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Isolation and Identification of Fungal Pathogen Associated with Post Harvest Deterioration of Cucumber (*Cucumis sativus* L.) Fruits in Three Selected Markets in Jos, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MI and AEA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors NJ and SAO managed the analyses of the study. Authors WCJ and OO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study was carried out to isolate, identify and characterize fungal pathogens associated with post-harvest spoilage of cucumber.
Study Design: This research study was done using random sampling technique.
Place and Duration of Study: The experiment was carried out from March to June, 2019 at biology laboratory Federal College of Forestry, Jos.
Methodology: Fungi isolates were obtained from diseased portions of the cucumber fruit samples cultured on PDA media and incubated at 28ºC±2 for seven days. The mycelial were identified macroscopically and microscopically. Pathogenicity tests were conducted for all the fungal

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pathogens identified by inoculating healthy cucumber samples and incubating for ten days with the readings being taken at two day intervals. The data obtained were analyzed using ANOVA and means were separated using LSD at $P \leq 0.05$.

Results: *Aspergillus fumigatus, Fusarium* sp., *Geotrichum candidum* and Yeast sp were isolated and identified. Result showed that Gada biu market was the most heavily infested location with all the fungal isolates. *Geotrichum candidum* constituted the highest (50%) occurrence of fungal isolates from all locations. All the fungal isolates were found to be pathogenic on cucumber fruits, with *Fusarium* being the most destructive, followed by yeast and *Geotrichum candidum* and the least was *Aspergilus fumigatus*.

Conclusion: The results obtained in this study showed the isolates identified were involved in Cucumber spoilage. Therefore, Careful handling of Cucumber fruits should be ensured to prevent the spread of these pathogenic fungi.

Keywords: Cucumber; pathogenicity; Aspergillus fumigates; Fusarium sp; Geotrichum candidum.

1. INTRODUCTION

Cucumber is a creeping vine that bears cylindrical fruits. It is known as *Cucumis sativus* L, it belongs to the gourd family cucubitacea. Other vegetables which belong to this family include Melon, squash, Watermelon and Pumpkins [1]. It originated from the Asia continent [2]. Cucumber plant can be cultivated in both temperate and tropical environment hence it is said to be a native of many regions of the world [3]. Cucumber (*Cucumis sativus* L.) is an important vegetable cultivated in Northern central, Nigeria both in rainy and dry season using irrigation. The fruits serves as refreshments during farming activities [4]. They fruit is normally consumed raw alone or eaten with other vegetables such as salad in Nigeria [5]. Cucumber is majorly cultivated because of its nutritional and medicinal relevance. Seed kernels are occasionally eaten [6].

Health wise, cucumber is said to give relief to people suffering from heart burn, acid stomach, gastritis, ulcer as well as lung, stomach and chest problems [7]. Field diseases and pests are major concern to cucumber cultivation and affect all stages of the crop development. Major leaf diseases that cause great losses are the fungal diseases downy mildew, powdery mildew, anthracnose, target leaf spots and gummy stem blight [8].

Diverse pathogenic fungi such as *Aspergillus* spp*., Alternaria tenuis, Penicillium oxalicum, Botrytis cinerea, Cladosporium tenuissimum, Alternaria alternate, corynespora cassiicola, Curvularia* spp*., Fusarium solani, Geotrichum candidum, Phytophthora capsici* and *Rhiophus nigricans* have been reported to induce infection on cucumber [9,10].

2. MATERIALS AND METHODS

2.1 Study Site

The research was carried out in the biological laboratory of the Federal College of Forestry Jos, Plateau State, Nigeria. The area lies in the northern savanna zone, on latitude 90º57'N and longitude 8º54' E with a height of about 118 m above sea level. The mean annual rainfall for the location is between 1200 mm and 1250 mm and mean temperature of 23ºC-25ºC the soil is sandy- loam light to darkish in color.

2.2 Collection of Sample

Thirty (30) infected cucumber fruits samples were collected from three cucumber selling points within Jos metropolis (Farin gada market, Terminus main market and Gada biyu market) all in Jos north LGA area of plateau state.

