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Effect of Fungi Contamination of Two Varieties of Cowpea Sold in Port Harcourt Metropolis

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Authors' contributions

This work was carried out in collaboration among all authors. Author NWEJ carried out the experimental analysis, performed the statistical analysis, wrote the protocol, managed the literature searches and wrote the first draft of the manuscript. Author ECC designed the study and together with author FWN supervised the study. All authors read and approved the final manuscript.

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ABSTRACT

The microbial contamination of two cowpea varieties sold in Port Harcourt was investigated. The fungi associated with the grains were isolated by placing the cowpea seeds in sterile Petri dishes containing sizeable cotton wool dampened with 5ml sterile distilled water for seven days to allow for the growth of fungi. Proliferated fungi were later grown on freshly prepared Sabouraud Dextrose Agar plates. Pathogenicity test was carried out by inoculating isolated microbes into healthy cowpea seeds. Three fungi belonging to the genera; *Mucor, Aspergillus, and Rhizopus* sp were identified. The pathogenicity test showed that the cowpea seeds were infected and the weight reduced drastically. The weight of the cowpea seeds (white) before inoculation was 0.24 ± 0.03. The weight after being inoculated with *Mucor* sp*, Rhizopus* sp*,* and *Aspergillus flavus* was 0.22 ± 0.12, 0.18 ± 0.09, and 0.25 ± 0.20, respectively. The weight of the cowpea seeds (brown) before inoculation was 0.18 ± 0.05. The weight after inoculation with *Mucor* sp*, Rhizopus* sp*,* and *Aspergillus flavus* recorded 0.25 ± 0.17, 0.18 ± 0.09, and 0.31 ± 0.12, respectively. The pathogenicity test showed that the microorganisms had effects on the cowpea seeds which was observed in the increased weight of the seeds. Furthermore, the microorganisms isolated in this study could be dangerous to health if proper measures are not taken during preparation.

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1. INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp.) is a member of the Phaseoleae tribe of the Leguminosae family. Members of the Phaseoleae include many of the economically important warm season grain and oilseed legumes, such as soy bean seeds (*Glycine max*), common cowpea seeds (*Phaseolus vulgaris*), and mung bean seeds (*Vigna radiata*). The name cowpea probably originated from the fact that the plant was an important source of hay for cows in the southeastern United States and in other parts of the world [1].

Cowpea are leguminous plants rich in crude protein and amino acid profile which makes them a potential substitute for other protein sources for human consumption. They are used for the production of fish meal and animal feeds. Animals require a supply of protein and energy to be in good health, and this promotes competition between men and animals over the limited source that is available [2].

Cowpea is widely cultivated in the humid tropics of South-western Nigeria; however, its cultivation is faced with several setbacks such as parasitic weeds, pests and diseases. The effect of field diseases on cowpea has led to significant reduction in yield of cowpea in the humid forest of Nigeria [3]. The major economic diseases of cowpea in the humid agroecologies of South-western Nigeria include brown blotch, anthracnose, cercospora leaf spot, choaniphora pod rot, false smut, web blight and sclerotium stem blight [4]. However, in Nigeria cowpea is majorly produced in the North in the savannah belt. Its yield in the South is affected by some environmental factors including rainfall hence it is seasonal. The high demand for this leguminous multipurpose crop plant is not met in the Southern part of Nigeria. The production of cowpea all year-round basis in all parts of Nigeria is expected to boost production, thereby improving nutrition, contributes to food security as well as increase in revenue of the producers. It also creates employment opportunities thereby enhancing the efficiency of utilization of labour [5]. Microbial diseases of cowpea could result in biodeterioration. Biodeterioration is the breakdown of food or any undesirable change in the property of a material caused by the vital activities of microorganisms, either directly or indirectly by products of their metabolism [6]. Biodeterioration of cowpea could lead to the loss of nutritional value, organoleptic and color changes, and most importantly, safety may become compromised. It could cause chemical changes in organic substances due to the action of specific enzymes produced by microorganisms, such as, moulds, bacteria, and yeasts which usually result in the breakdown of complex organic substances into simpler ones. A few species of moulds such as *Aspergillus oryzae* in soy sauce manufacture have been used in the production of fermented food. *Aspergillus* species compete with *Penicillium* and *Fusarium* species for dominance in foods and food plants while Aspergilla generally grow at higher temperatures or lower water activities than Penicillia [7]. There is dearth of information concerning the effects of microbial contamination of cowpeas. Thus, this study aimed to investigate the effects of fungi pathogens on two varieties of cowpea which are sold in Port Harcourt.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in four major markets located in two local government areas: Port Harcourt City Local Government Area and Obio-Akpor Local Government Area. These markets are known for high influx of traders who come from different localities to display and sell their produce. The map of the area under study is illustrated in Fig. 1.

