Quality Assurance and Safety of Crops & Foods, 2023; 15(4): 89–98

Surveillance of mycotoxin contaminants and mycotoxigenic fungi in agricultural produce

Sandhya Devi Takooree, Nooreen Mamode Ally, Ajjmeerah Bibi Ameerkhan, Vijayanti Mala Ranghoo-Sanmukhiya, Ramona Anaëlle Duchenne-Moutien, Hudaa Neetoo*

Department of Agricultural & Food Science, Faculty of Agriculture, University of Mauritius, Réduit, Mauritius

***Corresponding Author:** Hudaa Neetoo, Department of Agricultural & Food Science, Faculty of Agriculture, University of Mauritius, Réduit, 80837, Mauritius. Email: s.neetoo@uom.ac.mu

Received: 30 September 2023; Accepted: 10 October 2023: Published: 14 November 2023

ORIGINAL ARTICLE

Abstract

Food crops, including vegetables, are prone to attack by pathogenic and mycotoxigenic fungi and represent a food safety and public health risk. The study aimed to detect and quantify mycotoxins in vegetables widely consumed in Mauritius. Diseased samples of garlic, onion, potato, pumpkin and tomato were collected post-harvest. Following microscopic identification of the suspect pathogen(s), samples were tested for mycotoxins by ELISA. Results demonstrated a high mean level of citrinin in garlic (5,448.6 μg/kg) and ochratoxin in onion (9.25 μg/kg), which exceeded the permissible limits, thus pointing to potential health risks associated with the consumption of these vegetables.

Keywords: garlic; mycotoxins; onion; potato; pumpkin; tomato

Introduction

Mycotoxins are secondary metabolites naturally produced by fungi or moulds (WHO, 2018). They are small, highly toxic chemical products that can contaminate foods or feedstuffs at any stage along the food chain (WHO, 2018). In addition, mycotoxins are considered chemical hazards in foods (Cinar and Onbasi *et al.*, 2019) since they are known to have adverse health effects on humans and animals after ingestion, referred to as mycotoxicosis (WHO, 2018). Equally, the danger of mycotoxins should not be undermined since many mycotoxins have been reported to be carcinogenic (Sengül *et al.*, 2016).

Mycotoxin contamination of agricultural plant products has become a significant issue around the world (Luo *et al.*, 2021) due to post-harvest infection by mycotoxin-producing fungi such as *Aspergillus* spp., *Alternaria* spp., *Penicillium* spp. and *Fusarium* spp. (Barkai-Golan and Paster, 2008). Nevertheless, most past studies were mainly focused on cereals, grains, maise, bread, fruits, nuts, juices, sausages, infant cereals, dried fruits, legumes and dried spices (EU, 2006; de Medeiros *et al.*, 2012; Guo *et al.*, 2016; Eskola *et al.*, 2019; Cinar and Onbasi *et al.*, 2019; Kifer *et al.*, 2019). However, vegetables, which are considered essential commodities in the diet of people (Liu *et al.*, 2020), have also been found to be contaminated by mycotoxins (Gupta *et al.*, 2009). The latter included aflatoxin (AF), alternariol (AOH), citrinin (CIT), deoxynivalenol (DON), fumonisin (FUM), ochratoxin (OT), T-2/HT-2 toxin (T-2/HT-2) and zearalenone (ZEN) (Adegoke and Letuma, 2013).

However, since mycotoxins are heat stable, they cannot be destroyed easily during thermal processing or cooking (Liu *et al.*, 2020) and therefore represent a food safety risk. In Mauritius, vegetable crops which are widely produced and consumed include potato, tomato, onion, pumpkin and garlic. Till now, very few studies have recently been conducted on the detection or quantification of mycotoxin and its relative concentration in vegetables, for instance, potatoes (Youssef and Sabra, 2022), tomatoes (Ji *et al.*, 2023), garlics (Jeswel and Kumar, 2016), pumpkins (Sahar *et al.*, 2009) and onions (Gherbawy *et al.*, 2015). However, to our knowledge, no studies have been reported on detecting FUM in pumpkins. The objective of this study was, therefore, to determine the level of mycotoxin contamination on selected vegetable crops commercially available in the Mauritian market.

