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ORIGINAL ARTICLE

Induction of biodeterioration on vegetables by three fungal species

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Abstract
The effect of three fungal species on the nutrient profile of onions and cucumbers was studied. The species identities of the fungal isolates were initially determined based on macro- and micro-morphological characters as well as molecular confirmation, using the internal transcribed spacer region. The isolates were confirmed as *Neocosmospora ramosa* (MG682504), *Aspergillus tamarii* (MG682505) and *A. violaceofuscus* (MG682503). We then used each fungus to induce biodeterioration and performed proximate analysis of nutrient breakdown. We also assayed each fungus for the production of mycotoxins and performed anti-fungal susceptibility tests using fluconazole and voriconazole. We found that *N. ramosa* facilitated the highest rate of biodeterioration for onions and cucumber. Our anti-fungal tests revealed that non-aflatoxigenic *A. tamarii* was susceptible to voriconazole, but resistant to fluconazole. *A. violaceofuscus* (also non-aflatoxigenic) was found to be susceptible to both anti-fungals, while *N. ramosa* was resistant to both the anti-fungals tested. This study underscores the ability of fungi to degrade vegetables, and the need to focus on intervention through both chemical and best practices.

Keywords Biodeterioration · Fungi · Nigeria · Vegetables

Introduction

Biodeterioration is a process of chemical and physical spoilage, caused by the continuous growth of identifiable organisms called biodeteriogens that can be present in foods prior to packaging and on surfaces of packaging materials. These spoilage microorganisms can be acquainted with the product either (1) on the seed itself prior to planting, (2) through product development in the field, (3) during harvest/storage of the product, or (4) during conveyance/processing (Barth et al. 2009).

Onion (*Allium cepa* L.) is an important crop in Nigeria because of its domestic utilization and economic rewards to farmers (Roopa et al. 2014). It is widely cultivated and forms an essential ingredient in the diet of many people as sustenance, flavour enhancement or health improvement (Raju and Naik 2007). Cucumber (*Cucumis sativus*), reported to be one of the world's healthiest foods in 2015 (Szalay 2017), is the fourth most cultivated vegetable in the world. Usually consumed in its raw form (Chuku and Emelike 2014), it is known for its therapeutic potential. Spoilage of these important vegetables by fungi with the potential to produce mycotoxins, poses numerous risks to consumer health as well as trade, both locally and internationally. These vegetables may be salvaged by peeling or cutting away obvious mold growth, but this may not remove all of the contaminating organism, nor any potentially toxic compounds that may be secreted into vegetable tissue. It is recalled that *Fusarium* species, particularly *F. oxysporum*, are on record as the causal organism for basal rot of onion (Haapalainen et al. 2016; Javaid et al. 2017). The same *Fusarium* species may be harmful to cucumber by causing wilt disease (Zhao et al. 2017). Many species of *Aspergillus* have investigated and confirmed to be destructive to crops like onion and cucumber (De Lucca 2007; Ara et al. 2008). Not much has been studied on the direct pathological impact of our sampled fungi on the chosen vegetables. The aim of this study is to evaluate the effects of sampled biodeterioration fungi on the nutritional changes, metal content and possible mycotoxin contamination of

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onions and cucumbers in Nigeria, and to investigate the effects of two anti-fungal compounds as possible interventions.

Materials and methods

Twenty-five healthy samples each of onion and cucumber were purchased from marketplaces throughout Sagamu, Ogun State, in the southwestern region of Nigeria. A total of 50 healthy vegetable samples were placed in separate sterile plastic bags and transported to the laboratory for microbial analysis. All the experiments were performed in the post Graduate microbiology laboratory Babcock University, Ilishan, Ogun State. The culture media used were Potato Dextrose Agar (PDA), Wheat Waste Agar (WWA), Cocoyam Waste Agar (CWA) and Cassava Waste Agar (CWA). PDA was prepared according to manufacturer's instructions, while the infused media were prepared by soaking 200 g of each waste type in 1000 ml of distilled water for 30 min, in a water bath set at 100 °C, sieved to remove solid particles, and then 20 g of agar was added to the liquid portion and mixed thoroughly. All sterilizations were performed according to standard microbiological procedures.