2.2 Sample Collection

Cowpea seeds of different variety (brown and white) were bought from four major markets in Port Harcourt metropolis from different distributors. The major markets include; Mile III, Mile I, New market (Borikiri) and Rumuokoro market. The cowpea seeds were taken to the Department of Plant Science and Biotechnology, Rivers State University for confirmation. Confirmation included the verification of the sizes and colour of the different varieties. In the laboratory, the samples were sorted according to size, and wholesomeness for further analysis. The varieties of the cowpea are presented in Table 1.

2.3 Isolation and Identification of Fungal Isolates

The fungi associated with the cowpea seeds were isolated by placing the cowpea seeds in

sterile Petri dishes containing sizeable cotton wool dampened with 5 ml sterile distilled water. The petri dishes were covered and the plates were covered and allowed for seven days and monitored for the growth of fungi. The Petri dishes were left for seven (7) days to allow for the growth of fungi. The fungi which proliferated were later grown on Sabouraud Dextrose Agar plates which were freshly prepared. Fungal isolates were also preserved in Sabouraud Dextrose Agar slant bottles and used for further analysis. Fungal isolates were characterized based on macroscopic appearance on plates (spore form, texture, colour of spores and reverse) and microscopic appearance [8] and where compared with fungal charts by previous study [9].

2.4 Pathogenicity Test

Pathogenicity test as described in Koch's postulates was carried out. Fungal isolates from the infected cowpea seeds were inoculated onto healthy cowpea seeds. The inoculation was carried out by transferring 10 ml of respective fungal spores which had been suspended in sabouraud dextrose both for 48 hours into 90ml sterile distilled water. The water which contained the fungal isolate was sprayed directly on the healthy cowpea seeds which were kept in sterile Petri dishes. Prior to inoculation of the cowpea seeds, the weight of the healthy cowpea seeds was measured using electronic weighing balance. The weight of the sprayed cowpea seeds was monitored by weighing them on an

