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Research Article

Fungal Profile and Pathogenicity Indices Associated with Cucumber Fruits in Aba, Southeastern Nigeria

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ABSTRACT

The fungal profile and pathogenicity indices associated with cucumber fruits were determined using standard microbiological methods. A total of one hundred and twenty-five (125) samples were randomly picked from five (5) different markets/ hawking points, transported in ice bags to Laboratory for analysis, prepared and made ready for microbiological analysis. Also pathogenicity tests were conducted on the identified isolates to establish both severity and percent disease index in the Koch postulates of disease development. The mean fungal count ranged from 1.0 x 103 ±0.25/±0.32 to 5.4 x 103 ±0.12. Isolates were characterized colonially, microscopically, and morphologically with their identities confirmed with reference chart. The four (4) isolates include *Aspergillus* species, *Penicillium* species, *Fusarium* species, and Cladosporium species. Due to high microbial thresholds of associated genus, there is need for all round awareness to avoid health outbreaks and pathogenesis tendencies, as some isolated species could produce potent toxins that are linked with food-borne illnesses. Bruises, pierces and surface wounds should be avoided at all times, having known the PDI of some isolates. Infestation by rodents and insects during storage should be avoided as it may constitute serious health and deterioration exposures. Washing of fruits with salted-water before eating is most recommended, as transit eating of any kind should be discouraged.

Keywords: *Cucumber; Fungal Profile; Safety; Pathogenicity, Severity Index.*

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INTRODUCTION

Cucumber is a herbaceous vine of the botanical family *Cucurbitaceae* widely cultivated for its edible fruit. It is a dark green fruit, elongated and cylindrical with rounded ends and a slightly bumpy skin, and inside it is a crisp white flesh surrounding the seeded core. Cucumbers are eaten unripe since ripe specimens lose their crispness and become spongy and yellow (John & Mullinix, 2008). Cucumbers provide various nutrients but are low in calories, fat, cholesterol, and sodium with delicate flavour that makes it popular for salads and relishes. Cucumbers have a high-water content, which can help relieve dehydration and are pleasant to eat in hot weather. It is grown as crops in a light, rich, well-drained soil in sun or partial shade at a minimum temperature of 10OC. Cucumbers are well known at the dinner table, can be eaten in raw or cooked form. Its high-water content categorizes cucumber among the lowest calorie vegetables. However, it is the fourth most cultivated vegetable in the world, behind tomatoes, cabbage and onions (Adam, 2006).

Cucumber has a lot of dietary and health benefits ranging from weight loss to cancer treatment and even skin care. Cucumber and other green vegetables contain chlorophyll, a pigment that gives plants their green color. Research suggests chlorophyll may have anti-cancer properties, as people who eat diets rich in green vegetables may have lower incidence of

developing cancer. Cucumbers are now known to have a strong history in connection with reduced risk of cardiovascular disease as well as several cancer types, including breast, uterine, ovarian, and prostate cancers (Lee, 2010). Its high-water content promotes hydration which helps the body to flush out toxins and keep the skin healthy. Cucumbers are also rich in vitamin B, which can help with acne and other skin problems. Cucumber fruit (*Cucumis sativus*) contains vitamins, minerals, amino acids, phytosterols, phenolic acids, fatty acids, and cucurbitacins. Traces of essential oil, amino acids, pectin, starch, sugars, and vitamin C are found in cucumbers (Abiodun, 2010). Their high dietary and therapeutic value resides in their minerals, which are highly alkaline. They contain potassium, calcium, phosphorous, magnesium, and iron, as well as various trace elements, most notably sulfur (Singh, 2010). Hence, this work is aimed at evaluating the microbial profile of cucumber fruits sold in Aba, Abia State, Nigeria.

MATERIALS AND METHODS

Study area: The study area is Aba Metropolis, Abia State, in the South-Eastern Nigeria. The Aba town is known as a major commercial centre in Eastern Nigeria is of the Igbo tribe and inhabited by Ngwa people. The geographical coordinates are 5.1215ºN and 7.3732ºE.

Collection of samples: A total of one hundred and twenty-five (125) samples of cucumber fruits were bought at random from five different markets/ hawking points in Aba metropolis in the Southeastern Nigeria, and were transported in ice bags to Microbiology Laboratory for analysis.