2.3 Preparation of Medium Fungi Isolation

Potato Dextrose Agar was prepared following manufacturer's instructions. After heating to boiling, the medium was sterilized by autoclaving at 121ºC for 15 minutes. The medium was allowed to cool, then Gentamycin was introduced aseptically to inhibit bacterial growth, finally the medium was dispensed into sterile petri dishes.

2.4 Fungal Isolation

The infected cucumber samples were washed and surface sterilized in 75% ethanol for one minute. Then the samples were rinsed using sterile distilled water in three successive changes and blotted dry aseptically. Diseased portion of the samples were picked under aseptic condition and placed in sterile Petri dishes with the aid of a sterile scalpel and forceps initially dipped in a methylated spirit [10]. The picked portion were placed on solidified potato dextrose agar (PDA)

and incubated at 28 ± 2ºC in the dark for 5 days until visible growth was observed from the plates. The fungal colonies were subcultured until pure culture was obtained [4].

2.5 Identification of Fungi

The fungal mycelia were picked from the sub cultured plates in very small amounts with an inoculating needle and placed on a clean glass slide that already contained lacto-phenol cotton blue, it was covered with a cover slip and heat fixed by passing it through a naked flame thrice. The prepared glass slide was then placed on the microscope and observed under low and high power objectives using a light microscope before, the macroscopic and microscopic viewed were compared.

2.6 Pathogenicity Test

The isolated fungi were tested for their ability to induce rot in healthy cucumber fruits. Healthy samples of cucumber fruits were washed and surface sterilized with 75% ethanol and then washed distilled water. With the aid of a sterile Cork borer, a 5 mm diameter cylindrical hole was made into the healthy cucumber fruits and the plugs pulled out. In each hole, a 3mm diameter mycelia disc of pure culture of each of the fungal isolates were introduced into the hole. The plug was carefully replaced and the wounded area sealed with petroleum jelly. The inoculated fruits were incubated at room temperature (28 ± 2ºC) for 10 days. Inoculated cucumber fruits were observed for rot development at two, four, six, eight and ten days after inoculation. The degree of pathogenicity of each fungus on the inoculated samples was determined by measuring the length and width of rotten parts at two days intervals for ten days [4]. The measurement was taken by dividing the cucumber into two equal parts.

2.7 Statistical Analysis

The data obtained were analyzed using the analysis of variance (ANOVA) to test for significant difference for occurrence of different fungi and means were separated using LSD at P ≤ 0.05.

3. RESULT

The occurrence of fungal isolates in spoiled samples of cucumber fruits collected from three different markets in Jos north is presented in Table 1. Isolates from the spoiled cucumber fruits were *Aspergillus fumigatus, Geotrichum candidum, Fusarium,* and yeast. *Aspergillus fumigatus* was isolated from Terminus market, *Fusarium* was isolated from Farin gada and Gada biyu, Yeast was isolated from only Gada biyu market while *Geotrichum candidum* was isolated from both Farin gada, Gada biu and Terminus market. All the fruit collected from the various market showed to be infected by *Geotrichum candidum*.

Plate 1. Macroscopic view of *Aspergillus fumigatus*

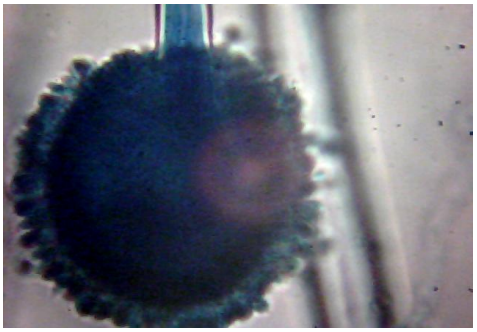

Plate 2. Microscopic view of *Aspergillus fumigatus*

Table 1. Occurrence of fungi isolate from spoiled cucumber fruit collected from three different locations in Jos North

