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Studies on Fungi Associated with Storage Rot of *Dioscorea alata* L. Roots in Odisha, India.

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ABSTRACT

An extensive survey on fungi associated with post-harvest decay of *Dioscorea alata* L. roots was conducted in different market places of Odisha, India, during 2014-15. Rotten samples of *D. alata* roots were collected from six different localities of Odisha. A total of five different genera of fungi such as; *Aspergillus niger, Fusarium oxysporum, Geotrichum candidum, Junghuhnia* sp. and *Neoscytalidium* sp. were isolated from rotten samples. As regards to the frequency of their occurrence, *Neoscytalidium* sp. recorded the highest percentage frequency followed *by Junghuhnia* sp., *Aspergillus niger*, *Geotrichum candidum* whereas *Fusarium oxysporum* revealed the least percentage frequency. The data on the pathogenicity tests revealed that all the isolated fungi were pathogenic to the host. However *Neoscytallidium* sp. was found most pathogenic leading to rapid disintegration of the infected tubers, while *Junghuhnia* sp. was found to be least pathogenic. The nutritional study on the effect of three solid nutrient media on these test fungi revealed that Sabouraud Dextrose Agar supported maximum growth followed by Czapek Dox Agar and Potato Dextrose Agar. The use of improved cultivars, good storage facilities and adequate control measures during transit and transport need to be encouraged in order to reduce storage rot of *D. alata* roots.

Keywords: Isolation, nutritional study, Pathogenicity, post-harvest disease.

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INTRODUCTION

Modified root crops are the energy-rich edible underground plant structures developed from modified roots [1]. Dioscorea, a genus of wild tuber crops is one of the major underground medicinal food sources among rural and tribal people of Odisha. It is reported that twelve species of *Dioscorea* are available in Odisha and most of which are considered to be wild except *Dioscorea alata* which is a cultivated one. *Dioscorea alata* are unique for their medicinal, food and economic values [2]. The syrup of the root has been used by the people of South Asia to reduce labour pain and also used against colic pain, high cough, asthma, rheumatism and gastric problem related to alcoholism. Powder of the tubers are used as an ingredient of medicines for cholera and constipation and plant juice used in the treatment of skin diseases, piles, intestinal worms and obesity [3,4]. *Dioscorea* species also have anti-microbial activities such as, anti-fungal, anti-bacterial and antiviral (cancer) due to the presence of secondary metabolites like steroid-saponins exerting a large variety of biological functions [5, 6].

Fungi are the primary causal agents for post-harvest storage losses of Dioscorea. There are several reports in the literature which indicates that fungi are the causal agents of Dioscorea rots [7-9]. Several fungi were found to be responsible for storage rot of dioscorea roots like *Alternaria solani*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Cephalsporium indicum*, *Curvularia pallescens*, *Curvularia lunata*, *Fusarium clamydosporum*, *Fusarium oxysporum*, *Fusarium solani* [10], *Aspergillus niger*, *Fusarium oxysporum*, *Fusarium solani* [11], *Aspergillus tamari* [12,13], *Botryodiplodia theobromae* [14,15], *Botrytis cinerea* [16], *Cylindrocarpon radicicola* [17], *Erwinia carotovora* [12], *Fusarium moniliforme* [18], *Fusarium subglutinans* [19], *Geotrichum candidum* [20].

The present study was carried out to isolate and identify fungi associated with storage rot of *D. alata* root 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

Collection of rotten samples

Dioscorea alata roots showing symptoms of rotting were randomly selected from different market places of Odisha like Bhubaneswar, Puri, Cuttack, Balasore, Bhadrak and Jajpur. The samples 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 roots of *D. alata* 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 hours [21].

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 Mycology and Plant Pathology, College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar, India.

Pathogenicity test

Fresh and healthy tuber samples 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 at regular intervals till the end of the incubation period [22].