Species identification Preliminary identification of the fungal cultures was based on macro- and micro-morphological characters and the use of dichotomous keys. Photographs of certain morphological characteristics were taken using a Nikon Digital Sight DS-5 M camera (Tokyo, Japan). This was followed by species confirmation using a molecular approach involving the internal transcribed spacer (ITS) region. This facet of the investigation was carried out by the Agricultural Research Service, United States Department of Agriculture, New Orleans, Louisiana, USA, and as stated in Fapohunda et al. (2012). Briefly, 400–600 μl of fungal spore suspensions (in 0.01% Triton) from each isolate were utilized to inoculate 75 ml of potato dextrose broth in separate 250 ml Erlenmeyer flasks. Flasks were placed in an orbital shaker (30 °C, 135 rpm) for 24–48 h. Once substantial spore germination occurred, the resulting mycelia were vacuum filtered through a Büchner funnel using Miracloth, to retain the mycelial tissue, and a wash was performed with sterile water. Mycelial tissue (200 mg) for each was collected into a 1.5 ml microcentrifuge tube, and a DNA extraction protocol was followed using the MasterPure Yeast Purification kit (Epicentre Biotechnologies; Madison, WI, USA). Optical density readings were performed on extracted genomic DNA using a Nano Drop (ND-1000) spectrophotometer (Wilmington, DE, USA), and dilutions (if necessary) were made in preparation for polymerase chain reaction (PCR) amplification of a portion of the internal transcribed spacer (ITS) region (~700 bp). For the PCR amplification reaction, 0.5 μl of ITS1 (CTTGGTCATTTAGAGGAAGTA) and ITS4 (TCCTCCGCTTATTGATATGC) were mixed with 12.5 μl of GoTaq Colorless Mastermix (Promega; Madison, WI, USA), 1 μl of genomic DNA and 10.5 μl of nuclease-free water. PCR cycling conditions included a 2 min initial denaturation at 95 °C, followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s and extension at 72 °C for 30 s. A final extension was added at 72 °C for 7 min. To test the success of PCR amplification, electrophoresis was performed using 5 μl of DNA on a 1.5% agarose gel in Tris-acetate-EDTA (TAE) buffer. DNA amplicons were purified using a QIA quick PCR purification kit (Qiagen Sciences; Germantown, MD, USA) before sequencing. Nucleotide sequences were cleaned and trimmed using Sequencher version 5.4.6 (Gene Codes Corporation; Ann Arbor, MI, USA) and then BLAST queried for molecular confirmation/ identification.

Pathogenicity All pathogenicity work was performed in a laminar flow chamber. Healthy vegetables from each sample were surface sterilized with 90% ethanol, and incisions were made using a sterile 3 mm cork-borer. Sterile cork-borers of the same size were used to cut pellets of PDA in cultures containing mycelia from each of the fungal isolates. These fungal plugs were then inserted into the holes created on the healthy vegetables (five samples for each fungus). The inoculate wound was sealed with petroleum jelly. Our controls involved inserting five plugs of non-inoculated medium into each sample. The inoculated vegetables were placed in clean polyethene bags (one vegetable sample per bag), each moistened with wetted balls of absorbent cotton wool to create a humid environment, and incubated at 28 °C for 10 days. The inoculated samples were observed every two days for symptom development (Adebayo-Tayo et al. 2012; Majdah 2015; Sani et al. 2015). On the 10th day, the deteriorated samples were oven-dried at 70 °C for another 4–5 days. The dried samples were then ground to fine powder using a blender and stored at −20 °C in airtight containers for further analyses.

Proximate analysis The proximate compositions of the dried samples were determined using standard analytical methods. All measurements were performed in duplicate and all values are presented as percentages. The ash and moisture contents were determined using the method described in AOAC (1995). Fat content for each sample was determined using the soxhlet fat extraction method (Onwuka 2005); crude fibre content was determined by Weende's method (James 1996); protein content was determined using the Kjeldahl method (AOAC 1990); carbohydrate content was determined by estimation using the arithmetic difference method (AOAC 1990): %CHO = 100 – (% fat. + % ash + % fiber + % moisture + % protein).