Isolates	Terminus	Gada Biu	Farin Gada	Percentage (%)
Aspergillus fumigatus	+	-	-	12.50
Geotrichum candidum	+	+	+	50.00
Yeast	-	+	-	12.50
Fusarium sp	-	+	+	25.0

Key: + = present, - = absent

Plate 3. Macroscopic view of *Fusarium spp.*

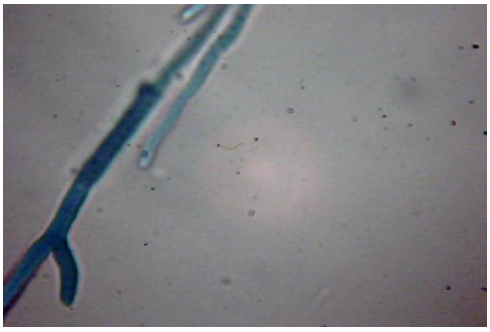

Plate 4. Microscopic view of *Fusarium spp.*

Plate 5. Macroscopic view of *Yeast spp.*

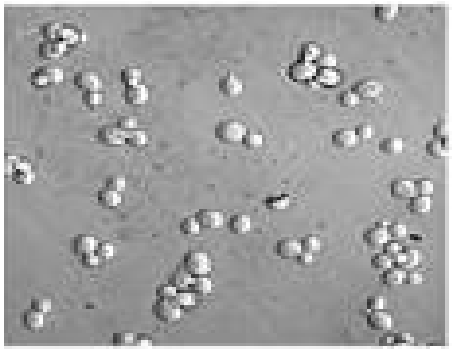

Plate 6. Microscopic view of *Yeast spp.*

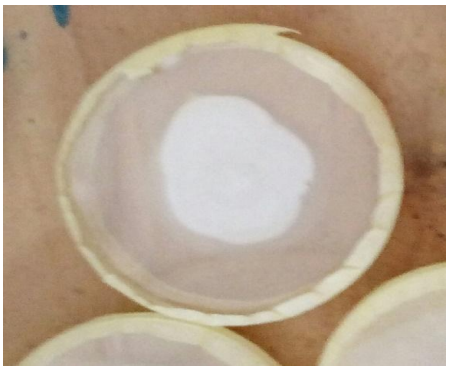

Plate 7. Macroscopic view of *Geotrichum candidum*

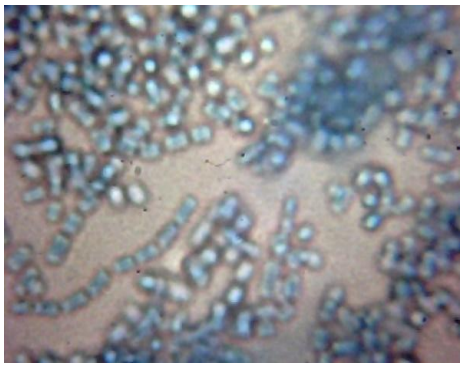

Plate 8. Microscopic view of *Geotrichum candidum*

Table 1 also shows the frequency of occurrence of fungal isolates from all the various locations. The percentage occurrence indicated *Geotrichum candidum* was the highest (50%). *Fusarium* occurred in 25% of all the samples collected from all the locations while *Aspergillus fumigatus* and *Yeast* had the lowest frequency of occurrence value of 12.5% each.

Table 2 shows the level of pathogenicity of the fungal isolate on fresh cucumber. The results in Table 2 show that the level of deterioration caused by fungal isolates were significantly different ($P \leq 0.05$) at two day intervals over the 10 days of data collection. Results of the study revealed that, mean values recorded for each treatment increased as the days of incubation increased from two to ten days and the highest mean value for each day interval was exhibited by *Fusarium* and the order of increase from second to the tenth days interval were *Fusarium* > Yeast > *Geotrichum candidum* > *Aspergillus fumigatus* > Control.

