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Isolation and Identification of Fungi Causing Postharvest Decay of Oranges (*Citrus sinensis*) During Storage in Makurdi

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RESEARCH ARTICLE

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ABSTRACT

Isolation and identification of fungi causing post-harvest decay of oranges during storage in Makurdi was investigated. Orange fruits showing symptoms of decay were collected in polythene envelops and taken to the botany laboratory of the Benue State University for isolation and identification of fungi pathogens. Small sizes were cut from the decaying orange fruits, surface sterilized in 1% of sodium hypochloride solution and rinsed in three changes of sterile distilled water. The excised sections were placed on solidified Potato Dextrose Agar (PDA) and observed for fungal growth. For pathogenicity, healthy orange fruits were plugged with pure cultures of the fungal isolates and disease incidence and severity were assessed. T-test revealed significant differences between the inoculated and the control at 5% level of probability in the pathogenicity of the fungal isolates. *Aspergillus niger, Penicillium chrysogenum, Rhizopus stolonifer, Fusarium oxysporum* and *Bipolaris harlodes* were isolated from the decaying orange fruits. Incidence of decay on healthy orange fruits was 100% for all fungal isolates while the control was 0%. Severity of decay ranged from 3 – 5 which indicated 41-100% of tissue damage. Pathogenic fungi on orange fruits are a potential health hazard to man and animals following ingestion.

Keywords: Postharvest, oranges, storage, decay, fungi

INTRODUCTION

Orange (*Citrus sinensis* L.) which belongs to the family Rutaceae is one of the most widely grown fruit crops in the world [1].The crop is mainly cultivated in the tropical and subtropical regions of the world in over 137 countries on six continents [2]. It is widely consumed both as fresh fruit and as juice.

Oranges are principal sources of important nutrients like vitamin C, flavonoids, pectin, folic acid, carotenoid, dietary fibre, potassium, selenium and a wide range of phytochemicals that are suggested to be responsible for the prevention of degenerative diseases [1,3].

In spite of the numerous benefits derived from oranges, pre- and postharvest diseases, improper handling and other conditions affect the quality and nutritional value of orange fruits. Improper handling, packaging, storage and transportation may result in decay and growth of microorganisms, which become activated because of the changing physiological

state of the fruits [1]. Citrus fruits, due to their low pH, higher moisture content and nutrient composition are very susceptible to attack by pathogenic fungi, which in addition to causing rots, may also make them unfit for consumption by producing mycotoxins [4].

Reports on post-harvest fungi rots of oranges are extensive and worldwide[1,5,6]. A number of fungal species including *Phythium, Phytophthora*, *Botryodiplodia*, *Aspergillus*, *Penicillium*, *Rhizopus*, *Glasdosporium, and Mucor* have been associated with orange fruit rot [5]. This study is therefore aimed at isolating and identifying fungi causing postharvest decay of oranges during storage in Makurdi with a view to add to the pool of information on fungal pathogens causing decay of orange fruits in Makurdi.

MATERIALS AND METHODS

Collection of Samples

Orange fruits showing symptoms of decay were collected in polythene envelops from the preserved samples and taken to the Botany Laboratory of the Benue State University for isolation of fungi pathogens.

Media Preparation

The medium used for isolation of fungi was Potato Dextrose Agar (PDA), which was prepared according to manufacturer's instruction. About 39 g of powdered PDA medium was dissolved in 1 litre of sterile distilled water and sterilized by autoclaving at 121^{O}C for 15 minutes under a pressure of 15 pounds per square inch (15lb/inch^{2}). After cooling, it was dispensed into sterile petri dishes. Streptomycin was added to the potato dextrose medium to prevent the growth of bacteria.

Isolation of fungi

Small sizes were cut from orange fruits infected with rot and surface sterilized by dipping in 1% sodium hypochloride (NaOCl) solution for one minute. They were removed and rinsed in three changes of sterile distilled water then placed on sterile paper towel to dry. They were then placed on solidified Potato Dextrose Agar medium. Three replications were made for each sample. The inoculated plates were incubated at room temperature and observations were made for microbial growth. After 6-7 days of growth, sub culturing was done to obtain pure cultures of the isolates as reported by [7].

Maize seed samples were collected in polythene envelopes from five markets namely; Wurukum, Wadata, Kanshio, Modern and high level in Makurdi and taken to the botany laboratory of the Benue State University for further studies.

