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Identification and pathogenicity of fungi causing disease of cucumber (*Cucumis sativus* L.) fruits on farmlands in Makurdi, Benue State, Nigeria

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ABSTRACT

Fungi causing disease of cucumber fruits on farmlands in Makurdi was investigated. Diseased cucumber fruits cultivated in farmlands in Makurdi were sampled using the quadrant method. The farmland was divided into four quadrants (A, B, C and D) and the diseased fruits were sampled. They were taken to the Botany Laboratory of the Rev. Fr. Moses Orshio Adasu University for isolation of fungi. Small pieces of decaying cucumber fruits were surfaced sterilized in 5% sodium hypochlorite for 30 seconds to 1 minute and plated on Potato Dextrose Agar (PDA) for fungi isolation. The pathogenicity of the isolated fungi was also carried out on healthy cucumber fruits. Data collected include incidence of fungi, occurrence of specific fungi, mean fungi count and area of rot. Data were analyzed using Chi square (χ^2) and Analysis of Variance (ANOVA). Means were separated using Fisher's Least Significant Difference at 5% level of significance. Two fungi were isolated namely; *Aspergillus niger* and *Rhizopus stolonifer*. Results obtained showed that out of 120 cucumber fruits examined in Quadrant A, 15 representing 12.50% were infected, 25 (16.70%) out of the 150 cucumber fruits examined in Quadrant B were infected. Also, 10 (10.50%) out of the 95 cucumber fruits examined at Quadrant C were infected while 30 (18.20%) of the 165 cucumber fruits examined in Quadrant D were infected. Cucumber fruits collected from quadrant D (2.67) had higher fungi count compared to quadrant B (2.00), quadrant A (1.67) and quadrant C (1.33). Eighty 80 (15.10%) out of the 530 cucumber fruits examined in this study were infected with fungi. There was no significant incidence of fungi on infected cucumber fruits across the quadrant ($\chi^2 = 3.69$; $df = 3$; $P = 0.29$). Cucumber fruits in quadrant D recorded the highest occurrence of fungi 8 (34.80%) followed by quadrant B 6 (26.10%), quadrant A 5 (21.70%) and the least was quadrant C 4 (17.40%) respectively. *Aspergillus niger* had higher occurrence 12 (52.20%), followed by *R. stolonifer* 11 (47.80%) respectively. Chi-square analysis showed that there was no statistically significant relationship in the occurrence of specific fungi on cucumber fruits based on location ($\chi^2 =$

1.15; $df = 3$; $P = 0.76$). The area of rot caused by *A. niger* (5.33) cm^2 and *R. stolonifer* (4.38) cm^2 was significantly higher compared to control (0.00) cm^2 . The occurrence of these pathogens varied within the farmland. The pathogenicity showed that the fungi were pathogenic on healthy cucumber fruits. Thus, farmers should ensure good agronomic practices on the field such as inspecting the field regularly for any symptoms of decay on the cucumber fruits.

Keywords: Identification, Pathogenicity, Fungi, Cucumber fruits, Farmlands, *Cucumis sativus*, Makurdi

1. INTRODUCTION

Cucumber (*Cucumis sativus* L.) is an important vegetable used to be cultivated once annually during the rainy season in Nigeria. It is an important vegetable cultivated in Northern central, Nigeria both in rainy and dry season using irrigation. The fruits serve as refreshments during farming activities (Ejimofo and Oledibe, 2021). Cucumber is a creeping vine that bears cylindrical fruits. It is known as *Cucumis sativus* L, it belongs to the family cucurbitacea. Other vegetables which belong to this family include Melon, squash, Watermelon and Pumpkins (Bello *et al.*, 2014).

It originated from the Asia continent. 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. The fruit is normally consumed raw alone or eaten with other vegetables such as salad in Nigeria. Cucumber is majorly cultivated because of its nutritional and medicinal relevance. Seed kernels are occasionally eaten (Aboloma *et al.*, 2009). Cucumbers have cooling properties and are extremely good for bringing relief to the eyes in summers (Jimeta *et al.*, 2022).

The fruit has high water content, about 96% water. Other than its use as a salad vegetable, cucumber fruit extracts are often incorporated as a primary ingredient in many topical skin preparations. Its deep cleansing action stems from its natural chemical constituent of glycolic, lactic and salicylic acids. These acids are known as organic or fruit acids because they contain one or more carboxyl radicals (COOH) in their structure (Jimeta *et al.*, 2022).