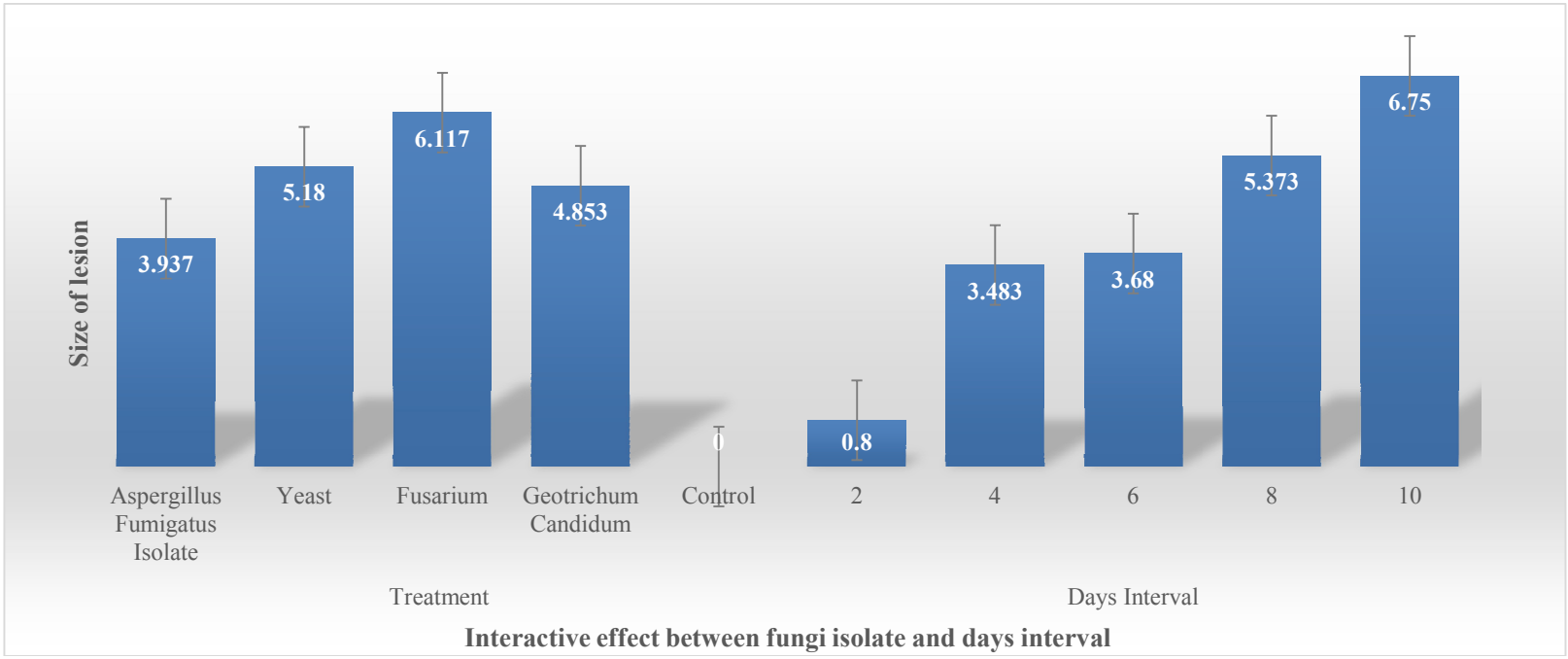

Fig. 1. Mean effects of the interaction between fungi isolates (treatment) and day's interval on size of lesion

Table 2. Mean effects of fungal isolate on the level of deterioration (cm) at different days interval

Treatment	Levels of deterioration (cm) and time				
	2D	4D	6D	8D	10D
Aspergillus fumigatus	1.783[a]	4.300[a]	3.533[a]	4.517[c]	5.550[c]
Yeast	0.317[ab]	4.883[a]	4.967[a]	6.950[b]	8.783[b]
Fusarium	1.900[a]	4.000[a]	4.633[a]	9.000[a]	11.05[a]
Geotrichum candidum	0.000[b]	4.233[a]	5.267[a]	6.400[b]	8.367[b]
Control	0.000[b]	0.000[b]	0.000[b]	0.000[d]	0.000[d]
SE	0.489	0.395	0.735	0.587	0.571