Identification of fungi

The method reported by [8] was used. Identification was done microscopically and macroscopically. For macroscopic characteristics, colony characteristics such as appearance, change in medium color and growth rate were observed. For microscopic characteristics, a drop of lactophenol cotton blue was placed on a grease free slide and a sterile wire loop was used to aseptically transfer a loopful of the fungal isolates onto the slide. A cover slip was placed on the slide in such a way as to prevent air bubbles. The slide was then viewed under a microscope at 10× and 40× objective. Shape of the conidia and conidiophores were taken note of. These features were matched with standards in [9].

Pathogenicity test

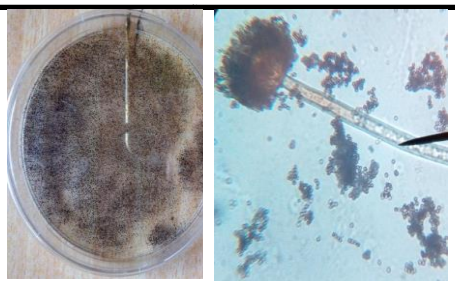
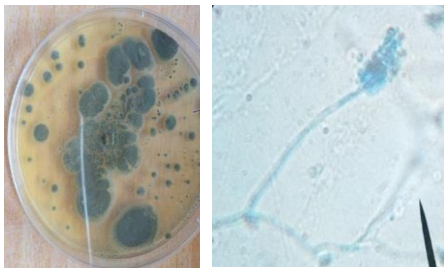
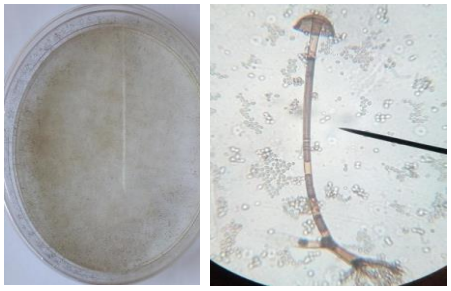
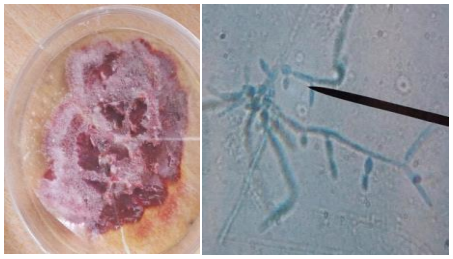
Mycelia plugs of the fungal isolates from 5 days old pure cultures were used to inoculate four orange fruits per pathogen. On appearance of symptoms, the tissues at the margin of the healthy and diseased parts were excised, sterilized and placed onto Potato Dextrose Agar (PDA) and incubated at room temperature for 5 - 7 days. At the end of the duration, morphological characteristics and growth patterns observed in each case were compared with the ones of the original isolates. One orange fruit each was used for each fungal isolate

which was replicated three times and arranged in completely randomized design. Controls were orange fruits inoculated with sterile PDA only. After 8-14 days of post inoculation, incidence was calculated by counting the number of fruits showing symptoms of decay divided by the total number of fruits per plot multiplied by 100 while decay severity index was assessed on a scale of 0 - 5 where 0 -no disease manifestation, 1 is 1-20% decay, 2; 21-40% decay, 3; 41-60% decay, 4; 61-80% decay, 5; 81-100% decay as reported by [7].

RESULTS AND DISCUSSION

Five fungi namely *Aspergillus niger, Penicillium chrysogenum, Fusarium oxysporum, Rhizopus stolonifer and Bipolaris harlodes* were isolated from the decaying orange fruits in storage. *For A. niger,* the colony was black to brown in colour. The colonies were well developed; hypha profusely branched, septate and hyaline. For *P. chrysogenum,* the colony grew rapidly and appeared green with sporulation and radial furrows on the surface and reverse. The conidia were in chains, columns and were ellipsoidal. For *F. oxysporum,* the colony colour was purple with a red tinge and a whitish peach. No distinct division in micro and macro conidia was observed. Conidiophores were slender and simple. Conidia were canoe shaped. For *R. stolonifer,* the colour of the colony appeared light grey and wooly. It grew extremely rapid and filling the Petri dish with dense cottony mycelium producing mass of sporangia. The sporangia were white at first, then black. Sporangia were globose to subglobose. Sporangiospores were regular in shape and were formed within the sporangium. The Sporangiosphores had branched rhizoides. For *B. harlodes,* the colony was dark, blackish brown and fluffy with a moderate to fast growth rate. Conidiophores were septate and cylindrical. Conidia were straight to slightly curved, brown and thick-walled. The basal septum was darker and thicker than the other septa as shown in Table 1. Incidence of decay on orange fruits inoculated with each test fungi was 100% respectively and this was significantly higher compared to the control which was 0%. Severity of decay ranged from 3 – 5 which indicated 41 – 100% of tissue damage and this was significantly higher than the control which showed 0% severity as shown in Tables 1 and 2.