Fruits and vegetables including cucumber are highly perishable products; the quality is affected by pre harvest and post-harvest handling, transportation, storage and marketing (Agrios, 2005). Cucumber (*Cucumis sativus* L.) is one of the most important economic vegetable crops all over the world. It is cultivated in open fields for both local consumption and export. Cucumber fruits grown in the field are affected by pre-harvest diseases leading to spoilage of the fruits.

The occurrence of fungal spoilage of fruits is recognized as a potential health hazard to man due to their production of mycotoxins (Chiejina *et al.*, 2013). Cucumber plants are subject to attack by several fungal diseases that affect the yield quantity and quality including *Alternaria tenuis*, *A. alternata*, *Botrytis cinerea*, *Choanephora cucurbitarum*, *Didymella bryoniae*, *Fusarium oxysporum*, *Geotrichum candidum*, *Penicillium oxalicum*, *Phytophthora capsici*, *Rhizopus nigricans* and *Sphaerotheca fuliginea* as reported by Ziedan *et al.* (2018).

Fruit rot pre and postharvest caused by *B. cinerea* (Soliman *et al.* 2015), *Geotrichum candidum* was reported as a fruit rot causal pathogen on carrot, cucumber, tomato and pumpkin in South Korea (Kim *et al.* 2011).

Considering the vast uses of cucumber especially its consumption as food and as condiments in preparation of other food such as salad, it is imperative that fungi causing its disease on the field which lead to reduced production be documented. This study will contribute to the understanding of fungal diseases affecting cucumber fruits in Nigeria, providing valuable information for the development of effective management strategies.

The findings of this study will also serve as a resource for farmers, researchers, and policymakers seeking to improve cucumber production in Nigeria. For farmers, it will enlighten them about fungi causing disease to cucumber fruits on the field, thereby enabling them to employ good agricultural practices that will reduce fungi contamination. For students, it will provide them with literatures for further studies and for consumers, it will educate them on the inherent dangers associated with consuming fungi contaminated produce. For researchers, it will provide the foundation for further research in this area of study.

2. MATERIALS AND METHODS

2. 1. Study Area

Makurdi, the capital city of Benue State is located in the North-Central region of Nigeria (Ocholi *et al.*, 2017). It lies between latitudes 7°43'N and 7°45'N, and longitudes 8°32'E and 8°35'E (Ogwu *et al.*, 2015). Makurdi is situated on the banks of the River Benue, which provides a significant source of water and fertile soil for agricultural activities (Afolabi *et al.*, 2013). Geographically, it falls within the Guinea Savannah region, characterized by a tropical savanna climate with two distinct seasons: wet and dry (Ocholi *et al.*, 2017). The city's elevation ranges from 100 to 150 meters above sea level, with undulating terrain and scattered hills (Ogwu *et al.*, 2015). Makurdi's climate is marked by high temperatures throughout the year, with an average annual temperature of 24.5 °C (Afolabi *et al.*, 2013). The city experiences significant rainfall during the wet season, which lasts from April to October, with an average annual rainfall of 1,200 mm (Ocholi *et al.*, 2017).

2. 2. Collection of Samples

Cucumber fruits showing symptoms of disease were collected from farmlands in Makurdi. Collection of samples was done using the quadrant sampling method. In this method, the field was divided into smaller sections or blocks and ensuring each block was relatively homogenous consisting of diseased and healthy cucumber fruits. A quadrant size of 1m × 1m was selected for sampling. Cucumber fruits with symptoms of disease were collected from each quadrant within the farmland.

The number of diseased and healthy cucumber fruits were recorded for each quadrant. Samples collected from each quadrant were packaged separately in a polythene bags, labelled appropriately and transported to the Botany Laboratory of the Rev. Fr. Moses Orshio Adasu University for fungi isolation. Disease incidence was assessed by expressing the number of infected cucumber fruit as a percentage of the total number of cucumber fruit per quadrant.

These were applied in the formula proposed by Akhtar and Alam (2007).

$$\text{Disease incidence (D1)} = \frac{X}{N} \times 100\%$$

where: X = number of infected plants and N = total number of plants sampled.

2. 3. Preparation of Culture Media

The Potato Dextrose Agar (PDA) was prepared according to the manufacturer's recommended procedures and used for isolation of fungi. About 39.6g of powdered PDA medium were dissolved in 1000 ml of sterile distilled water and stirred vigorously to homogenize.