Nutritional study

A comparative nutritional study was conducted to know the effect of three different solid nutritional media on the mycelial growth of six fungal species causing storage decay in *D. alata*. The test solid media were: Sabouraud Dextrose Agar (SDA), Czapek Dox Agar (CZA) and Potato Dextrose Agar (PDA). The incubation period of *Aspergillus niger, Fusarium oxysporum, Geotrichum candidum, Junghuhnia* sp. and *Neoscytalidium* sp. was 8, 10, 11, 5 and 3 days respectively.

RESULTS

Isolation of fungi from rotten tubers of *Dioscorea alata*

The data presented in Table 1 revealed that 5 genera comprising of 5 species of fungi were isolated from 58 samples of rotten tubers of *Dioscorea alata* in varying frequencies. The frequency of occurrence of different genera of fungi varied from 5.17 to 34.49 %. The isolated fungi were identified as *Aspergillus niger*, *Fusarium oxysporum*, *Geotrichum candidum*, *Junghuhnia* sp. and *Neoscytalidium* sp. (Figure 1).

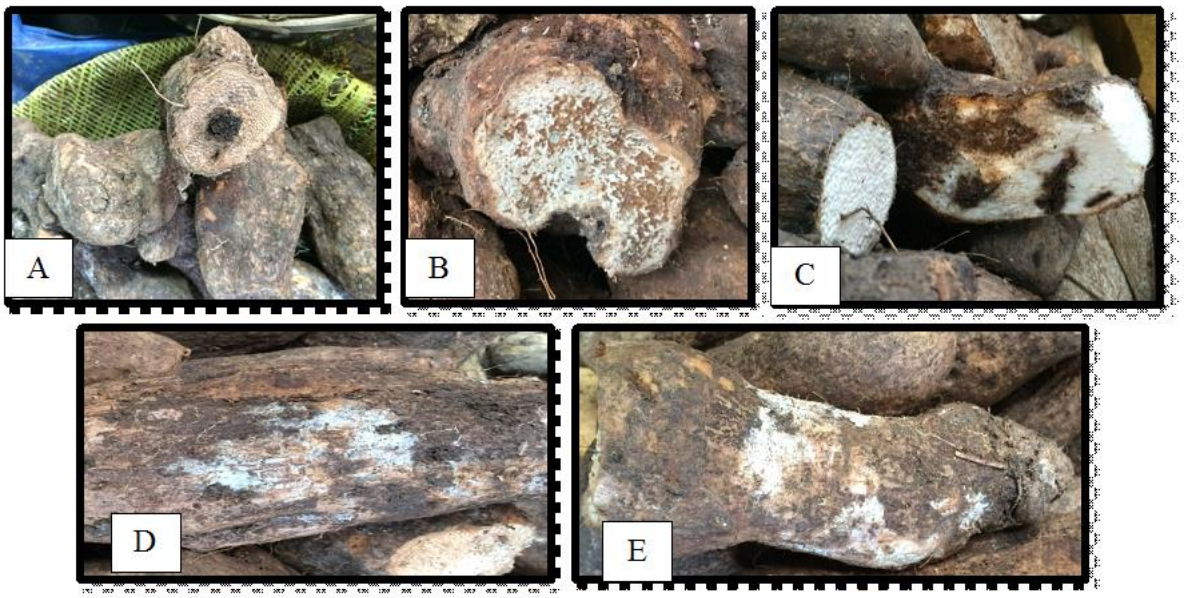

Figure 1: Naturally infected *Dioscorea alata* roots.

A= Natural infection of *Aspergillus niger*, B= Natural infection of *Geotrichum candidum*, C= Natural infection of *Neoscytallidium* sp., D= Natural infection of *Fusarium oxysprum*, E= Natural infection of *Junghuhnia* sp.

As regards to the distribution of test isolates in the samples collected from different market places, all the five species were isolated from samples collected from Puri, 4 fungal species from samples of Cuttack, 3 species each from samples in Bhubaneswar and Balasore and 2 species from samples collected from markets of Jajpur while no test fungi in the samples from Bhadrak. The maximum percentage of incidence was recorded in *Neoscytalidium* sp. followed by *Junghuhnia* sp., *Aspergillus niger*, *Geotrichum candidum* and *Fusarium oxysporum*. Their percentage of incidence was 34.49, 27.59, 20.69, 12.06 and 5.17 respectively (Table 1 & Figure 2).