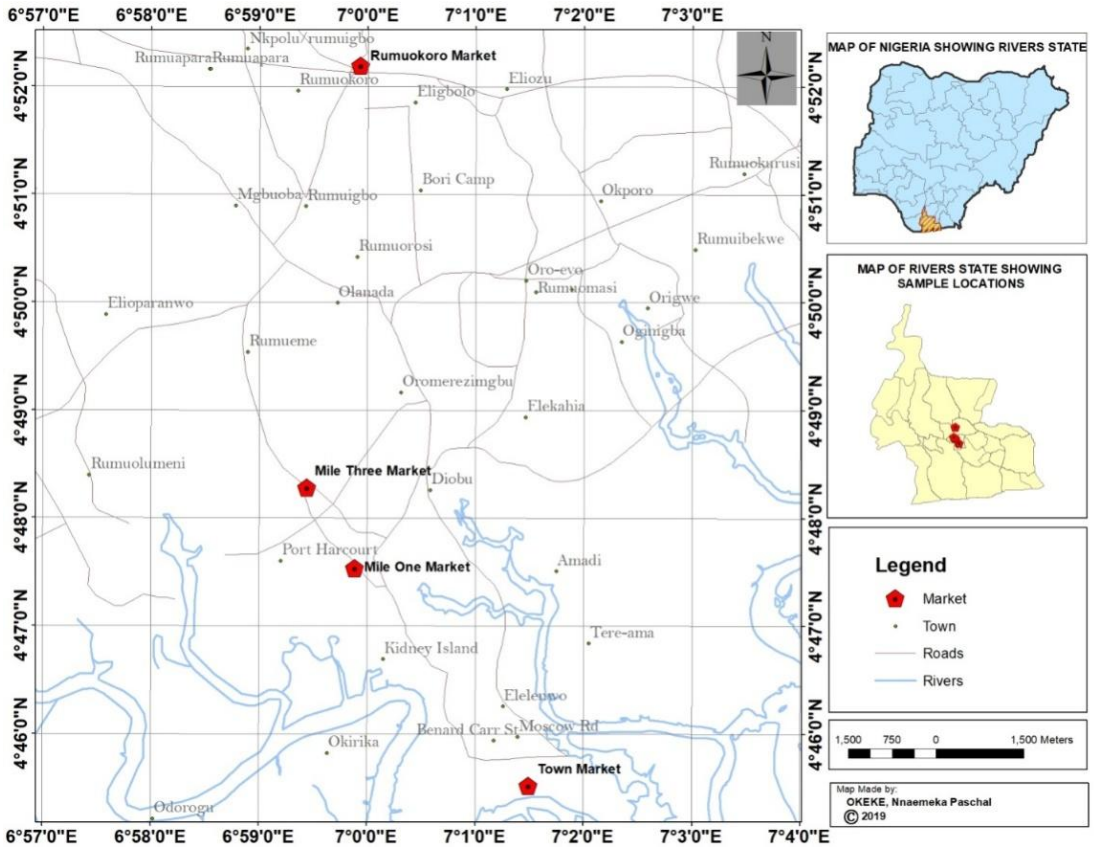

Fig. 1. A map showing the area under study

Table 1. Cowpea variety under study

S/N	Cow pea variety	Seed size	Representing key
1	IT/2246	Big	Big Brown (BB)
	IT/2246	Small	Small Brown (SB)
2	IT/84E	Big	Big White (BW)
	IT/84E	Small	Small White (SW)

electronic weighing balance (Denver instrument, Germany-090111M). After seven days of inoculation of healthy cowpea seeds with fungal isolates, infections were observed on the seeds. There were changes in the physical nature of the seeds which were a deviation from the healthy form. The infected cowpea seeds were analysed using microbiological techniques to determine the presence of the inoculated organisms. This was done by transferring 10 g of infected seeds into 90 ml sterile normal saline. Aliquot from 10^{-1} and 10^{-2} dilution were cultivated on sterile dried SDA plates and incubated at room temperature. This was done to ensure that the infections on the cowpea seeds were as a result of the fungal isolates inoculated on the cowpea seeds. The Pathogenicity was carried out at different period. This different period was classified as season one and season two.

2.5 Data Analysis

Weights of the cowpea seeds that were recorded were presented in Microsoft excel (2016 version). One-way ANOVA in SPSS was used in getting the mean and standard deviation of the weights and the Tukey-Kramer HSD was used for comparisons for all pairs of the mean. The $P < 0.05$ was considered significant in the weight of the cowpea seeds.

3. RESULTS AND DISCUSSION

The fungi identified in the cowpea seeds were *A. flavus, A. niger, Mucor,* and *Rhizopus* sp.

3.1 Pathogenicity Test

The pathogenicity test showing the effect of *Aspergillus flavus, A. niger* and *Rhizopus* sp on the weight of the big brown cowpea seeds, big white cowpea seeds, small brown cowpea seeds and small white cowpea seeds in season one (Table 2). The result showed that the fungal isolates affected the weight of the different cowpea seeds by causing loss of weight. Despite the loss in weight of the cowpea seeds, there were no significant difference between the weight before inoculation, the weight of the control and the weight of the inoculated cowpea seeds.

In season two, the weight of the cowpea seeds before and after treatment with fungal isolates such as *A. niger, Mucor* sp and *Rhizopus* sp is illustrated in Table 3. The results showed that all the fungal isolates had negative impact on the various cowpea seeds thereby causing a loss in weight of the cowpea seeds. Irrespective of the weight lost of the various cowpea seeds after treatment with the respective fungal isolates, Findings showed that there were no significance differences between the weight of the cowpea seeds before inoculation, the control and the weight after inoculation of the fungal isolates.

Cowpea (cowpea seeds) are leguminous plants which are rich in crude protein and amino acid profile which makes them a potential substitute for other protein sources for human consumption, production of fish meal and animal feeds. Animals require a supply of protein and energy to

Table 2. Pathogenicity test for fungi on different varieties of cowpea (season 1)

Fungi	Variety	Weight (g)			
		Control	Before inoculation	After inoculation	Weight loss
Aspergillus flavus	BB	0.38±0.07[a]	0.39±0.07[a]	0.51±0.43[a]	0.12±0.36
	BW	0.41±0.03[a]	0.43±0.04[ab]	0.62±0.16[b]	0.19±0.16
	SB	0.17±0.02[a]	0.19±0.01[a]	0.31±0.2[a]	0.13±0.16
	SW	0.19±0.04[a]	0.2±0.04[a]	0.25±0.2[a]	0.05±0.15
Aspergillus niger	BB	0.37±0.03[a]	0.39±0.03[a]	0.61±0.3[a]	0.22±0.26[a]
	BW	0.44±0.03[a]	0.46±0.03[a]	0.69±0.28[a]	0.18±0.22[a]
	SB	0.15±0.05[a]	0.17±0.05[a]	0.23±0.22[a]	0.09±0.18[a]
	SW	0.19±0.04[a]	0.21±0.03[a]	0.25±0.08[a]	0.06±0.08[a]
Rhizopus spp	BW	0.4±0.07[a]	0.42±0.07[a]	0.34±0.06[a]	0.07±0.07[a]
	BW	0.43±0.05[a]	0.45±0.06[a]	0.58±0.26[a]	0.21±0.24[a]
	SB	0.15±0.04[a]	0.17±0.05[a]	0.21±0.13[a]	0.03±0.05[a]
	SW	0.17±0.03[a]	0.19±0.03[a]	0.13±0.06[a]	0.06±0.05[a]