Table 1: Cultural and Morphological characteristics of isolated fungi from orange fruits during storage

Microscopic/Macroscopic Characteristics	Photomicrograph	Probable Organism
Conidia have a black color in the culture. The colonies are well developed; hypha profusely branched, septate and hyaline		*Aspergillus niger*
It grows rapidly and appears green with sporulation and radial furrows on the surface and reverse. The conidia is in chains, columns and is ellipsoidal		*Penicillium Chrysogenum*
The color appears light grey and wooly. It grows extremely rapid and filling the Petridish with dense cottony mycelium producing mass of sporangia. The sporangia are white at first, then black. Sporangia are globose to subglobose. Sporangiospores are regular in shape and are formed within the sporangium. The sporangiophores have branched rhizoides.		*Rhizopus stolonifer*
The colony colour is purple with a red tinge and a whitish peach. No distinct division in micro and macro conidia. Conidiophores are slender and simple. Conidia are canoe shaped.		*Fusarium oxysporum*

Colony is dark, blackish brown and fluffy with a moderate to fast growth rate. Conidiophores are septate and cylindrical. Conidia are straight to slightly curved, brown and thick-walled. The basal septum is darker and thicker than the other septa.			*Bipolaris harlodes*

Table 2: Incidence of decay on healthy orange fruits after artificial inoculation with test fungi

Test fungi	*Aspergillus niger*	*Rhizopus stolonifer*	*Penicillium chrysogenum*	*Bipolaris harlodes*	*Fusarium Oxysporum*
Treatment	100.0	100.0	100.0	100.0	100.0
Control	0.00	0.00	0.00	0.00	0.00
T-test	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**

Table 3: Severity of decay on healthy orange fruits inoculated with test fungi

Fungal isolates	*A. Niger*	*P.chrysogenum*	*F.oxysporum*	*B.harlodes*	*R. stolonifer*
Severity	5.00	4.00	3.00	5.00	5.00
Control	0.00	0.00	0.00	0.00	0.00
LSD(0.05)	**0.93**	**0.91**	**0.92**	**0.00**	**0.00**

Severity scale: 0=No infection
1=1-20% decay
2=21-40% decay
3=41-60% decay
4=61-80% decay
5=81-100% decay

This study revealed that five fungi namely *Aspergilus niger*, *Penicillum chrysogenum*, *Fusarium oxyporum*, *Rhizopus stolonifer* and *Bipolaris harlodes* were associated with decay of orange fruits (*Citrus cinensis*).These organisms have been associated with post-harvest rot during storage as reported by [8,10]. Also, the authors in [7] reported similar organisms to be associated with post havest decay of yams during storage. Additionally, [10] reported that *Aspergillus flavus*, *Aspergillus. niger*, *Fusarium solani*, *Penicillium digitatum*, *Rizopus.*

Stolonifer and yeast were found in fruits sold in major markets in Ibadan, Oyo State, South Western Nigeria.

The pathogenicity test revealed that healthy orange fruits showed symptoms of rot after 5 - 7 days of inoculation with fungi mycelia.This was due to the ability of the fungal pathogens to utilize the nutrients of the oranges as a substrate for growth and development [7]. The occurrence of these organisms may be attributed to their ability to produce resistant spores from the field as reported by[12]. *Aspergillus* species have been implicated in the spoilage of fruits and vegetables in Nigeria [13]. Similar results were reported by [14] who isolated similar organisms in cowpea. The study also agrees with findings of [7-8] that isolated similar organisms from yam and oranges respectively.

CONCLUSION

Aspergillus niger, *Penicillium chrysogenum*, *Fusarium oxysporum*, *Rhizopus stolonifer* and *Bipolaris harlodes* are fungi pathogens responsible for decay of orange fruits during storage in Makurdi. Pathogenic fungi on orange fruits are a potential health hazard to man and animals following ingestion.

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