The flask content was heated on a heating mantle until the solution became clear. After heating, the flask was covered with foil paper and autoclaved at 121 °C for 15mins at 760 mmHg. The sterile medium was allowed to cool to a temperature at which it could be held with hands and two to three drops of streptomycin sulphate were added to inhibit bacterial growth. The medium was poured into Petri dishes and allowed to solidify before being used for culturing fungi.

2. 4. Isolation of Fungi from Cucumber Fruits showing Symptoms of Disease

Fungi infecting cucumber fruits were isolated by cutting small sections along the margin of the diseased fruits. The sections were surfaced sterilized in 5% sodium hypochlorite solution for 30 seconds 1 minute (Ekhuemelo and Otor, 2021).

Afterwards, they were rinsed in three changes of sterile distilled water to remove residuals of the sodium hypochlorite and blotted dry on sterile Whatman's filter papers. They were then placed on solidified PDA medium.

Three replicates were made for each sample. The inoculated Petri plates were incubated at ambient temperature and observations were made daily for possible microbial growth. Data collected include:

(i) Mean Fungi Count

Mean fungi count was determined by counting the number of fungi that occurred in each quadrant.

(ii) Percentage occurrence of specific fungi

Plates were observed for fungi growth and the occurrence of specific fungi was determined by counting the number of times each individual fungus occurred divided by the total number of fungi and expressed as a percentage using the formula adopted by Liamngee *et al.* (2016).

$$\text{Percentage occurrence of fungi} = \frac{\text{Number of times each fungus occurred}}{\text{Total number of fungi per plate}} \times 100\%$$

2. 5. Subculturing of Fungi isolates

After 5-7 days of growth, subculturing was done to obtain pure cultures of the isolates as reported by Liamngee *et al.* (2023). To subculture, a sterilized inoculation needle was used to pick a little quantity of the fungal growth on the old culture and transferred to the centre of a freshly prepared PDA in another Petri dish. Sub-culturing was done repeatedly until pure cultures of each fungal isolate was obtained as reported by Liamngee *et al.* (2023)

2. 6. Identification of Fungi

Macroscopic and microscopic identification was carried out. For macroscopic identification, the colour, nature of growth and growth rate of the fungi were observed on the PDA in Petri plates. Microscopic identification was done by staining a glass slide with a drop of Lactophenol in cotton blue and with the aid of inoculation needle, a small quantity of the fungal colony was placed on the stained-glass slide, covered with a cover slip and viewed under the 40× objective lens of the light microscope. The observed characteristics of the fungi were compared with a standard chart for identification by Barnett and Hunter (1972) as reported by Liamngee *et al.* (2016).

2. 7. Pathogenicity Test on Healthy Cucumber Fruits

Pathogenicity test was carried out on healthy cucumber fruits to confirm the pathogenicity of the isolated fungal organisms. The pathogenicity of the isolated organisms was tested *in vivo* on healthy cucumber fruits using agar plug method of inoculation (Ekhuemelo and Yaaju, 2017). Healthy fruits were surface sterilized with 5% Sodium hypochlorite for one minute and washed in three changes of sterile distilled water and allowed to dry at room temperature. A 3mm cork borer was used to bore into the healthy cucumber fruits and the bored tissues were removed.

With the aid of forceps, small quantity of each pure cultures of the fungi were used to inoculate the cucumber fruits (three cucumber fruits were inoculated per replicate of each test fungus) and the tissue replaced. The inoculated fruits were incubated for 5-7 days at ambient conditions of light and temperature.

After 5-7 days of incubation, with the aid of a sharp knife, the margin along the decayed area of the cucumber fruits were cut open and the lesion length and diameter were measured using a metre rule. The area of rot was calculated using the formula adopted by Ezeibekwe and Ibe (2010) as:

$$\text{Area of rot} = \pi dl$$

where: $\pi = 22/7$, d = diameter, l = depth

2. 8. Data Analysis

Data obtained from this study were analyzed using Analysis of Variance and Chi-square. Means were separated using Fisher's Least Significant Difference at 95% level of significance.

3. RESULTS

The fungal organisms isolated from diseased cucumber fruits were *Aspergillus niger* and *Rhizopus stolonifer*. The macroscopic and microscopic views of the organisms are presented in Plates 1a - 2b. *Rhizopus stolonifer* colony on PDA was white in colour with cottony appearance (Plate 1a).