Materials and Methods

Sample collection and fungal isolation

Samples of diseased garlic (*Allium sativum*), onion (*Allium cepa*), potato (*Solanum tuberosum*), pumpkin (*Cucurbita moschata*) and tomato (*Solanum lycopersicum*) suspected to be infected by pathogenic fungi were collected at the postharvest stage from open-fields, packhouses and markets of Mauritius from February 2019 to September 2021. A total of 287 samples of vegetables were collected: garlic (n=20), onion (n=40), potato (n=125), pumpkin (n=10) and tomato (n=92). To identify the fungal agent, the diseased samples were placed in moist chambers for 24 h to favour the development of fungal mycelia and subsequently examined by microscopy using a bright-field microscope.

The following day, eight squares with dimensions of 1 cm x 1 cm were cut to include a margin of the diseased and healthy tissue from the different samples. The cut pieces were gently flushed with continuous water for at least 2 h and then surface-disinfected for 30 s with sodium hypochlorite solution (1:3 sodium hypochlorite to water) and left to dry on sterile tissue paper. The samples were then transferred on Potato Dextrose Agar (PDA) amended with chloramphenicol. The inoculated plates were incubated at room temperature for 7 days in the dark. After incubation, observation of the macroscopic morphology of the colonies and microscopic examination of the fungi were conducted. If the culture obtained corresponded to the suspected fungus, it was sub-cultured using the hyphal tip method on a new PDA plate to get a pure culture (Takooree *et al.*, 2021).

Molecular characterisation of the etiological agent of diseased samples

After 7 days of incubation, fungal isolates presumptively identified on PDA were selected for molecular identification. Briefly, mycelial growth was scraped from the plate, weighed, and ground with liquid nitrogen (Takooree *et al.*, 2022). DNA was extracted by the Cetyl Trimethyl Ammonium Bromide (CTAB) method (Madarbokus and Ranghoo-Sanmukhiya, 2012) and subsequently subjected to PCR analysis. ITS regions of rDNA were amplified using the ITS primers, ITS5 (5'-GGAAGTAAAAGTC GTAACAAGG-3') and ITS4 (5'-TCCTCGCTTATTGAT ATGC-3') (Ristaino *et al.*, 1998). The thermal cycling parameters comprised of initial denaturation at 94°C for 3 min followed by 30 cycles consisting of denaturation at 94°C for 1 min, annealing at 52°C for 1 min, and extension at 72°C for 2 min followed by a final extension at 72°C for 10 min. DNA sequencing reactions were done using a Big Dye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems) following the protocol outlined by the manufacturers. Sequencing reaction products were purified by the ExoSAP method. They were directly sequenced in both directions using an automated sequencer (ABI 3500 DNA sequencer (Applied Biosystems) at Inqaba Biotechnical Industries (Pty) Ltd, South Africa, using the same primers for amplification. Forward and reverse sequences were assembled and edited using CLC Main Workbench Version 22.0 (https://www.qiagenbioinformatics.com/). Consensus sequences were computed using the ClustalW (Thompson *et al.*, 1994), integrated in MEGA11 software (Tamura *et al.*, 2013), and deposited in GenBank (http://www.ncbi. nlm.nih.gov). All generated sequences were compared by calculating nucleotide (nt) similarities. Moreover, they were compared with previously deposited *Fusarium* spp. and *Alternaria* spp. isolates available in GenBank (Table S1), using the similarity search tool BLAST. The phylogenetic tree was made using PAUP Version 4.0 beta for the Parsimony Method, and the reliability was confirmed by bootstrapping using 1000 random replicates. *Fusarium nygamai* and *Alternaria gypsophila* were designated as the fungal outgroup to validate the obtained results.