Determination of mineral content The mineral contents (e.g. calcium, potassium, magnesium, iron, zinc and phosphorus) were determined by Flame Atomic Absorption Spectrophotometry (FAAS). Ash samples were digested with 10 ml of 0.1 N HCl. The digested samples were filtered, and those filtrates were made up to 25 ml with distilled water. The filtrates were used for atomic absorption spectrophotometry using filters that matched the different elements. The concentrations of the minerals were determined with their calibration curves prepared with the standards solutions. The percentage values were later calculated by multiplying the concentration by 100 (AOAC 1990). Phosphorus content was determined by the Molybdate method. About 0.5 ml of the digest and 9.5 ml of 10% trichloroacetic acid were put into a test tube. This was followed by agitation for 5 min, and then filtration using Whatman No. 1 filter paper. Five ml of the filtrate was then measured into a cuvet. Five ml of trichloroacetic acid and 5 ml of the working standards were also measured into two other cuvets, which served as a blank and a standard, respectively. About 0.5 ml of molybdate reagent was then added to each test tube and mixed. Similarly, 0.2 ml of sulphuric acid reagents were mixed and allowed to stand for 10 min. The absorbance of sample and standard were read in a Spectrophotometer at 660 nm with blank set at zero (Oko et al. 2012). The percentage of phosphorus present was then calculated using standard procedures. All data were expressed as mean ± SEM. Statistical analyses were carried out by one way analysis of variance ANOVA with $P < 0.05$ regarded as significant.

Antifungal susceptibility The identified fungi were subcultured on PDA at 25 °C for five days. Spore suspensions were prepared from five-day old cultures by transferring a loopful into 5 ml distilled water and vortexed, and 0.1 ml of the suspension was inoculated onto prepared Malt Extract Agar and spread using a sterile rod. Each disc of fluconazole (25 μg/disc) and voriconazole (1 μg/disc) was placed on the plates and incubated at 25 °C for 72 h, and readings were taken by visual inspection for 100% growth inhibition of fungi at 24 h intervals (NCCLS 1998; Therese et al. 2006).

Aflatoxin analysis Aflatoxin analysis was performed using High Performance Liquid Chromatography (HPLC) at the Centre of Biological Engineering, Braga, Portugal. The extraction protocol used was the recommended by VICAM Aflatest extraction protocol. Briefly, a mix of 50 g of each ground fungal sample with 5 g of common salt (NaCl) was blended in a jar with 100 ml of methanol:water (80:20) at high speed for 1 min. The extract was filtered and diluted by pipetting 10 ml and adding 40 ml of purified water. The diluted extract was poured through a glass microfibre filter and 10 ml (10 ml = 1.0 g sample equivalent) was passed completely through AflaTest®-P affinity column at a rate of 1–2 drops/s until air comes through column, and 10 ml of purified water was passed through the column at a rate of about 2 drops/s. The affinity column was eluted by passing 1.0 ml HPLC grade methanol through the column, at a rate of 1–2 drops/s, and then collecting all of the sample eluate (1 ml) in a vial.

Quantification of aflatoxins was performed as described elsewhere (Rodrigues et al. 2009). Samples were analysed using a HPLC equipped with a Jasco FP-920 fluorescence detector (365 nm excitation wavelength; 435 nm emission wavelength), using a photochemical post-column derivatization reactor (PHRED unit Aura Industries, USA). Chromatographic separations were performed on a reverse phase C18 column (Waters Spherisorb ODS2, 4.6 mm × 250 mm, 5 μm), fitted with a pre-column with the same stationary phase. Six standards from a mix of AFB1, AFB2, AFG1, AFG2 (1 ng/ml, 2 ng/ml, 5 ng/ml, 10 ng/ml, 20 ng/ml and 40 ng/ml) were prepared in order to obtain a calibration curve.

