Asian Journal of Research in Crop Science

1(2): 1-6, 2018; Article no.AJRCS.40642

Isolation and Identification of Fungi Causing Decay in Pepper (*Capsicum spp*) from Selected Markets in Makurdi

B. K. Akinyemi[1*] and K. Liamngee[1]

[1]Department of Biological Sciences, Center for Food Technology and Research (CEFTER), Benue State University Makurdi, Benue State, Nigeria.

Authors' contributions

This work was carried out by both authors. Authors BK and KL were the supervisors and reviewers of the work. Authors BK and KL performed laboratory experiments and wrote the manuscript. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJRCS/2018/40642
Editor(s):
(1) Deligios Paola Antonia, Professor, Department of Agriculture, Università degli Studi di Sassari, Italy.
Reviewers:
(1) Ahmed Karmaoui, Southern Center for Culture and Sciences, Morocco.
(2) Gwa, I. Victor, Federal University, Nigeria.
(3) Umoru S. Amodu, University of Uyo, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/24040

Original Research Article

Received 26th January 2018
Accepted 1st April 2018
Published 7th April 2018

ABSTRACT

Isolation and identification of fungi causing decay in pepper fruits from four markets in Makurdi, Benue state, Nigeria namely Northbank, Wurukum, High level and Wadata was carried out. Samples were collected in polythene envelopes and taken to the laboratory for fungal isolation. They were surfaced sterilized in 5% NaOCl solution for 1 minute, rinsed in several changes of sterile distilled water and plated on Potato Dextrose Agar in Petri dishes. After 5-7 days of growth, subculturing was done to obtain pure cultures. Identification of the isolates was made macroscopically and microscopically. Colony characteristics such as appearance, change in medium colour and growth rate were observed. Shapes of the conidia and conidiophores were also taken note of. Five fungi, namely *Aspergillus niger*, *Fusarium moniliforme*, *Fusarium oxysporum*, *Colletotrichum asianum* and *Bipolaris zeicola* were isolated with 31.52%, 29.76%, 5.93%, 20.68% and 12.11% as their percentage occurrence respectively. Pathogenicity of isolates ranged from 4 - 5 respectively which indicated 61-100% of decay. The presence of these fungi in pepper could lead to severe health implications when consumed.

**Corresponding author: E-mail: akemfortco@gmail.com;*

Keywords: Isolation; fungi; identification; decay; pepper; Makurdi.

1. INTRODUCTION

Pepper (*Capsicum spp*.) is one of the most commercially important crops grown in Nigeria. Peppers are currently the object of much attention due to possible links to the prevention of a certain type of cardiovascular diseases, atherosclerosis, hemorrhage, delaying of the ageing process, improving physical resistance and increasing appetite [1]. Additionally, peppers are remarkable vegetables because of their significant provitamin A concentration, through their concentration of carotenoids such as beta-carotene [2]. Peppers are one of the few foods that contain lycopene, a carotenoid whose consumption has been inversely correlated with cancer [3]. Consumption of vitamin C, beta-carotene, and folic acid, all found in peppers is associated with a significantly reduced risk of cancer [4]. However, since peppers are highly perishable, they encounter several problems in their transportation, storage and marketing [5]. Owing to lack of information on appropriate postharvest treatments, packaging, storage conditions, the fruits not only lose their quality but also encounter substantial postharvest losses [6].

Pathogenic fungi such as *Alternaria*, *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus*, and *Trichoderma* have been implicated in crop spoilage [7]. Fungi contamination of many agricultural products, including pepper starts in the field [8]. Both the biological and physical damages during the harvest and transportation phases coupled with a significant amount of water and soft endocarp makes pepper very susceptible to spoilage by fungi [9,10]. Few studies on fungi associated with pepper spoilage are available in Nigeria [11]. The present study was carried out to isolate and identify fungi species associated with spoilage of pepper from four markets in Makurdi, Benue State, Nigeria.

2. MATERIALS AND METHODS

2.1 Sample Collection

Pepper fruits showing symptoms of decay were procured from four markets in Makurdi namely, Northbank, Wurukum, High level and Wadata. They were transported in polythene envelops to the Botany laboratory of the Benue State University, Makurdi, Nigeria for fungal isolation. Makurdi town is located at 7°44^{1}50" North, 8^{0}32^{1}10" East on the geographical map of Nigeria.