Means not connected by same letter across the rows are significantly different (P < 0.05)
Keys: BB: Big brown; BW: Big white; SB: Small brown; SW: Small white

Table 3. Pathogenicity test for fungi on different varieties of cowpea (season 2)

Fungi	Variety	Weight (g)			
		Control	Before inoculation	After inoculation	Weight Loss
Aspergillus niger	BB	0.423±0.10[a]	0.44±0.11 [a]	0.62±0.53 [a]	0.18±0.42 [a]
	BW	0.42±0.04 [a]	0.44±0.04 [a]	0.49±0.16 [a]	0.05±0.11 [a]
	SB	0.15±0.02 [a]	0.165±0.02 [a]	0.25±0.30 [a]	0.09±0.28 [a]
	SW	0.16±0.04 [a]	0.175±0.04 [a]	0.14±0.05 [a]	0.04±0.01 [a]
Mucor spp	BB	0.34±0.04 [a]	0.36±0.04 [a]	0.46±0.17 [a]	0.10±0.13 [a]
	BW	0.40±0.08 [a]	0.41±0.08 [a]	0.56±0.23	0.15±0.15 [a]
	SB	0.20±0.03[a]	0.21±0.03 [a]	0.25±0.17	0.04±0.14 [a]
	SW	0.2±0.014 [a]	0.21±0.02 [a]	0.22±0.12 [a]	0.01±0.10 [a]
Rhizopus spp	BB	0.40±0.08 [a]	0.39±0.08 [a]	0.55±0.11 [a]	0.16±0.03 [a]
	BW	0.41±0.04 [a]	0.42±0.04 [a]	0.44±0.32 [a]	0.02±0.28 [a]
	SB	0.16±0.01 [a]	0.17±0.01 [a]	0.15±0.05 [a]	0.02±0.04 [a]
	SW	0.35±0.30 [a]	0.19±0.06 [a]	0.16±0.02 [a]	0.03±0.04 [a]

Means not connected by same letter across the rows are significantly different (P < 0.05)
Keys: BB: Big brown; BW: Big white; SB: Small brown; SW: Small white

be in good health, and this promotes competition between men and animals over the limited source that is available [2]. The fungi identified in this study are amongst the fungal isolates reported by Gabriel and Ruth [2] on jack cowpea seeds. Also, early work done on cowpea seeds by previous authors have reported the presence of *A. niger, A. flavus, Mucor* and *Rhizopus* sp [10,11]. The microbial isolates identified in this study on the cowpea varieties could be normal flora of the cowpea or they could be contaminant that settled on the cowpea seeds surfaces. All the microbial isolates affected the weights of the cowpea varieties thereby causing a loss in the weight as compared to the original weight. Though the controls which were not inoculated or treated with any microbial inoculant had slight weight loss but the weight loss were not as those observed with the microbial treated seeds. The reduced weight observed in this study after treatment with microbial inoculants could be attributed to these microorganisms which have caused the cowpea seeds to deteriorate. Deterioration of food materials as a result of microorganisms could lead to loss of the physical properties of the food material. This agreed with Hocking et al. [7] where biodeterioration of food materials lead to the loss in the food physical and chemical properties. On the other hand, Nwachukwu [9] posited that plant pathogens cause disease in economic plants, resulting in devastating, damage, losses in crop yield, reduced quality, and disfiguring of food products. Thus, this statement agreed with findings in this current study which showed eight loss in cowpea seeds affected with fungi pathogens. Douglas and Robinson [8] in a study characterized most of the fungi in this current study and suggested that they could be pathogenic. Thus, pathogenicity of these fungal isolates in this current study may not just be plant pathogens but could cause diseases or allergic reactions to man.

4. CONCLUSION

Pathogenicity of these isolates on human and plants are well documented. Thus, these isolates could be pathogenic. More so, the fungal isolates affected the different cowpea seeds thereby causing great weight loss. *A. niger* and *Rhizopus* sp caused more weight loss to the cowpea seeds than other fungi. The nutrients in the different cowpea seeds in respect to this study have shown that nutritional contents varied and one could choose on the cowpea variety that suits his/her desired nutrition.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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