Assessment of mycotoxin level in samples

On each sampling occasion, infected vegetable samples were examined, and sections of the diseased parts of the samples were aseptically cut with a knife and examined microscopically. It was observed that the different vegetables were found to be susceptible to infection by other mycotoxin-producing fungal agents. Table 1 thus indicates the corresponding mycotoxins for which the various vegetables were analysed. For all vegetables except for pumpkin, five cut sections of the same vegetable kind were pooled to form a composite sample, which was subsequently investigated. Pumpkin sections were analysed individually due to the limited availability of diseased samples. Each composite sample was blended to produce a slurry, which was later analysed by Enzyme-Linked Immuno-Sorbent Assays (ELISA) in duplicate. The quantitative analysis of AOH, AF, CIT, DON, FUM, OT, T-2/HT-2 and ZEN was carried out using ELISA; Beacon Analytical Inc. Alternariol Plate Kit (Cat. # 20-0288),

Table 1. Mycotoxins of relevance in vegetables based on susceptibility to infection by certain mycotoxigenic fungal species.

Vegetable	*Alternaria* spp.	*Aspergillus* spp.		*Fusarium* spp.				*Penicillium* spp.	
	AOH	AFL	OT	DON	FUM	T-2/HT-2	ZEN	CIT	OT
Garlic	–	✓	✓	–	–	–	–	✓	✓
Onion	–	✓	✓	✓	–	✓	✓	✓	✓
Potato	✓	–	–	✓	✓	✓	✓	–	–
Pumpkin	✓	–	–	–	✓	–	–	–	–
Tomato	✓	–	–	✓	✓	✓	✓	–	–

Ridascreen® TOTAL Aflatoxin (Art. No. R4701), Ridascreen® Fast Citrinin (Art. No. R6302), Ridascreen® DON (Art. No. R5906), Ridascreen® Fumonisin (Art. No. R3401) or Ridascreen® Fast Fumonisin (Art. No. R5602), Ridascreen® Ochratoxin A 30/15 (Art. No. 1312), Ridascreen® T-2/HT-2 Toxin (Art. No. R3805) and Ridascreen® Zearalenon (Art. No. 1401) test kits (R-Biopharm, Germany). All mycotoxin extraction and analyses were conducted according to the manufacturer's instructions. Results were obtained by reading the absorbances of standards or samples at 450 nm using an ELISA microplate reader (Rida® Absorbance 96, Germany). Calculations for the concentration of each mycotoxin level were done according to the instructions. The limit of detection of the assays of AOH, AF, CIT, DON, FUM, OT, T-2/HT-2 and ZEN were: not available, 1.75 μg/kg, 15 μg/kg, 3.7-18.5 μg/kg, 25 μg/kg or 222 μg/kg, 0.4-1.6 μg/kg, 12-33 μg/kg and 0.00005- 0.00175 μg/kg respectively. In addition, to validate the ELISA assay, a standard sample suspected to be contaminated with FUM was additionally tested by both ELISA and LC-MS/MS, and the results were compared.

Data analysis

One-way analysis of Variables (ANOVA) and Tukey's multiple comparisons test were done to determine if there was a statistically significant difference ($P < 0.05$) in the prevalence of mycotoxins among the samples chosen for the study. The Tukey test was the post-hoc test of choice as it is more conservative than other tests.

Results and Discussion

Morphological identification

Vegetables such as garlic, onion, potato, pumpkin and tomato were found to be infected by *Alternaria* spp., *Aspergillus* spp., *Fusarium* spp. or *Penicillium* spp. from different regions of Mauritius. Microscopic analysis at the site of infection and from PDA cultures was conducted to identify the causative agent (Figure 1) presumptively. The suspected fungal isolates infecting each crop were thus confirmed based on their morphological characteristics, which included mainly the colour and shape of colonies grown on PDA, shape, colour and branching pattern of the spores or conidia or conidiophores and the presence or absence of septa (Meena *et al.*, 2017). Each isolate of the *Alternaria* spp., *Aspergillus* spp., *Fusarium* spp. or *Penicillium* spp. demonstrated variations in culture morphology as shown in Figure 1. It can be observed that the colour of isolated *Alternaria* species varies, from olive-brown green or pale brown [a(i)] to white colony [b(i)], as reported by Meena *et al.* (2017). As for *Fusarium* isolates, they also vary in colony morphology, such as white to pink cottony, with purple tinge [c(i)]; orange [c(ii)]; and pale yellow and white cottony [c(iii)], similar to past studies (Nirmaladevi *et al.*, 2016; Swamy *et al.*, 2020)