2.2 Media Preparation

The media used for isolation of spoilage organisms was Potato Dextrose Agar (PDA), which was prepared according to the manufacturer's instruction. About 40 g of powdered PDA was dissolved in 1000 milliliters of sterile distilled water and sterilized by autoclaving at 121°C for 15 minutes before pouring carefully into sterile Petri dishes.

2.3 Isolation of Fungal Pathogens from Decaying Pepper Fruits

Small sizes were cut from pepper fruits infected with rot. They were first surfaced sterilized by dipping them in a concentration of 5% NaOCl solution for 1 minute. The pieces were then removed and rinsed in several changes of sterile distilled water and placed on sterile paper towels to dry for 5 minutes. They were then placed on solidified PDA medium. Three replicates were made for each sample. The inoculated Petri plates were incubated at room temperature and observations were made daily for possible microbial growth. After 5-7 days of growth, subculturing was done to obtain pure cultures of the isolates as reported by Liamngee et al. [12].

2.4 Identification of Fungal Isolates

The identification of the isolates was done by examining the isolates macroscopically and microscopically. Colony characteristics such as colony appearance, change in medium colour and growth rate were observed. Shapes of the conidia and conidiophores were taken note of. These structural features were matched with standards in [13] as reported by Liamngee et al. [12].

2.5 Pathogenicity Test

To confirm the pathogenicity of fungal isolates from the pepper fruits, the method reported by Liamngee et al. [12] was used. Pure cultures of the isolates from 5–7 days old cultures were used to inoculate healthy pepper fruits using a cork borer. On appearance of symptoms, the tissues at the margins of the healthy and diseased parts were surfaced sterilized, excised

and plated on Potato Dextrose Agar (PDA) and incubated at room temperature for 6-9 days. At the end of this period, morphological characteristics and growth patterns observed in each case were compared with the ones of the original isolates. Four pepper fruits were used for each fungal isolate respectively, replicated three times and arranged in complete randomized design. Controls were pepper fruits inoculated with sterile PDA only.

3. RESULTS

3.1 Characterization of Isolates

3.1.1 *Aspergillus niger*

Colony grows slowly, consisting of compact, faintly yellow basal mycelium which bears abundant and usually crowded conidia structures, typically carbon black. Conidia heads are typically large, black and spherical. Conidiophores smooth, hyaline or faintly brownish and up to 3µm in length.

3.1.2 *Fusarium moniliforme*

Colony grows rapidly with white aerial mycelium often tinged with purple. Mycelium has a powdery appearance due to the presence of Chains of micro-conidia. Abundant micro-conidia are formed and are hyaline and usually one celled. They are oval and slightly flattened at each end.

3.1.3 *Colletotrichum asianum*

The colony grows slowly and is mint cream to light orange in colour. The reverse is light brown to orange colour. Conidia produced were hyaline and cylindrical in shape with obtuse to slightly rounded ends.

3.1.4 *Fusarium oxysporum*

The colour of the colony is light to dark violet purple with a cottony mycelium. The reverse colour is red. Macroconidia are produced on branched conidiosphores and are kidney shaped. They are smooth and sickle shaped with pointed ends.

3.1.5 *Bipolaris zeicola*

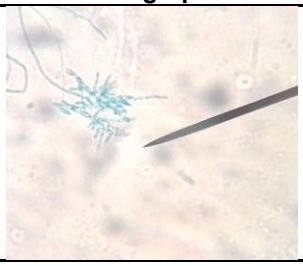
Colony is covered by very dark brown to black mycelium which gives it a characteristic charcoal appearance. Conidiophores are single, mid to dark brown, septate and cylindrical. Conidia are cylindrical but usually broad in the middle and tapering towards the rounded ends.