The data collected was analysed using Microsoft Excel 2007 and Statistical Package for Social Science (SPSS) version 21.

Results and discussion

Species identifications We found that Potato Dextrose Agar (PDA) and Wheat Waste Agar (WWA) supported the best growth for the fungi. The three species we isolated, *A. tamarii* (GenBank accession No. MG682505), *A. violaceofuscus* (MG682503) and *N. racemosa* (MG682504) were identified according to Fapohunda et al. (2012). It is known that *Neocosmospora* genus is affiliated with the *Fusarium* species complex, specifically being a component of the *Fusarium solani* complex. Their respective ITS sequences were deposited in GenBank of the US National Institute of Health. At the morphological level, distinguishing morphological characters for the isolate, preliminarily identified as *Aspergillus tamarii*, included a rough colony texture and army green colour (macro), while the large thick-walled rough conidia are a distinguishing micro-morphological character (Fig. 1a, b). The *A. violaceofuscus* identification was based on colonies that appeared purplish-black (macro), but its most distinguishing features are micro-morphological such as being uniseriate and producing black spinose conidia (Fig. 1c, d). We first identified this isolate as *A. japonicus*, based on available dichotomous keys for *Aspergillus* species as well as the NCBI database, a name which has recently been reported as a synonymous taxon that should be replaced with *A. violaceofuscus* since it was the first name assigned to this fungus (Varga et al. 2011). The preliminary identification of *F. chlamydosporum* resulted from colonies that appeared fluffy white with a reddish pigment secreted into the medium (macro), and there was an observed micro-morphological

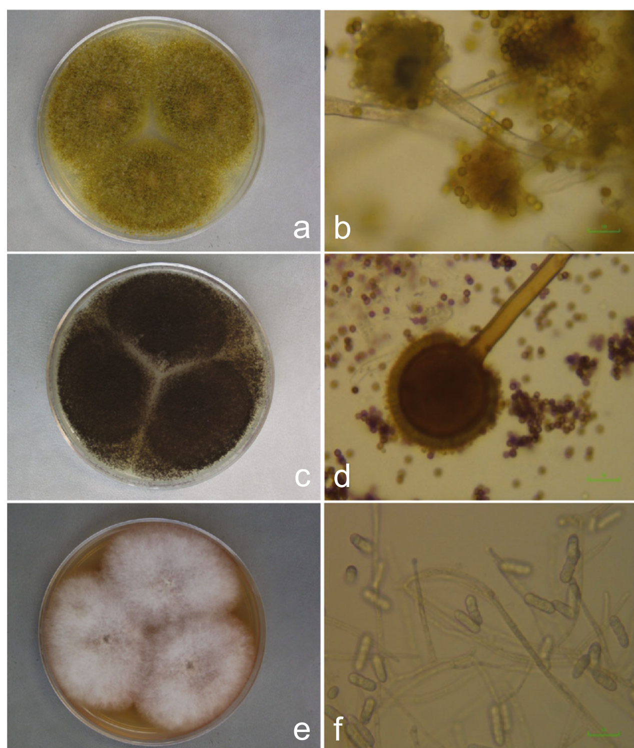

Fig. 1 Macro- and micro-morphological observations for sampled fungi. 1a is an *A. tamarii* macro-morphological observation (army green) for a colony grown on PDA after 7 days incubation. 1b shows *A. tamarii* micro-morphological characters including a conidiophore and some conidia. 1c shows *A. violaceofuscus* colony macro-morphology (dark green) on PDA after 7 days incubation. 1d shows *A. violaceofuscus* micro-morphology including conidial head and clusters of black spinose conidia. 1e shows a *N. racemosa* colony (whitish grey/fluffy white) grown on PDA for 7 days. 1f shows *N. racemosa* micro-morphology including falcate macroconidia and a few microconidi