The sporangia were large, round with enclosed spores borne on a transparent sporangiospores (Plate 1b). The colony of *A. niger* on PDA was black (Plate 2a).

Microscopically, *A. niger* conidia were dark in colour borne on a smooth, long and transparent conidiophores (Plate 2b).



Plate 1(a). Macroscopic view of *R. stolonifer*.

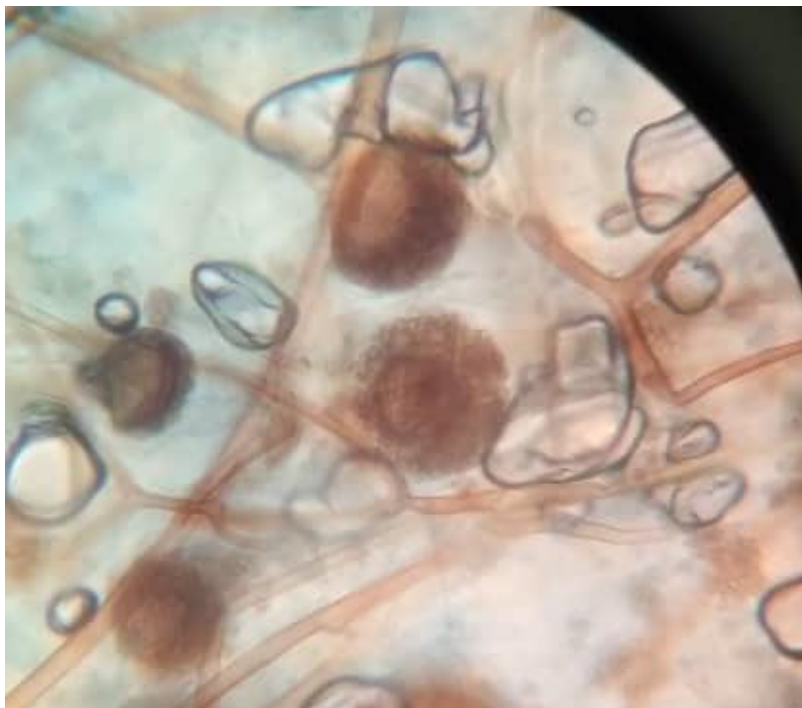


Plate 1(b). Microscopic view of *R. stolonifer*.



Plate 2(a). Macroscopic view of *A. niger*.



Plate 2(b). Microscopic view of *A. niger*.

3. 1. Incidence of Disease on Cucumber Fruits in Farmlands in Makurdi

The incidence of disease on cucumber fruits in farmlands in Makurdi is presented in Table 1. Findings of the study showed that out of 120 cucumber fruits examined in Quadrant A, 15 representing 12.50% were infected, 25 (16.70%) out of the 150 cucumber fruits examined in Quadrant B were infected. Also, 10 (10.50%) out of the 95 cucumber fruits examined in Quadrant C were infected while 30 (18.20%) of the 165 cucumber fruits examined in Quadrant D were infected. Overall, 80 (15.10%) out of the 530 cucumber fruits examined in this study were infected with fungi. There was no significant relationship in the incidence of fungi on infected cucumber fruits across the quadrants ($\chi^2 = 3.69$; df = 3; P = 0.29)

Table 1. Incidence of Disease on Cucumber Fruits in Farmlands in Makurdi.

Location	Number of fruits Examined	Number of fruits with symptoms of disease (%)
Quadrant A	120	15 (12.5%)
Quadrant B	150	25 (16.7%)
Quadrant C	95	10 (10.5%)
Quadrant D	165	30 (18.2%)
Total	530	80 (15.1%)

$\chi^2 = 3.69$; df = 3; P = 0.29

3. 2. Mean Fungi Count of Cucumber Fruits Collected on Farmlands in Makurdi

The mean fungi count of cucumber fruits on farmlands in Makurdi is shown in Table 2. Results obtained showed higher fungi count of cucumber fruits collected from quadrant D (2.67) compared to quadrant B (2.00), quadrant A (1.67) and quadrant C (1.33). There was no significant difference in fungi count of cucumber fruits between the quadrants.

Table 2. Mean Fungi Count of Cucumber Fruits Collected on Farmlands in Makurdi.