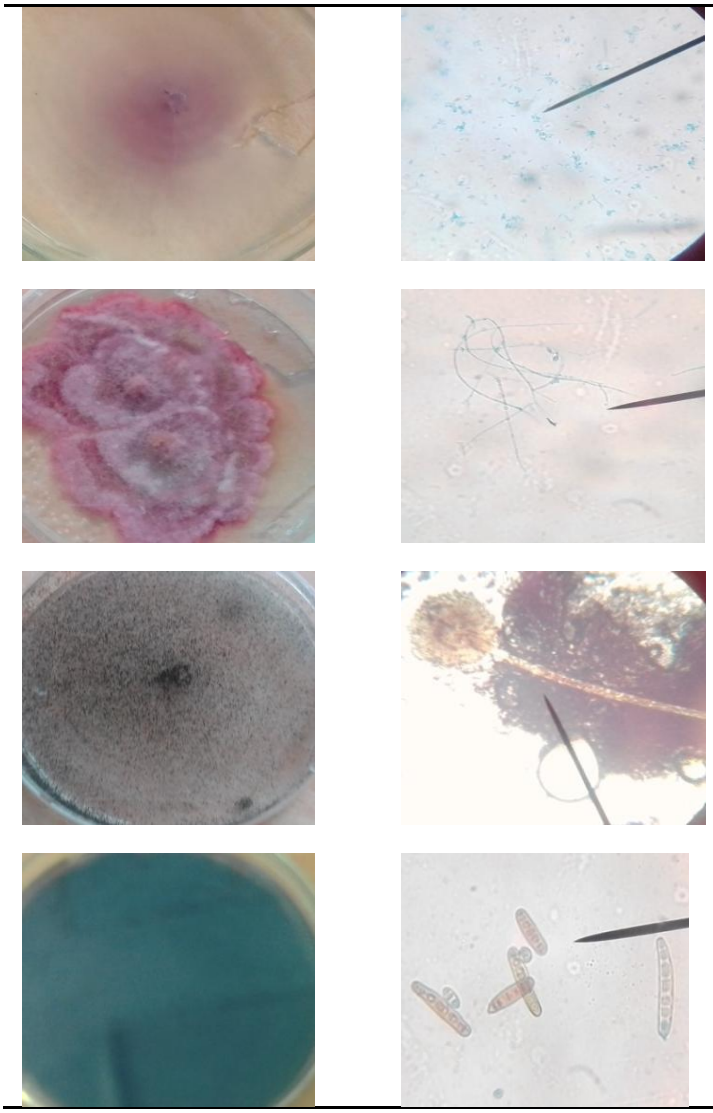
4. DISCUSSION

A total of 793 fungi were isolated. They were *Aspergillus niger* 250 (31.52%), *Fusarium moniliforme* 236 (29.76%), *Colletotrichum asianum 164* (20.68%), *Bipolaris zeicola* 96 (12.11%) and *Fusarium oxysporum* 47 (5.93%) as shown in Table 2.

In this study, *Aspergillus niger, Fusarium moniliforme, Colletotrichum asianum, Fusarium oxysporum, Bipolaris zeicola* were isolated from the pepper samples. This is similar to a study carried out on fungi associated with the spoilage of post-harvest pepper fruits reported by Ugwu et al. [14]. They discovered six species of fungi; *Candida tropicalis, Penicillium notatum, Aspergillus niger, Fusarium oxysporum, Absidia corynbifera, Rhizopus stonolifer* and four species of bacteria namely *Escherichia coli, Klebsiella spp., Salmonella spp. and Pseudomonas aeruginosa.* Several studies have also reported that *Aspergillus spp*. are associated with spoilage

Table 1. Appearance of Isolates on PDA and their photomicrograph

Appearance on PDA	Photomicrograph	Probable organism
		*Colletotrichum asianum*



Fusarium moniliforme

Fusarium oxysporum

Aspergillus niger

Bipolaris zeicola

Table 2. Percentage occurrence of fungi isolates from decaying pepper fruits

Fungi Species	Number of Isolates	% Frequency
Aspergillus niger	250	31.52
Fusarium moniliforme	236	29.76
Colletotrichum asianum	164	20.68
Bipolaris zeicola	96	12.11
Fusarium oxysporum	47	5.93
Total	793	100

of tomatoes, pepper, apricot, orange, lemon, peach, apple, kiwi, mango [15]. [10] reported that *Aspergillus* had the highest decay diameter among other fungi associated with pepper spoilage. The occurrence of *Aspergillus* in rotten peppers could pose a serious health risk, especially when the peppers are not well cooked. Some molds are capable of producing more than one mycotoxin and some mycotoxins are produced by more than one fungal species [16].