presence of both macro- and micro-conidia that are straight and falcate to obvate in shape (Fig. 1e, f). From record, these fungi have a history of importance in agriculture, medicine and industry. For example, *A. tamarii* causes human infections such as nasal polyposis (Kamble 2015). It also has the potential to produce mycotoxins such as cyclopiazonic acid and kojic acid (Goto et al. 1997). *A. tamarii* and *A. violaceofuscus* are used to produce industrial enzymes such as tannase, amylase and lipase (Costa et al. 2008). The genus *Fusarium* comprises a diverse array of fungi members which are phytopathogens capable of infecting wide range of agricultural products and in some occasions as emerging problems. *F. chlamydosporum* has been reported as pathogenic for plants and humans (Siddiquee et al. 2010; Popovski and Celar 2013). BLAST query of the sequences from ITS region for each isolate confirmed our preliminary morphological species identifications for the *A. tamarii* and *A. violaceofuscus* isolates, but revealed the *F. chlamydosporum* isolate to be *N. ramosa*, which has recently been determined a phylogenetically-distinct lineage of fungus that happens to share cladal associations with *Fusarium* species (Lombard et al. 2015). O'Donnell (1996) reported that the sexual morphotype of *Neocosmospora* were species of *Fusarium* that could produce microconidia, but had lost the ability to produce macroconidia and septate ascospores. Its basionym is a fungal species known as *Hyaloflorea ramosa*, first characterized in 1955 (Batista and Maia 1955). Given the very recent naming of *N. ramosa*, and its lack of support for our morphological identification as *F. chlamydosporum*, additional examination of several other conserved loci may be required for this isolate.

Pathogenicity Table 1 lists the observed signs for each sample treatment, based on 5- and 10-day post-inoculation time points. There were no signs of spoilage in the control materials, however the fungi expressed varying degrees of biodeterioration ranging from softening, through greenish discoloration, to the presence of wrinkled, water-soaked depressions as shown in Table 1; Figs. 2 and 3. This study implicated the three fungal species in the biodeterioration of cucumbers with the associated tissue softening and discolouration (Huang 1990; Josiah and Efiuvwevwere 2012; Yaji et al. 2016; Watson and Napier 2009). Confirming findings from a previous study, *Aspergillus violaceofuscus* did not result in any sign of spoilage in onions (Umesh and Hemalatau 2012). Externally for all, and particularly with *A. tamari*, the spoilage did not express massive destruction, but analysis of the samples showed extensive internal biodegradation.

Proximate analysis For the proximate analysis (Table 2), the ash, fibre, fat and moisture contents for onion samples inoculated with the *A. tamarii* and *N. ramosa* fungi were relatively low compared to the control samples, while the same contents for onion samples inoculated with *A. violaceofuscus* were similar to those of the control samples. There was also no significant difference observed between the control and treated cucumber samples. The ash, fibre, fat and protein and mineral contents of control samples for both vegetables are similar to the values obtained by Norman (1992), Shovon et al. (2013). Also the values of ash, fat, moisture and protein of in the control samples of cucumbers agree with previous reports (Abulude and Adeleke 2010; Okoye 2013; Onimisi and Ovansa 2015).

Determination of mineral content Table 3 lists the percentages of mineral content observed during our study. Compared to the controls, the vegetable samples inoculated with *A. tamarii* exhibited some reduction in mineral levels, with the exceptions of phosphorus (onion) and calcium (cucumber). Inoculation with the three fungi resulted in significant reduction in magnesium content in onion compared to the control. Reductions were also observed in

Table 1 Signs of fungi-induced bio-deterioration on samples, based on inoculation treatment, over 5- and 10- day observations