Moreover, microscopic examination revealed the presence of conidia of *Alternaria* spp. appearing brown and oval in shape with septa [a(ii) & b(ii)], macroconidia of *Fusarium* spp. demonstrating three to five septa together with cells appearing pointed on both ends and having a foot-like shape [c(iv)], conidiophores of *Penicillium* spp. with some oval small spores formed in chains [d(ii)] and round conidiophores of *Aspergillus* spp. with numerous oval-shaped spores [e(ii)] (Figure 1). Those microscopic observations are congruent with microscopic features observed by other authors for *Alternaria* spp. (Troncoso-Rojas and Tiznado-Hernández, 2014), *Fusarium* spp. (Thrane, 1999; Nirmaladevi *et al.*, 2016; Swamy *et al.*, 2020), *Penicillium* spp. (Palou, 2014) and *Aspergillus* spp. (Plascencia-Jatomea *et al.*, 2014).

Molecular characterisation of the etiological agent of diseased samples

After sequencing the PCR products and obtaining the consensus sequences for each isolate, the BLAST analysis revealed the fungal species to be *Fusarium oxysporum*, *Fusarium equiseti*, *Fusarium graminearum*, *Alternaria alternata* and *Alternaria solani*, which showed 100% identity with GenBank sequences. Submission of the