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Table 1- Incidence of fungi from rotten *Dioscorea alata* collected from six localities of Odisha.

FUNGI	*Localities							
	I	II	III	IV	V	VI	Total	%
Aspergillus niger	2	6	-	1	3	-	12	20.69
Fusarium oxysporum	-	-	-	2	1	-	3	5.17
Geotrichum candidum	1	1	-	3	2	-	7	12.06
***Junghuhnia* sp.**	-	3	3	10	-	-	16	27.59
***Neoscytallidium* sp.**	7	3	9	1	-	-	20	34.49
Total	10	13	12	17	6	-	58	100

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

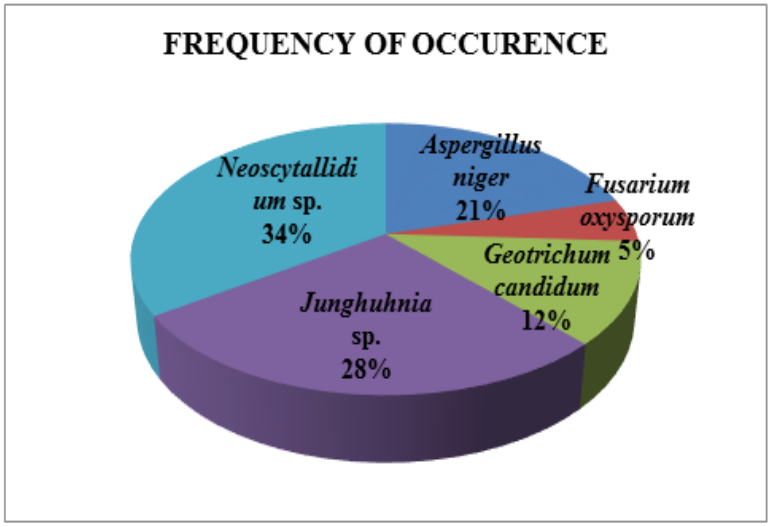

Figure 2: Incidence of fungi from rotten *Dioscorea alata* collected from six localities of Odisha.

Pathogenisity test

The pathogenicity test revealed that all the fungal isolates were pathogenic to the host under study. The percentage of pathogenicity was recorded varying from 11 % to 80 %. Of all the fungi isolated during the present investigation, *Neoscytalidium* sp. was found to be most pathogenic followed by *Aspergillus niger*, *Geotrichum candidum*, *Fusarium oxysporum* and *Junghuhnia* sp. All the inoculated pathogens on pathogenicity test found to cause the rotting in *D. alata*. The extent of rotting of tubers was estimated to be 80 %, 56 %, 46 %, 24 % and 11 % respectively. Upon re-isolation, the rotten tissues yielded a fungus which was identical with the original fungus inoculated (Table 2 & Fig 3, 4).

Table 2: Pathogenicity of the isolates on *Dioscorea alata*

Sl. No.	Fungi	Percentage of rotting
1	*Aspergillus niger*	56
2	*Fusarium oxysporum*	24
3	*Geotrichum candidum*	46
4	*Junghuhnia* sp.	11
5	*Neoscytallidium* sp.	80

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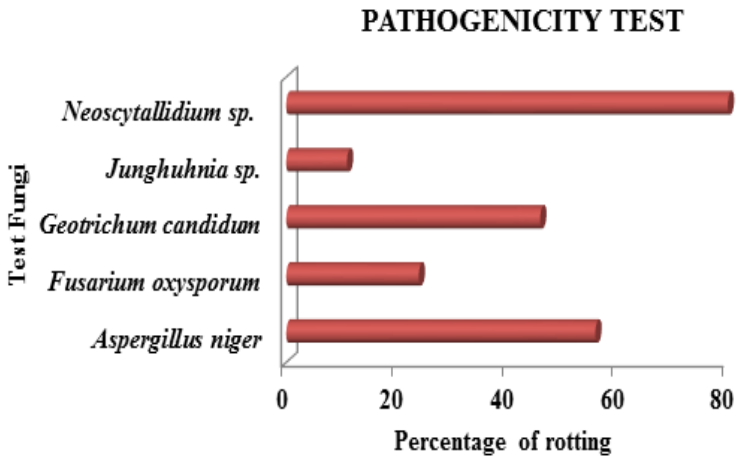