	A. tamarii	*A. violaceofuscus*	*F. chlamydosporum/ N. ramosa*	Control
Cucumber_Day 5	Sunken tissue around bore site	Black discolouration around bore site associated with fungal conidiation	Watery, wrinkled, sunken tissue with whitish discolouration, associated with fungal mycelia, around bore site	No sign of spoilage around bore site
Cucumber_Day 10	Increase in observed D5 signs; spread of tissue softening and greenish discolouration, associated with fungal conidiation, beyond bore site	Increase in observed D5 signs; tissue softening beyond bore site, with little sunken tissue observed	Increase in observed D5 signs; tissue softening beyond bore site	No sign of spoilage around bore site
Onion_Day 5	Watery, sunken tissue with whitish discolouration, associated with fungal mycelia, around bore site	No sign of spoilage around bore site	Watery, wrinkled, sunken tissue with whitish discolouration, associated with fungal mycelia, around bore site	No sign of spoilage around bore site
Onion_Day 10	Increase in observed D5 signs	No sign of spoilage around bore site	Increase in observed D5 signs; tissue softening beyond bore site	No sign of spoilage around bore site

potassium regarding samples inoculated with the three isolates as control values were higher. In cucumber, the highest levels of potassium was observed both in control and experimental.

Antifungal susceptibility Our antifungal susceptibility test results correlate with previous studies (Therese et al. 2006; Patel et al. 2011; Kazemi et al. 2012) by confirming that *Aspergillus* species are susceptible to voriconazole and fluconazole. *Aspergillus tamarii* was found to be resistant only to fluconazole. This is not totally unexpected because, as of recent, the emerging trend is the global prevalence of azole resistance to *Aspergillus* (CDC 2017). *Aspergillus tamarii* is a confirmed trigger for onychomycosis (Samson et al. 2014). The *A. violaceofuscus* isolate showed susceptibility to both fluconazole and voriconazole. A case of diminished in vitro susceptibility has earlier been reported in *Aspergillus*-induced morbidities such as aspergillosis (Gregson et al. 2013).

Our findings on the morphological identification of *F. chlamydosporum* also seem to correlate with *Fusarium* spp. expressing drug-resistance (Popovski and Celar 2013). This isolate (molecularly determined to be *N. ramosa*) was found to be resistant to both fluconazole and voriconazole. This was in agreement with Mellado et al. (2004) and Al-Hatmi et al. (2016), previously confirming that *Fusarium* species are intrinsically resistant to azole antifungals. It has been shown that at least some *Fusarium* species have cyto-chrome P450 sterol 14a-demethylase (CYP51) paralogs, and that the genes exhibit a high degree of variation, which suggests the possibility of resistance (van Diepeningen et al. 2015). With an increasing incidence of fusariosis (Crabol and Lortholary 2014), it is important to pay attention to candidate hosts of

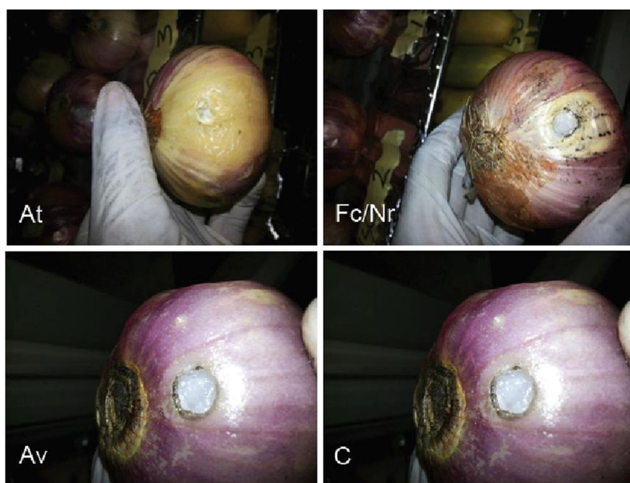

Fig. 2 Onion bulb and the effect of inoculating with various fungi of interest: At = *A. tamarii*, Av = *A. violaceofuscus*, Fc/Nr = *F. chlamydosporum/N. ramosa*, C = Control