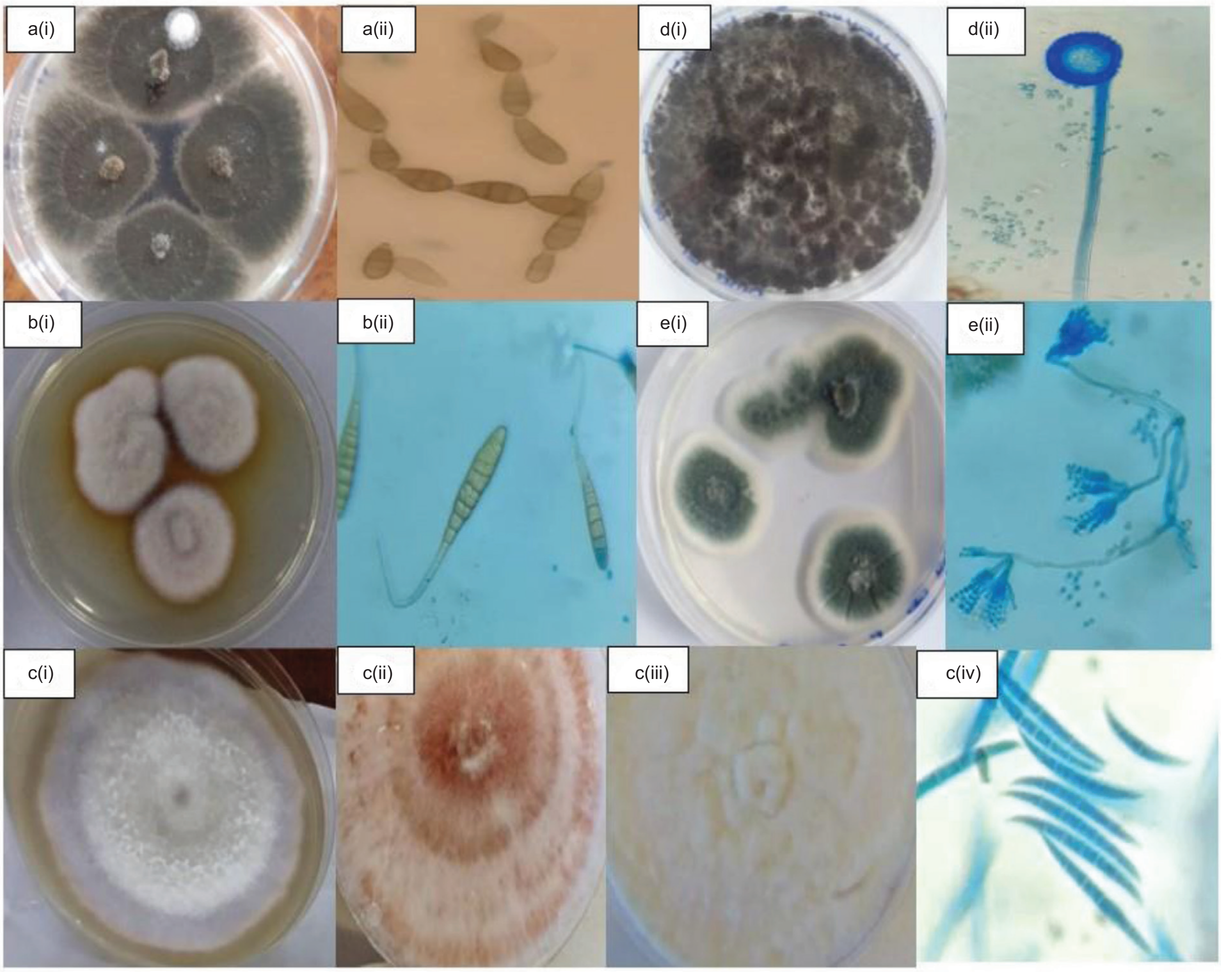

Figure 1. Fungal isolates from infected crops showing colony morphology, spores or conidia, conidiophores, hyphae and reproductive structures stained with cotton blue lactophenol dye using light microscopy, 40X: a(i) & a(ii) *Alternaria alternata*; b(i) & b(ii) *Alternaria solani*; c(i) *Fusarium oxysporum*; c(ii) *Fusarium graminearum*; c(iii) *Fusarium equiseti*; c(iv) macroconidia of *Fusarium* spp.; d(i) & d(ii) *Aspergillus* spp.; e(i) & e(ii) *Penicillium* spp.

sequences to NCBI GenBank was carried out, and accession numbers were obtained, as shown in Table S2.

A phylogenetic tree was constructed to illustrate the possible genetic relatedness among *Fusarium* and *Alternaria* spp. isolates from onion, potato, pumpkin and tomato samples, and several mycotoxigenic fungi already published in the literature (Figure 2). The tree revealed four main clades, with two outgroups (*Fusarium nygamai* and *Alternaria gypsophilae)* occurring in separate clades. Among the clade of *Fusarium* species, the sequences of *F. oxysporum*, *F. equiseti* and *F. graminearum* were observed to form separate subclusters. *A. alternata* and *A. solani* formed the other clade. Indeed, it can be observed from the resulting phylogenetic tree that all the isolates of *Fusarium* spp. and *Alternaria* spp. were clustered together with 100% bootstrap value, supporting their correct identification.

Among the eight *Fusarium* species, two *F. oxysporum* strains from potatoes (P2 and P198) clustered well with the reference sequence (Accession No. KC478622). The latter strain has been reported to be highly virulent, causing severe *Fusarium* wilt in tomato plants in India (Murugan *et al.*, 2020), thus pointing to the potentially high virulence of the two local potato strains. The remaining three strains of *F. oxysporum* isolated from potato (P124), tomato (T1) and onion (G11) were found to cluster with the reference sequence (Accession No. KP719138). Stefańczyk *et al.* (2016) reported that the latter *Fusarium* species were more frequently observed than other species, such as *F. avenaceum*, *F. solani* and *F. sambucinum.* However, according to the author, *F. oxysporum* was not found to be pathogenic in the laboratory, causing dry rot symptoms in potato tubers. Nevertheless, Stefańczyk *et al.* (2016) indicated that the agent can still present a disease risk in field or storage areas.