Figure-3: Pathogenicity of the isolates on *Dioscorea alata* roots

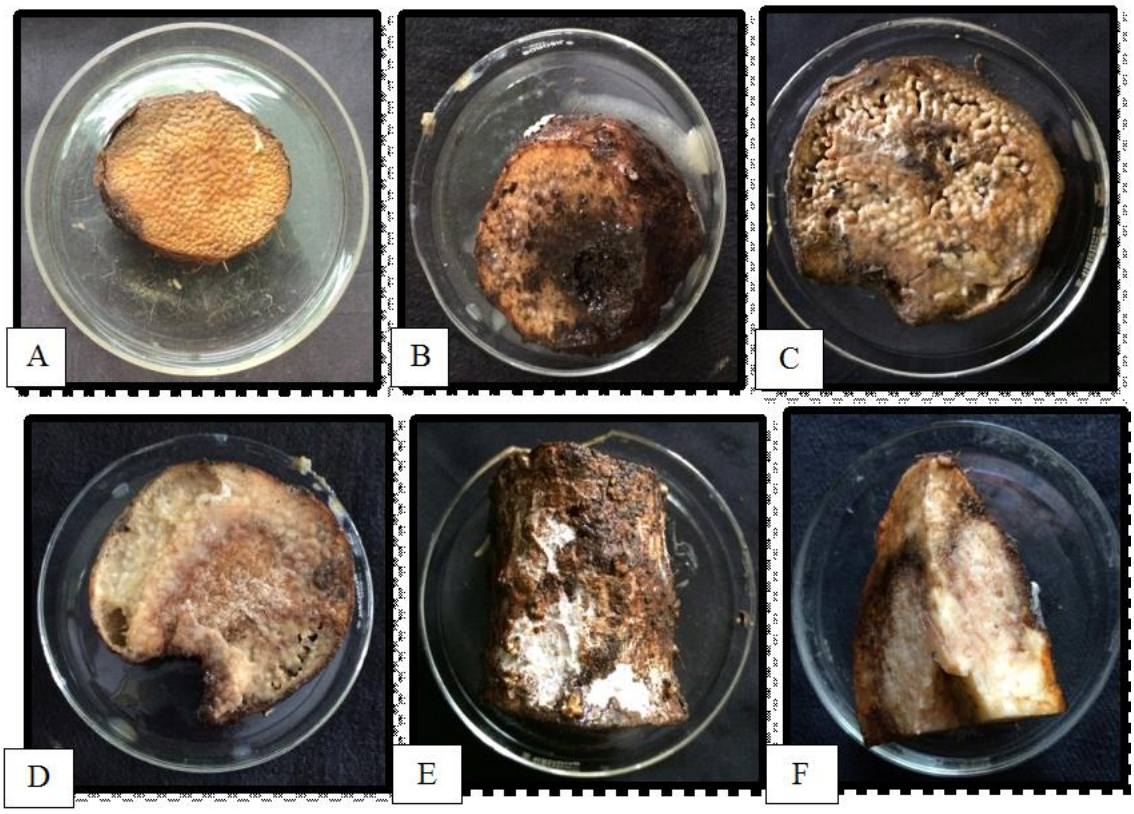

Figure 4: Pathogenicity of the isolates on *Dioscorea alata* roots

A= Uninoculated, B= Inoculated by *Aspergillus niger*, C= Inoculated by *Fusarium oxysporum*, D= Inoculated by *Geotrichum candidum*, E= Inoculated by *Junghuhnia* sp., F= Inoculated by *Neoscytallidium* sp.