Sample preparation: The cucumber samples prior to culturing were all-surface-swapped and disengaged in a test tube containing diluent as stock and serially diluted using the ten-fold serial dilution method. Ten-fold serial dilutions of the cucumber samples were done using sterile peptone water as diluent. One milliliter (1 mL) each of the samples was aseptically transferred into a sterile test tube containing nine milliliters (9 mLs) of sterile peptone water, stirred with sterile glass rod and shaken vigorously to ensure adequate disengagement of entrapped microorganisms to obtain 10-1 dilution. Serial dilutions of the homogenates were continued and made step-wisely till the fourth (4th) tube, to obtain dilutions of 10^{-1} to 10^{-4}.

Microbiological analysis of samples: Spread plate techniques of (Cappucino & Sherman, 2010) were used to isolate fungi in the samples. One milliliter (1 mL) each from the dilution was plated in replicates using sabouraud dextrose agar (SDA) fortified with streptomycin sulfate for enumeration. The plates were incubated at 25±2 °C for 120 hours for growths. Growths were sub-cultured using streak method until pure isolates were obtained. Slant universal bottles were used to preserve the pure cultures of the pathogen and stored in the fridge at 4°C for identification. The purified fungal isolates were identified on the basis of macroscopic and microscopic characteristics by slide culture technique and lactophenol staining. The schemes of Kidd *et al*. (2005) and Watanabe, (2010) were used for the identification.

Preparation of conidial suspension: Fourteen-day-old pure cultures in SDA were flooded with 5 ml of sterile distilled water (DW). A sterile wire loop was used to scrape off the conidia and bring them to suspension. The suspension was filtered through a sterile double-layer muslin cloth and the collected filtrate diluted serially to 1×10^5. A haemocytometer was used to adjust the spore concentration.

Pathogenicity Test: In establishing Koch's postulates, isolated microbes were subjected to pathogenicity test as described by Freeman *et al*. (1998) and modified by Twizeyimana *et al*. (2013). Healthy cucumber fruits were purchased and washed with clean tap water to remove any soil debris. The fruits were surface-sterilized by dipping in 75% ethanol for about three minutes, rinsed with distilled water, and then air-dried. Each of the isolates was subjected to two methods of inoculation.

A sterile cork borer (5 mm diameter) was used to create wound on the surface skin of each fruit and mycelial discs of equivalent diameter obtained from the edge of actively growing pure cultures were placed on the wound. Six inoculated fruits for each pathogen and six control fruits inoculated with plain PDA were arranged on individual trays and covered with cling film to conserve moisture and avoid contamination. The fruits were incubated at room temperature of 25°C ± 1.

his test could also be done using this alternative method where one (1) ml conidial suspension (5 × 10−5 conidial/ml) was placed on a pierced wound on the surface skin of each fruit and covered with cling film. Six inoculated fruits for each pathogen and six control fruits inoculated with distilled water were arranged in individual trays and covered with cling film. The inoculated fruits were incubated at 25 ± 1°C.

Evaluation was done by monitoring symptom development daily till the last day. Disease and symptom development was evaluated beginning at 2–4 days after inoculation by measuring the length of the lesion discolouration that formed at the inoculation site. At the end of the pathogenicity test, re-isolation from the symptomatic cucumber fruits was made, and the re-isolated fungal colonies were compared morphologically to the original isolates (Guarnaccia *et al*. 2016 & Twizeyimana *et al*., 2013).

Disease severity: Disease severity on fruit parts was recorded using a four (4) point rating scale based on the percentage of fruit area affected by the disease, as presented below.

Fruit area affected	Grade
No infection	0
1 – 24.9 percent	1
25 – 49.9 percent	2
50 – 74.9 percent	3
75 & above percent	4

Percent disease index (PDI): At the 10th day, cutting of the fruits longitudinally were done to determine the degree of pathogen severity from the visible dimensional checks. Severity of fungi rots on cucumber fruits was calculated using the formula as described by Lakshmi *et al*. 2011. Based on the numerical ratings given above a 'Percent disease index' for

fruit rot was calculated using the formula (Mayee and Datar, 1986) given below:

Percent disease index (PDI)

$$= \frac{\text{Sum of numerical ratings x 100}}{\text{No. of units (fruit surface) examined x Maximum grade}}$$

Statistical analyses: All obtained data in this study were analyzed using analysis of variance (ANOVA). Descriptive statistics in form of mean, standard deviation, and Duncan post hoc were also used to assess the data, and analyses were done using SPSS version 20 (Statistical Product and Service Solutions).