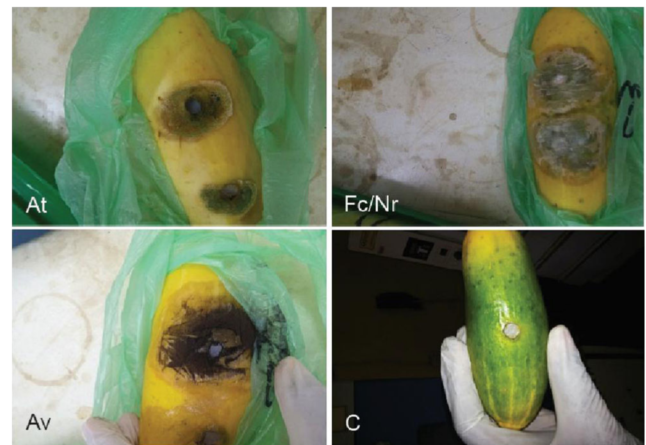

Fig. 3 Cucumber and the effect of inoculating with various fungi of interest: At = *A. tamarii*, Av = *A. violaceofuscus*, Fc = *F. chlamydosporum/N. ramosa*, C = Control

Table 2 Proximate analysis of biodeteriorated cucumber and onion samples with the 3 fungi

Sample/Content		*A. tamarii (%)*	*A. violaceofuscusus (%)*	*F. chlamydosporum/ N. ramosa (%)*	Control (%)
Cucumber	Ash	16.4 ± 0.98	15.6 ± 0.38	15.8 ± 0.89	17.7 ± 0.93
	Fibre	9.9 ± 0.12	11.6 ± 0.85	9.6 ± 0.23	12.3 ± 0.03
	Fat	17.3 ± 0.95	16.9 ± 0.58	14.9 ± 0.39	18.7 ± 0.89
	Moisture	93.7 ± 1.96	96.1 ± 1.65	96.9 ± 1,49	89.6 ± 0.96
	Protein	10.7 ± 0.06	11.2 ± 0.59	12.9 ± 0.94	10.6 ± 0.95
Onion	Ash	3.1 ± 0.01	3.6 ± 0.03	3.1 ± 0.02	3.8 ± 0.02
	Fibre	4.8 ± 0.04	5.9 ± 0.05	5.7 ± 0.02	6.7 ± 0.36
	Fat	2.4 ± 0.02	3.3 ± 0,01	2.9 ± 0.02	3.4 ± 0.02
	Moisture	85.0 ± 0.59	87.9 ± 0.58	87.8 ± 0.5–91	87.9 ± 0.84
	Protein	8.8 ± 0.31	9.8 ± 0.26	8.8 ± 0.04	8.9 ± 0.06

Data expressed as mean ± SEM; $P < 0.05$

contamination (i.e. vegetables), which most of time are not exposed to the best of hygiene in developing countries. Even though voriconazole was reported to have a superior efficacy than many antibiotics on some filamentous fungi (Karina et al. 2013), this is in contrast to our observations. Control samples had full growth, interpreted as covering more than 95% of the experimental dish. The close phylogenetic association of *Fusarium* and *Neocosmospora* spp. (Lombard et al. 2015) could indicate that antifungal resistance is possible for both genera, although further research involving the effect of antifungal compounds on the type strain of *N. ramosa* should be carried out to confirm or refute this.

Aflatoxin analysis There was no direct link between the isolates and aflatoxin profile of the contaminants on the two vegetables. The aflatoxin analysis showed positive results in onion samples including control. There is the suspicion of primary contamination from the source due to improper storage regime (Sahar et al. 2009). The ability of these fungi to incite biodeterioration is now confirmed. The process is likely to invite changes in metabolism resulting in abnormal values (Aboloma et al. 2012; Onifade and Jeff-Agboola 2003). No aflatoxin was detected in both the control and treated cucumber samples (Table 4). Cucumber displayed resistance to aflatoxin invasion while onion harboured B_1, B_2 and G_1 in various quantities. Cyclopiazonic acid, a mycotoxin normally produced by strains of *A. tamarii* was not tested in this investigation. Aflatoxin G_2 was not detected in any of the samples.