Nutritional study

The data presented in (Table 3) revealed that there was no significant difference in the mycelial growth under the impact of different solid nutrient media. 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 *Fusarium oxysporum* and *Geotrichum candidum* was obtained on Czapek Dox, while maximum growth of *Junghuhnia* sp. was observed on Potato Dextrose Agar (Table 3 & Figure 5).

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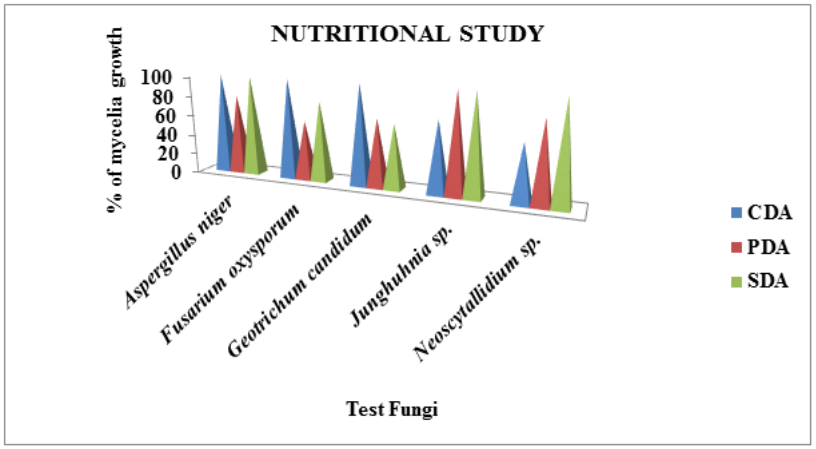
Table 3: Effect of three solid media on the growth of isolated fungi

Test organisms	Percentage of growth of fungal mycelia		
	CDA	PDA	SDA
Aspergillus niger	100	80	100
Fusarium oxysporum	100	58.75	80
Geotrichum candidum	100	67.5	63.75
Junghuhnia sp.	71.25	100	100
Neoscytallidium sp.	57.5	80	100

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



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

Figure 5: Effect of three solid nutrient media on the growth of ten test fungi.

DISCUSSION

During present investigation, five species of fungi namely *Aspergillus niger*, *Fusarium oxysporum*, *Geotrichum candidum*, *Junghuhnia* sp. *and Neoscytalidium* sp. were found to be associated and cause of storage decay in *Dioscorea alata* roots in Odisha, India. According to some earlier reports, storage rots of yam was caused by *A.* niger [9, 11, 16, 19, 22-29]; by *Fusarium oxysporum* [11,27]; and by *Geotrichum Candidum* [20]. From the present study it was found that a species of *Junghuhnia* and *Neoscytalidium* were responsible for post harvest storage decay of *D. alata* roots which is a new host record in the country. However there was a report on *Junghuhnia vincta* which is casual agent of a root disease of *Pinus radiate* [30]. Besides *P. radiata*, *Junghuhnia vincta* is associated with a root disease of other coniferous hosts and also with other dicotyledonous trees [31]. So *Junghuhnia* sp. is described here for the first time as a pathogen of *D. alata* root causing storage rot in Odisha, India. *Neoscytalidium* is a genus of fungi in the *Botryosphaeriaceae* family with two species namely *Neoscytalidium dimidiatum* and *N. novaehollandiae* and reported to cause diseases [32]. In Australia, Ray et al., 2010 [33] reported dieback of *Ficus carica* by *Neoscytalidium dimidiatum* and dieback of *Mangifera indica* by *N. novaehollandiae*. *Neoscytalidium dimidiatum* was also found to cause stem canker on red-fleshed dragon fruit (*Hylocereus polyrhizus*) plantations in Malaysia [34] but there is no report on storage rot of *D. alata* by any species of *Neoscytallidium*. So storage rot of *D. alata* roots by *Neoscytalidium* sp. was found to be a new host record in India.

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