RESULTS

The result of the mean fungal count (MFC) for cucumber samples sold in Aba metropolis is shown in Figure 1. The highest fungal count was observed in samples obtained from Ariaria market (C5) 5.4 $x10^3$±0.12 while the lowest fungal count was observed in samples obtained from Afule market (C5) 1.0 $x10^3$ ±0.25 and Ahiaohuru market (C1) 1.0 $x10^3$ ±0.32. Four (4) fungal isolates were identified with cucumber fruits to include *Aspergillus* species, Penicillum species, *Fusarium* species and *Cladosporum* species. All data obtained were statistically analyzed and compared, with values of same alphabets as not significant ($p>0.05$), while those with different alphabets as significant ($p<0.05$).

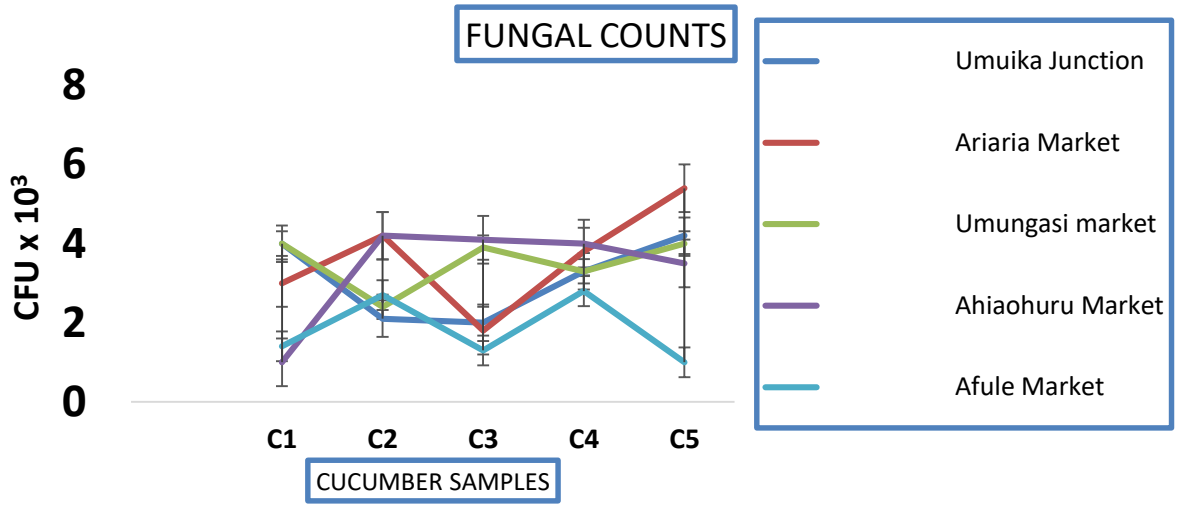

Figure 1.
Mean fungal counts of cucumber fruits
Recommended Standard Counts: aerobic bacteria count (abc) = ≤ 10^5/g, Fungal count (fc) = ≤ 10^4/g (FSANZ, 2018; PHLS, 2000).
Key: C = cucumber sample

Table 1:
Pathogenicity parameter indexes of cucumber fruits

S/N	Isolate/ Replicate ID	HR Development (DAI)	Symptoms (Length-cm) – DAI[10]		Sum of Severity Values (SV)		MG (SV)/NE	Percent Disease Index (PDI)	
			x	$\overline{x}$	x	$\overline{x}$		x	$\overline{x}$
1	AsA	3	4.27[a]	3.76[a]	30	24.67	4/9	83.33[b]	82.10[a]
	AsB	2	3.68[b]		23		3/9	85.19[a]	
	AsC	3	3.32[c]		21		3/9	77.77[d]	
2	PeA	4	3.29[c]	3.39[b]	24	24	4/9	66.67[g]	73.46[b]
	PeB	3	3.71[b]		26		4/9	72.22[e]	
	PeC	3	3.16[d]		22		3/9	81.48[c]	
3	FuA	3	3.12[e]	3.10[d]	21	20	4/9	58.33[j]	60.80[d]
	FuB	3	2.98[f]		17		3/9	62.96[h]	
	FuC	4	3.19[d]		22		4/9	61.11[i]	
4	ClA	4	3.33[c]	3.18[c]	23	20	4/9	63.89[h]	66.98[c]
	ClB	3	3.11[e]		18		3/9	66.67[g]	
	ClC	4	3.09[e]		19		3/9	70.37[ef]	

***Legend**: HR- hypersensitivity reaction, DAI- days after incubation, MG- maximum grade, NE-number of fruits examined. Values with different alphabets along columns were significant, while those with same alphabets were not significant*

Table 1 showed the pathogenicity indexes of cucumber fruits. Mean results of replicates showed "As" samples had the highest dimensional measurement (cm) and severity values of 3.76/24.67, followed by "Pe" samples – 3.39/24 and "Cl" samples – 3.18/20 and the least with "Fu" samples – 3.10/20. In the same trend, PDI had "As" samples with the highest percentage (%) of 82.10, followed by "Pe" samples 73.46, "Cl" samples 66.98 and the least with "Fu" samples 60.80. Also from the table there were different days of incubation for the initiation of symptoms appearance.