Quadrant	Fungi Count
Quadrant A	1.67
Quadrant B	2.00
Quadrant C	1.33
Quadrant D	2.67
FLSD (0.05)	NS

Key: FLSD: Fisher's Least Significant Difference; NS: Not Significant.

3. 3. Occurrence of Specific Fungi on Cucumber Fruits Based on Quadrant

The occurrence of specific fungi causing disease on cucumber fruits on farmlands in Makurdi based on quadrant is presented in Table 3. *R. stolonifer* had the highest occurrence with 3 (60.00%) on cucumber fruits in quadrant A, followed by *R. stolonifer* with 2 (40.00%). At quadrant B, *R. stolonifer* and *A. niger* had equal occurrence each with 3 (50.00%). *Aspergillus niger* had the highest occurrence in quadrant C with 3 (75.00%) followed by *R. stolonifer* with 1 (25.00%) respectively. In quadrant D, *A. niger* and *R. stolonifer* had equal occurrence 4 (50.00%) respectively. Overall, *A. niger* had higher occurrence 12 (52.20%), followed by *R. stolonifer* 11 (47.80%) respectively. Cucumber fruits in quadrant D recorded the highest occurrence of fungi; 8 (34.80%) followed by quadrant B; 6 (26.10%), quadrant A; 5 (21.70%) and the least was quadrant C; 4 (17.40%) respectively. Chi-square analysis showed that there was no statistically significant relationship in the occurrence of fungi on cucumber fruits based on location ($\chi^2 = 1.15$; df = 3; P = 0.76)

Table 3. Occurrence of Specific Fungi on Cucumber Fruits Based on Quadrant.

	Fungi Species		
Location	<i>A. niger</i>	<i>R. stolonifer</i>	Total (%)
Quadrant A	2 (40.0)	3 (60.0)	5 (21.7)
Quadrant B	3 (50.0)	3 (50.0)	6 (26.1)
Quadrant C	3 (75.0)	1 (25.0)	4 (17.4)
Quadrant D	4 (50.0)	4 (50.0)	8 (34.8)
Total	12 (52.2)	11 (47.8)	23 (100.0)

$\chi^2 = 1.15$; df = 3; P = 0.76

3. 4. Pathogenicity of Fungi Isolates on Healthy Cucumber Fruits

The pathogenicity of fungi isolates on healthy cucumber fruits in Makurdi is presented in Table 3. The result showed that rot caused by *A. niger* (5.33) cm² and *R. stolonifer* (4.38) cm² was significantly higher compared to control (0.00) cm².

Table 4. Pathogenicity of Fungi Isolates on Healthy Cucumber Fruits.

Fungi	Area of Rot
<i>A. niger</i>	5.33
<i>R. stolonifer</i>	4.38
Control	0.00
FLSD (0.05)	3.56

Key: FLSD: Fisher's Least Significant Difference

Table 5. Morphological Description of rot Induced by Fungal Isolates on Healthy Cucumber Fruits.

Fungal Organisms	Nature of Rot
<i>Aspergillus niger</i>	The rot caused a soft rot and watery texture in infected tissues producing an unpleasant odour (Plate 3)
<i>Rhizopus stolonifer</i>	The infected tissues produced dry rot along the margin of inoculation (Plate 4)



Plate 3. Soft rot produced by *Aspergillus niger*.



Plate 4. Dry rot produced by *R. stolonifer*.

4. DISCUSSION

The study investigated fungi causing disease of cucumber fruits in farmlands in Makurdi. Two fungi namely; *Aspergillus niger* and *Rhizopus stolonifer* were isolated. This is similar to the work of Jimeta *et al.* (2022) who isolated *Rhizopus stolonifer*, *Aspergillus brasiliensis* and *Aspergillus flavus* from cucumber fruits obtained from retail outlet in Jimeta markets, Yola State. Also, Jidda and Muhammad (2017) isolated *A. niger*, *Saccharomyces cerevisiae*, *A. flavus*, *S. resus*, *A. ustus*, *A. wentii* and *A. oryzae* from cucumber fruits obtained from retail outlets in Maiduguri. The observed differences in the isolated fungi in this work and other reported works could be due to the samples of cucumber collected as most of their work collected cucumber samples from markets whereas this present study deals with isolation of fungi causing fruit decay of cucumber on the field. Although, *Aspergillus niger* and *R. stolonifer* isolated in this work have been reported by Jimeta *et al.* (2022). The isolation of these organisms on cucumber fruits obtained in the market shows that the inoculum of these pathogens might have been present on the fruits from field which continues to manifest in storage and in market places.