Means on the same column with the same superscript do not differ significantly from each other (P = 0.05). SE = Standard error; D = Days post inoculation

The results on treatment and time interval shown on Fig. 1 demonstrated significant difference ($p \leq 0.05$), likewise the interaction between treatments and the level of deterioration over the 10 day interval also revealed a significant difference. From the results in Table 3, *Fusarium* showed the highest effect on level of deterioration as measured by lesion size post inoculation and the order of increase at this stage of incubation measured was *Fusarium*>*Yeast*>*Geotrichum candidum* > *Aspergillus fumigatus*> Control. Subsequently, the result on day intervals revealed increase effect as the days after inoculation increased from day two to ten and the highest mean value for each stage was obtained from day.

4. DISCUSSION

The isolation and identification of deteriorated cucumber fruit samples shows that the samples were contaminated with *Aspergillus fumigatus, Fusarium*, *Geotrichum Candidum* and *yeast* species. This findings is contrary to study of Jidda and Muhammad [4], who reported that their samples in Maiduguri, Nigeria were contaminated with *Zygosacchar omycesbaili, Aspergillus flavus*, *Aspergillus niger, A. Oryzae, A. wentii, Saccharomyces cerevisiae* and *Saccharomyces resus*. The most heavily infested location was Gada biu market which revealed cucumber fruits with all the fungal isolates. This might not be unconnected with the market being in one of the major highways where different fruits and vegetables are being transported through and to other places. Cucumbers from different sources may serve as the source of various fungal pathogens associated with postharvest diseases and also contribute to rots of various other fruits. The least infested location was the Terminus market with only two out of the four fungi detected. The distribution of fungi showed that *Geotrichum candidum* constituted the most frequent (50%), followed by *Fusarium* (25%) from all the locations. This is contrary to work of Oviasogie, et al. [11] who reported that *Aspergillus* species were the most frequent occurring fungal isolate. The least isolated fungi from the three locations were *Aspergillus fumigatus* and yeast with 12.5% frequency each. This result is similar to Yaji, et al. [12] who reported the presence of *Aspergillus* spp. In all their surveyed locations. Rotten cucumber fruits were similarly reported by Al-sadi, et al. [10] to be host to *Aspergillus spp*. Akinmusire [13] found *Aspergillus species* as the most dominant fungal isolate in tomato, orange, onions and other spoiled fruits in Nigeria.

All the fungi found in this study were pathogenic to fresh cucumber fruit samples. *Fusarium* was the most pathogenic isolate with the highest level of deterioration with mean effect of 6.117 cm lesion size. The rot occurred as a result of inoculation with each of the pathogens. The findings disagreed with the report of Akinmusire [13] that *Aspergillus* species were more pathogenic to their tested fruits (Pawpaw, Orange, Pineapple and Tomato). *Aspergillus* specie were however, found to be weakly pathogenic to cucumber fruits.

The yeast and *Geotrichum candidum* were the second most pathogenic fungi with lesion size of 5.18 cm and 4.85 cm respectively. The yeast has been found to be major cause of food spoilage in many different products including fruit juice, sauces, carbonated soft drinks and ketchup. Yeast have a reputation of being able to grow on a very high concentration of sugars [14] and have caused significant economic losses to the food industry [15]. Yeast is used in the fermentation of many crops such as millet, guinea corn and potato to make beer and has been implicated spoilage of orange, mango,

banana and tomato fruits [16,17]. In general, data analysis of the pathogenicity tests for all the fungi species showed that there was significant difference between individual fungi at different day intervals after inoculations.