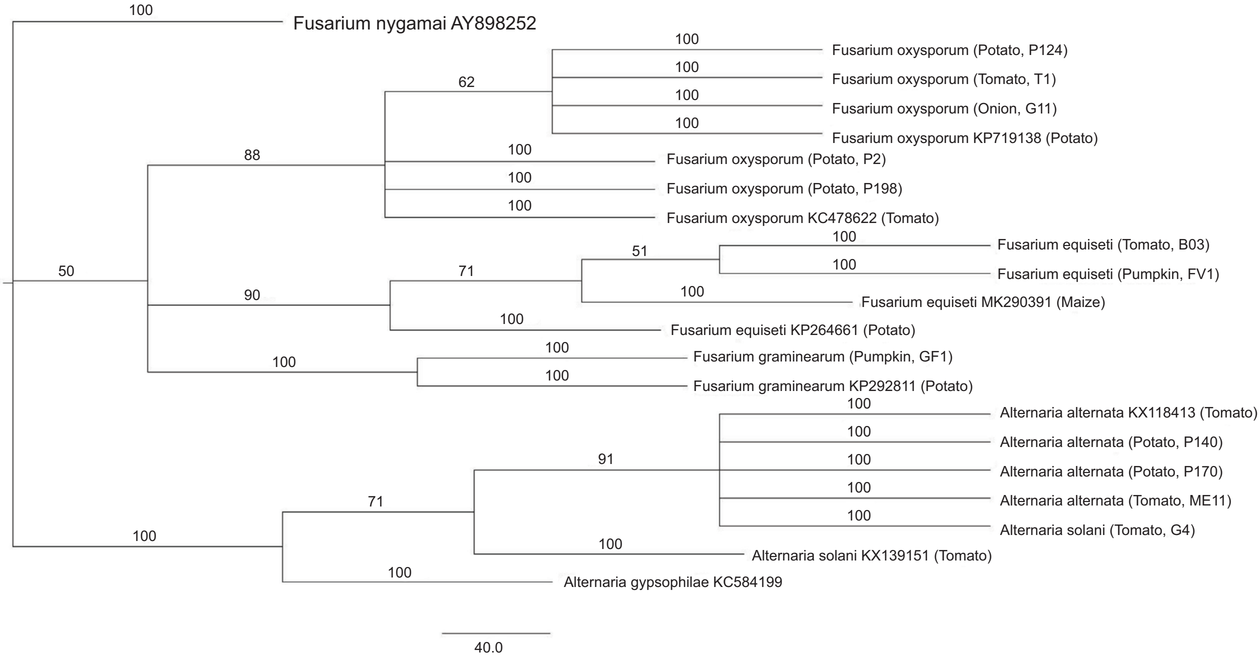

Figure 2. Phylogenetic tree of *Fusarium* spp. and *Alternaria* spp. isolates from mycotoxin-contaminated vegetable samples compared with reference sequences obtained from NCBI.

Table 2. Concentration (µg/kg) of AF, AOH, CIT, DON, FUM, OT, T-2/HT-2 and ZEN toxins in vegetables sampled from different storage areas of Mauritius, compared with the safe limits.

Mycotoxin	Vegetable	Number of samples tested	Concentration of mycotoxin			Safe limits	
			Mean (µg/kg)	Maximum (µg/kg)	Minimum (µg/kg)	Concentration (µg/kg)	Reference
AOH	*Potato*	57	1.02	5.28	LOD-0.02	Still under consideration	van Egmond and Jonker, 2008
	Pumpkin	5	0.50	0.90	LOD-0.13		
	Tomato	49	1.21	3.00	0.04		
AF	*Garlic*	4	1.27	1.49	1.04	Max. 10	Food Regulations, 1999
	Onion	35	2.03	3.31	0.57		
CIT	*Garlic*	20	**5,448.55**	37,179.60	12.90	Max. 2,000	EC, 2006
	Onion	40	455.46	3,008.00	1.35		
DON	*Onion*	5	15.45	17.00	13.90	Max. 750 Max. 50 (in potato)	EC, 2006; van Egmond and Jonker, 2008
	Potato	52	4.27	14.40	0.25		
	Tomato	43	18.79	107.44	0.99		
FUM	*Potato*	31	740.14	**5,531.00**	0.62	Max. 4,000	EC, 2006
	Pumpkin	5	16.42	32.90	3.80		
	Tomato	33	533.32	1,071.71	5.67		
OT	*Garlic*	4	0.0045	0.005	0.004	Max. 5	EC, 2006
	Onion	20	**9.25**	40.00	0.0006		
T-2/HT-2	*Onion*	10	18.92	32.30	2.36	200-1,000	EU, 2013
	Potato	50	98.73	340.5	0		
	Tomato	43	12.81	64.52	1.00		
ZEN	*Onion*	15	40.59	62.30	37.30	Max. 350	EC, 2006; de Medeiros *et al.*, 2012
	Potato	51	0.40	14.80	0.004		
	Tomato	19	1.60	2.48	0.63		