Aspergillus niger recorded the highest frequency of occurrence on cucumber fruits compared to *R. stolonifer* in this study. This could be due to the cosmopolitan nature of *Aspergillus niger*. Also, the higher frequency of *Aspergillus niger* in the decay of cucumber fruits may be attributed to its ability to grow rapidly on moist surfaces and produce abundant spores that can disperse easily (Kumar *et al.*, 2017). Additionally, *Aspergillus niger* may have a competitive advantage over *Rhizopus stolonifer* due to its ability to produce mycotoxins that can inhibit the growth of other microorganisms (Pitt *et al.*, 2013). This contradicts the findings by Jimeta *et al.* (2022) who reported *R. stolonifer* as the most occurred fungus on decay of cucumber fruits.

The study recorded variations in the occurrence of specific fungi. *Rhizopus stolonifer* had the highest occurrence in quadrant A, equal occurrence of *A. niger* and *R. stolonifer* in quadrant B while *A. niger* had higher occurrence in quadrant C and equal occurrence of *R. stolonifer* and *A. niger* in quadrant D. This may be due to environmental factors such as temperature and climate, soil and nutrient availability (soil type and pH levels).

The pathogenicity test revealed that *Aspergillus niger* and *Rhizopus stolonifer* were able to infect healthy cucumber fruits. This agrees with the findings by Jidda and Muhammad (2017); Jimeta *et al.* (2022) and Al-Sadi (2011) who all reported that isolates of *Aspergillus niger*, *A. flavus*, *Saccharomyces* sp., *Rhizopus stolonifer*, *A. ustus* and *A. wentii* were pathogenic on healthy cucumber fruits. The ability of *A. niger* to induced higher rot suggests that *Aspergillus niger* is a more aggressive pathogen that can cause more extensive damage to healthy cucumber fruits. *Aspergillus niger* is a saprophytic fungus that can grow on a wide range of organic substrates, including plant tissues (Kumar *et al.*, 2017). It is known to produce mycotoxins, such as ochratoxin A, which can contaminate food and pose health risks to humans (Pitt *et al.*, 2013). On the other hand, *Rhizopus stolonifer* is a phytopathogenic fungus that can cause soft rot and decay in various plant tissues, including fruits and vegetables.

The fungi organisms produced distinctive symptoms when inoculated on healthy cucumber fruits as revealed by the pathogenicity. *Aspergillus niger* produced soft rot due to the production of cell degrading enzymes like pectinases, cellulases and proteases which allow *A. niger* to break down cell walls resulting in softening of the tissue, creation of watery texture and release of cellular contents.

This process enables the fungus to obtain nutrient from the plant tissues facilitating its growth and colonization. *Rhizopus stolonifer* produced dry rot due to its unique infection mechanisms such as enzymatic degradation producing cellulase and proteases which break down cellulose and protein respectively. *Rhizopus stolonifer* absorb water from the plant tissues, causing dehydration and desiccation leading to dry appearance.

5. CONCLUSION

This study has shown that diseases on cucumber fruits collected from farmlands in Makurdi were caused by *Aspergillus niger* and *Rhizopus stolonifer*. The occurrence of these pathogens varied within the farmlands. Cucumber plants in quadrant D had the highest number of infected fruits while cucumber plants in quadrant C had the lowest number of infected fruits. *Rhizopus stolonifer* had the highest occurrence in quadrant A, equal occurrence of *A. niger* and *R. stolonifer* in quadrant B while *A. niger* had higher occurrence in quadrant C and equal occurrence of *R. stolonifer* and *A. niger* in quadrant D. The pathogenicity showed that the fungi were pathogenic on healthy cucumber fruits. *Aspergillus niger* induced more rot area than *Rhizopus stolonifer* on the fruits.

Recommendations

Based on the findings of the study, the following recommendation are made;

- Farmers should ensure and engage in good agronomic practices on the field such as inspecting the field regularly for any symptoms of decay on the cucumber fruits.
- Farmers should engage in good sanitation practices by consistent removal of infected fruits, dispose of infested debris, and disinfect tools and equipment.
- Sellers should carry out proper cleaning of the cucumber fruits from storage house to markets so as to remove fungi inoculum that can cause decay of the fruits.

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