DISCUSSION

Cucumber fruits are hawked along major highways and junctions and most often sold in urban and rural markets because of its dietary and health benefits. It is characterized with deterioration from environmental and atmospheric elements, hence grouped as perishable goods. Most of the deterioration are caused by microbial contamination and invasion. Most of those that patronizes the hawked fruits especially travelers, eat this fruit raw while on transit. The public health concern is majorly because they are consumed raw by travelers while on transit. The high microbial loads recorded in the obtained results of this study points to many diseases associated with such consumption such as like cholera, diarrhea, typhoid etc.

Fungi has been accused to be responsible for the deterioration and spoilage of most fruits. The pH of fruits were always supporting the activities of these fungi. The presence of the isolates is an indication of poor storage conditions. The presence of *Aspergillus*, and *Penicillium* species points to surrounding environmental flora as was reported by Ike *et al*. (2017). *Aspergillus* have been reported to produce potent mycotoxins responsible for various mycotoxicosis in humans (Abbey, 2006; Efiuvuwevwere, 2000). The surrounding environment played a role on the isolated fungal species in this work, and such results obtained here were similar to previous reports of Ike & Ogwuegbu, 2020; Ike *et al*. 2017 & 2015. Jay *et al*. (2005) reported that fungi could survive in low pH conditions, and this may be the reason for the recorded counts, deterioration activities and recorded isolates in the study.

It does not come as a surprise that food-borne disease-causing microorganisms were isolated from the cucumber fruits because of the prevailing environmental conditions under which these cucumbers were stored, displayed and hawked. Some of these markets are so dirty and most times are surrounded and littered with decomposing dumps. The results of this study have revealed the unsafe status and public health concern of most transit-consumed hawked fruits unless properly washed with salt water. Fresh cucumber is a transient host to variety of microorganisms, some of which are pathogenic and knowledge of the consequences could prevent food poisoning resulting from unwholesome consumption.

After incubation of the inoculated fruits, dark brown discoloration to black rot developed on the fruits, with fungal mycelia occasionally observed on the fruit surface. An internal discoloration of the vascular bundles was observed when cut open. The inoculation with either mycelia or spore suspensions in conducting pathogenicity test showed that both methods developed similar symptoms as was initially reported by Wanjiku *et al*. (2020). However, inoculation with spore suspension seems to express more severity properties when compared with mycelia inoculation because of surface area coverage, ease of flow and assimilation to various areas. The initiation of symptoms occurred at different days after incubation (DAI) for the isolates. Severity of infection was determined using procedures elucidated in 2.5.1. After incubation, *Aspergillus* had the highest dimensional measurement and that of PDI showing that it had the ability to elucidate diseases and deteriorate fruits. Second to *Aspergillus* in severity and PDI elucidation was *Penicillium*, while others to follow same trend were Cladosporium and *Fusarium*. The recorded indices here are the measurements index which determines the extent of disease spread and severity. From the overall analysis, it can be deduced that most deterioration activities encountered during storage of cucumber fruits are emanating from such genus of organisms with high PDI and severity index. The re-inoculation of these isolates into fresh healthy cucumber fruit, with each showing the capability to infect host and cause same deterioration symptoms, has proved and established Koch postulate of diseases.

In conclusion, considering the high rate of fruits consumption especially in shedding of weights and maintaining healthy living, unhygienic cucumber fruits are potential sources of various microbial pathogens. Due to high microbial thresholds of associated genus, there is need for control and management of counts proliferation to avoid health outbreaks and pathogenesis tendencies. Bruises, pierces and surface skin wounds should be avoided at all times, having known the PDI of some isolates. Infestation by rodents and insects during storage should be avoided as it may constitute serious health and deterioration exposures. Washing of fruits with salted-water is most recommended before being consumed. Transit eating of any kind should be discouraged.

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