Conclusion

Our discordant findings for the morphological and molecular identification of the *Fusarium*/*Neocosmospora* isolate

Table 3 Mineral profile of biodeteriorated cucumber and onion samples with the 3 fungal isolates

Sample/Content (mg/L)		*A. tamarii (%)*	*A. violaceofuscus (%)*	*F. chlamydosporum/ N. ramosa (%)*	Control (%)
Cucumber	P	70.16 ± 1.05	84.04 ± 1.06	86.23 ± 1.07	77.96 ± 1.07
	Ca	1.68 ± 0.07	1.86 ± 0.02	0.56±	1.65 ± 0.02
	Mg	12.29 ± 1.06	14.21±	8.05 ± 0.06	12.61 ± 0.56
	K	1091.30 ± 20.00	1053.18 ± 17.04	1572.86 ± 15.06	1310.34 ± 14.09
	Fe	0.00	0.00	0.20 ± 0.00	0.00
	Zn	0.01 ± 0.00	0.00	0.02 ± 0.00	0.07 ± 0.00
Onion	P	95.12 ± 1.23	91.10±	93.21±	95.01 ± 1.24
	Ca	24.65 ± 0.89	43.92±	49.30±	53.34 ± 0.59
	Mg	14.05 ± 0.02	6.51±	6.95±	38.88 ± 0.96
	K	167.38 ± 10.07	206.86 ± 9.07	186.09 ± 9.65	249.96 ± 12.04
	Fe	0.10±	0.41 ± 0.00	0.37 ± 0.01	0.22 ± 0.01
	Zn	0.50 ± 0.02	0.47 ± 0.01	0.46 ± 0.01	0.68 ± 0.03

Data expressed as mean ± SEM; $P < 0.05$

Table 4 Aflatoxin chemotype profiles for biodeteriorated samples

Sample type	Fungal species	AFB1 (ng/μL)	AFB2 (ng/μl)	AFG1 (ng/μl)	AFG2 (ng/μl)
Onions	*A. tamarii*	2.26	0.27	0.00	0.00
		0.96	0.62	0.00	0.00
Onions	*A. violaceofuscus*	0.46	0.13	0.00	0.00
		0.00	0.00	0.00	0.00
Onions	*Fusarium chlamydosporum*	0.26	0.24	0.00	0.00
		0.00	0.00	0.00	0.00
Onions	*A. tamarii*	0.30	0.14	0.00	0.00
		0.65	0.00	1.50	0.00
Onions	Control	1.45	0.00	2.75	0.00
		2.19	0.00	4.76	0.00
Cucumber	*A. tamarii*	0.00	0.00	0.00	0.00
Cucumber	*A. violaceofuscus*	0.00	0.00	0.00	0.00
Cucumber	*Fusarium chlamydosporum*	0.00	0.00	0.00	0.00
Cucumber	*A. tamarii*	0.00	0.00	0.00	0.00
Cucumber	Control	0.00	0.00	0.00	0.00

underscore the importance of utilizing and refining the holistic approach to species identification. We show that morphology alone, or molecular analysis alone, could result in conflicting identifications. This work also showed that *N. ramosa* has the ability to deteriorate both onions and cucumber than *Aspergillus tamarii* and *A. violaceofuscus* with effects on the proximate composition and mineral contents of the two vegetables. Reports on the effect of these species on the deterioration of cucumber and onion have been scant in literature. Although they can incite decay and infection, their growth can also be inhibited by fluconazole and voriconazole. This is an important information for plant and human health in relation to infectious diseases. The detection of aflatoxin even without any link to the fungi shows the ever-present risk consumers are exposed to in the open markets. The risk of contamination is always there. Unintended fungal contaminants and their products are critical sources of health concern to human and animals. The aflatoxin content detected was not traceable to the inoculated fungi. Aflatoxin B1 is listed as type 1 carcinogen by the International Agency for Research on Cancer and it is of great health concern to developing countries due to economic challenge and lack of enlightenment. This toxin and its relationslike aflatoxin B2, are therefore the likely points of investigation in any situation of fungal invasion of food materials. It is therefore important to state that non-hygienic regimes, unrelated to the experiment, were likely the source of contamination. Even when cucumber seemed to have the capacity not to accommodate aflatoxin contamination, good agricultural practices that stress harvest and storage hygiene are still recommended as effective intervention against fungal invasion of vegetables in Nigeria.

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