5. CONCLUSION

The present study has shown that *Aspergillus fumigatus, Fusarium, Geotrichum candidum* and *Yeast* were not only associated with diseased cucumber samples but actually caused the spoilage of cucumber. The isolation and identification of the fungal pathogen showed that *Geotrichum candidum* had the highest frequency of distribution (50%). The pathogenicity test shows that *Fusarium* is the most pathogenic with rapid lesion development. Based on the results obtained from frequency distribution and pathogenicity, it could be concluded that *Geotrichum candidum* even with the highest frequency, is not the most pathogenic. Identifying fungi that cause spoilage to cucumber fruits will help to adopt appropriate preventive and control measures that will help minimize fungi infection on cucumber fruits.

6. RECOMMENDATION

Further research should be carried out on the particular effective control for the various pathogens identified in this research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Bello MO, Owoeye G, Abdulhammed M, Yekeen TA. Characterization of gourd fruit (Cucubitaceae) for dietary values and anti-nutrition constituent. Journal of Pharmaceutical Biology Chemistry Science. 2014; 6:7575-7585.
2. Abulude OA, Adeleke KO. Comparative studies on nutritional composition of four melon seeds varieties. Pakistan Journal of Nutrition. 2010;9:905-908.
3. Mortimore M. Dry land developer's success stories from West Africa environment. Journal of Biological Science. 2015;45:10-21.
4. Jidda MB, Muhammad MM. Assessment of fungal pathogens associated with spoilage of cucumber (*Cucumis sativu* L.) fruits. International Journal of Current Microbiology Applied Science. 2017;6(3):510-516.
5. Ibrahim M, Sada MD. Yeasts associated with spoilage of some selected fruits in Sokoto Metropolis; 2015. Available:http://scienceq.org/Journals/JALS.php (Accessed 10th September, 2019)
6. Aboloma RI, Onifade AK, Adetuyi FC. Fungi associated with the deterioration of some fruits of the family Cucurbitaceae. Nigerian Journal of Mycolog. 2009;2:229-236.
7. Bates DM, Richard WR, Jeffrey C. Biology and utilization of the Curcurbitaceae. Cornell University Press. 1990;52.
8. Imre JH. Fungal, disease Management in environmentally friendly cucumber production. A review sustain Agriculture. Review. 2009;2:219-292.
9. Gevens AJ, Ando K, Lamour KH, Grumet R, Hausbeck MK. A detached cucumber fruit method to screen for resistance to *Phytophthora capsici* and effect of fruit age on susceptibility to infection. Plant Disease. 2006;90:1276–1282.
10. Al-Sadi AM, Al-Said FA, Al-Kaabi SM, Al-Quraini SM, Al-Mazroui SS, Al-Mahmooli IH, Deadman ML. Occurrence, characterization and fruit rot of immature cucumbers under greenhouse conditions in Oman. Phytopathologia Editerranea. 2011;50(3): 421−429.
11. Fawole MOB. Laboratory manual of microbiology. Spectrum Books Limited, Ibadan. 2012;26-31.
12. Oviasogie FE Ogofure AG, Beshiru A, Ode JN, Omeje FI. Assessment of fungal pathogen associated with orange spoilage. African Journal of Microbiology Research. 2015;9(29):1758 -1763.
13. Yaji ME, Aerman PT, Sule M. Micro-organisms associated with the spoilage o cucumber, garden egg and pawpaw in Makurdi metropolis, Benue Nigeria. Intentional Journal of Recent Research Life Science. 2016;3(1):11-18.
14. Akinmusire OO. Fungal species associated with the spoilage of some edible fruits in Maiduguri Northern Eastern Nigeria. Advance Environmental Biology. 2011; 5(1):157-161.
15. Pitt JI, Hocking AD. Fungi and Food Spoilage, 3rd edition. Springer, Derdrecht/ Heidelberg. 2009;519:2(3):61-68.

16. Anonymous. *Zygosaccharomyces bailii* - food spoilage yeast; 2016.
17. Mbajiuka SC, Enya E. Isolation of microorganisms associated with deterioration of tomato (*Lycopersicon esculentum*) and pawpaw (*Carica papaya*) fruits. International Journal of Current Microbiology Applied Science. 2014;3(5): 501-512.

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