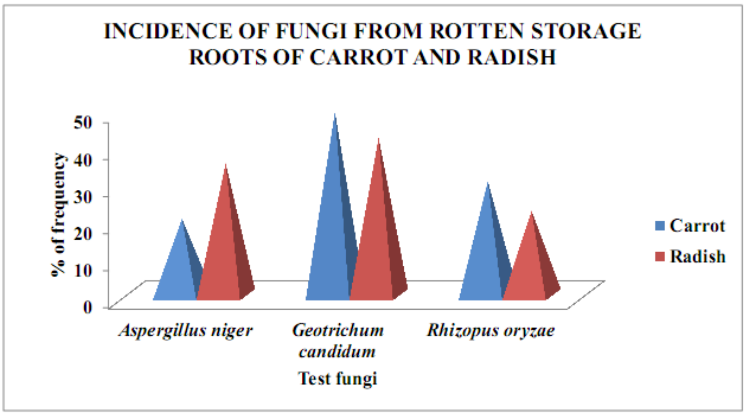

Fig-1: Incidence of fungi from rotten roots of carrot and radish collected from six localities of Odisha

Pathogenisity test

Table 3: Pathogenicity of the isolates on carrot and radish roots

Sl. No.	Fungi	Percentage of rotting in carrot	Percentage of rotting in radish
1	*Aspergillus niger*	86	87
2	*Geotrichum candidum*	78	68
3	*Rhizopus oryzae*	92	35

The pathogenicity test of carrot revealed that all the fungal isolates were pathogenic on carrot roots. The percentage of pathogenicity ranged from 78 % to 92 % (Table 3). Among all the pathogenic fungi, *Rhizopus oryzae* was found to be comparatively most virulent followed by *Aspergillus niger* and *Geotrichum candidum*. The inoculated pathogens on pathogenicity test cause the rotting of respective host roots. Their percentage of rotting was 92 %, 86 % and 78 % respectively (Table 3 & Fig 2, 3).

The pathogenicity test revealed that all the fungal isolates were pathogenic on radish roots. The percentage of pathogenicity was varied from 35 % to 87 %. Among all the pathogenic fungi, *Aspergillus niger* was found to be comparatively most virulent followed by *Geotrichum candidum* and *Rhizopus oryzae*. The inoculated pathogens on pathogenicity test caused the rotting of roots. Their percentage of rotting was 87 %, 68 % and 35 % respectively (Table 4 & Fig 2, 4).

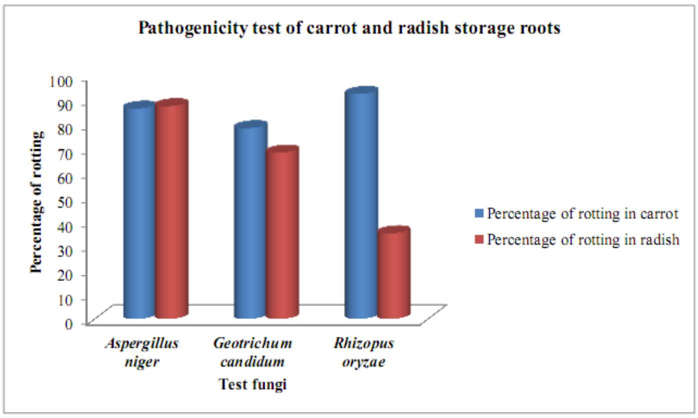

Fig-2: Pathogenicity of the isolates on carrot and radish roots

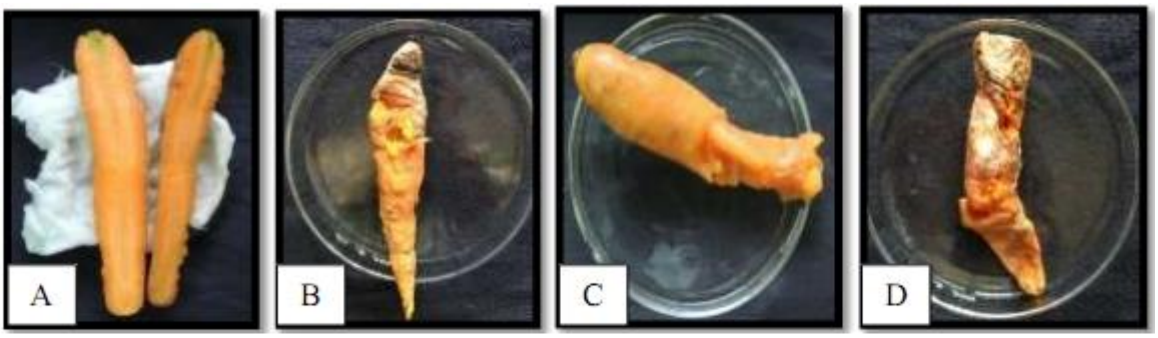

Fig-3: Pathogenicity of the isolates on carrot (A= Uninoculated, B= Inoculated by *Aspergillus niger*, C= Inoculated by *Geotrichum candidum*, D= Inoculated by *Rhizopus oryzae*)

Fig-4: Pathogenicity of the isolates on radish (A= Uninoculated, B= Inoculated by *Aspergillus niger*, C= Inoculated by *Geotrichum candidum*, D= Inoculated by *Rhizopus oryzae*)

Nutritional study

It might be seen from Table 18 that there was no significant difference among the media tested, on mycelial growth of three test fungal species. Among the three solid nutrient media tested, Czapek Dox Agar supported complete radial growth of all the fungi tested followed by Sabouraud Dextrose Agar and Potato Dextrose Agar (Table 4 and Figure 5).

Table 4: Effect of three solid media on the growth of isolated fungi

Test organisms	Percentage of growth of fungal mycelia		
	CDA	PDA	SDA
Aspergillus niger	100	80	100
Geotrichum candidum	100	67.5	63.75
Rhizopus oryzae	100	100	100

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

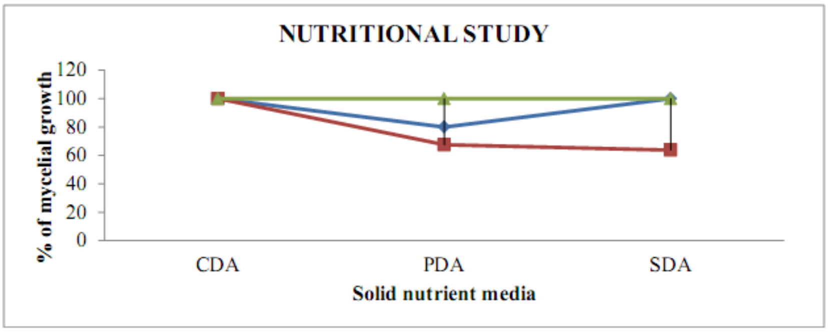

Fig-5: Effect of three solid nutrient media on the growth of three test fungi

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

CONCLUSION

The present study revealed that three fungi have been found to cause storage rot of carrot and radish roots during storage condition in Odisha. These pathogens lead to enormous loss of these tubers 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 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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