Table 3. Pathogenicity of fungal isolates on healthy pepper fruits

Fungal isolates replicates	R1	R2	R3	C
Aspergillus niger	5	4	5	0
Fusarium moniliforme	5	5	5	0
Fusarium oxysporum	4	4	5	0
Colletotrichum asianum	4	5	5	0
Bipolaris zeicola	5	5	5	0

Key: C = Control, R1 = Replicate1, R2 = Replicate2, R3 = Replicate3
Severity scale, 0 = No infection, 1 = 1 - 20% of decay, 2 = 21 - 40% of decay, 3 = 41 - 60% of decay
4 = 61 - 80% of decay, 5 =81 - 100% of decay

Table 4. Analysis of variance in the pathogenicity of fungal isolates

Fungal Isolates	*A. niger*	*F. moniliforme*	*F. oxysporum*	*C. asianum*	*B. zeicola*
Pathogenicity	5.00^{a}	5.00^{a}	4.00^{a}	5.00^{a}	5.00^{a}
Control	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}
LSD (0.05)	0.93	0.93	0.91	0.00	0.00

5. CONCLUSION

This research work identified fungi namely *Aspergillus niger, Fusarium moniliforme, Fusarium oxysporum, Colletotrichum asianum* and *Bipolaris zeicola* in decaying peppers sold in four different markets in Makurdi. The disease causing potential of the fungal isolates ranged from 4-5 which indicated a 61-100% of decay. Some of these organisms have been implicated in mycotoxin production and their presence in peppers could lead to severe health implications when consumed. It is therefore recommended that pepper fruits showing symptoms of decay should not be consumed but discarded.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Marin A, Ferreres F, Tomas-Barberan, GIL M. Characterization and quantitation of antioxidant constituents of sweet pepper (*Capsicum annum* L.). Journal of Agriculture. Food Chem. 2008;52:3861-3369.
2. Duthie G, Crozier A. plant-derived phenolic antioxidants. Curr. Opin. Lipidol. 2000;11: 43-47.
3. Tonya Zavasta. Beautiful on raw uncooked creations. BR Publishing P.O. Box 623. 2005. Conlova IN 380 880623.
4. Mateljan G. The world's healthiest foods. Way of eating. GMF Publishing; 2007. Available:http://www.whfoods.com
5. Banaras M, Bostand DW, Lowuds NK. Effects of harvest time and growth conditions on storage and post-storage quality of fresh peppers (*Capsicum annum).* PAL. J, Bot. 2007;37:337-344.
6. Nasrin TAA, Molla MM, Alamgir Hussuen MS Alan, Yasmin. Effect of pH treatments on shelf life and quality of tomato. Bangladesh Journal of Agril. Res. 2008; 33(3):579-585.
7. Beuchart LR. Pathogenic microorganisms associated with fresh produce. Journal of Food Production. 1995;50(2):204-216.
8. Aran N, Alperden I, Topal O. Mould contamination problem in tomato paste and risk analysis system in the critical control place. Journal of Food Industry. 1987;2(3):43-47.
9. Asan A, Ekmeki S. Contribution to the colonial and morphological characteristics of some Aspergillus species isolated from soil. Journal of Faculty of Science. 2002; 25:121-139.
10. Onuorah S, Orji MU. Fungi associated with the spoilage of post-harvest tomato fruits sold in major markets in Awka, Nigeria. Universal Journal of Microbiology Research. 2015;3(2):11-16.
11. Wogu MD, Ofuase O. Microorganisms responsible for the spoilage of tomato fruits, Lycopersicum esculentum, sold in markets in Benin City, southern Nigeria. Scholars Academic Journal of Bioscience. 2014;2(7):459-466.
12. Liamngee K, Onah DO, Zakki YH. Evaluation of three plant extracts in the control of fungal pathogens isolated from Garri (fried mashed fermented cassava) in

Makurdi, Nigeria. Arch. Appl. Sci. Res. 2015;7(9):6-12.
13. Barnett HL, Hunter BB. Illustrated genera of imperfect fungi, 3rd edition, Burgess publishing company, NY. 1972;21-56.
14. Ugwu CO, Chukwuezi FO, Ozougwu VCO. Microbial agents of tomato spoilage in Onitsha metropolis. Advances in Biological Research. 2014;8(2):87-93.
15. Rashad RA, Ahmed RA, Saleh AM. Isolation and identification of some fruit spoilage fungi: Screening of plant cell wall degrading enzymes. African Journal of Microbiology. 2011;5(4):443-448.
16. Zain ME. Impact of mycotoxins on humans and animals. Journal of Saudi Chemical Society. 2011;15:129-144.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/24040