*LOD: limit of detection, low.
Values in bold indicate levels exceeding safe limits.

In addition, the other two isolates of *F. equiseti* (BO3 and FV1) clustered with the reference sequence (Accession No. MK290391). Since BO3 and FV1 strains both originated from the region of Moka (Supplementary Table 2), their geographical proximity could explain their genetic relatedness. It is worth mentioning that the reference strain was previously associated with causing a relatively high disease incidence in maize in South India (Swamy *et al.*, 2020). Equally, the other reference isolate (Accession No. KP264661) was found to be a producer of trichothecene and ZEN (Stefańczyk *et al.*, 2016). Finally, one isolate of *F. graminearum* from pumpkin (GF1) clustered well with the reference sequence (Accession No. KP292811). The latter strain was reported to be pathogenic in potatoes in Poland and also can produce trichothecene and ZEN (Stefańczyk *et al.*, 2016). Hence, the isolates in this study are indeed a potential threat to the crops in Mauritius in terms of both their occurrence and ability to produce high levels of different mycotoxins.

The four recovered species of *Alternaria,* three *A. alternata* (P140, P170 and ME11) and one *A. solani* (G4), were observed to be clustering with the reference sequence (Accession No. KX118413). This strain was previously reported to be highly pathogenic and elaborated a high level of mycotoxins. Meena *et al.* (2017) said that this strain was highly toxigenic and elaborated all three mycotoxins (tenuazonic acid, AOH and alternariol monomethyl ether) produced by *Alternaria* spp. Hence, this could adversely affect the tomato plant and reduce the quality and safety of the edible fruits (Meena *et al.*, 2017). In addition, the isolate G4 (*A. solani*) was observed to be clustering with a very pathogenic and mycotoxigenic isolate from potato leaf (reference sequence, Accession No. KX139151), with significantly high level of mycotoxins (tenuazonic acid: 14.98 ± 1.43 μg/ml, AOH: 26.43 ± 4.52 μg/ml and alternariol monomethyl ether: 46.18 ± 4.12 μg/ml) (Meena *et al.*, 2017). Thus, this study indicates that the fungal isolates from vegetable crops of Mauritius also have the potential to elaborate a high level of mycotoxins.

Level of mycotoxin in the vegetable samples

Most infected vegetable samples tested for different mycotoxin levels had mean concentrations within safe limits (Table 2). However, garlic and onions had a mean level of CIT (5,448.55 μg/kg) and OT (9.25 μg/kg) higher than their respective safe limits. One garlic sample had a CIT level as high as 37,179.60 μg/kg. In a previous study in Egypt, some infected onion samples were found to have a mean concentration of 30,000 μg/kg of CIT, thereby also exceeding the tolerable level (Zohri *et al.*, 1992). Moreover, in this study, among the onion samples tested for OT, one onion sample had a higher value of 40 μg/kg compared to the safe limit of 5 μg/kg. Nevertheless, some previous studies reported that OT was below the limit of detection in infected onion samples collected from Belgium, Brazil, Egypt, India (van der Perre *et al.*, 2013) and Saudi Arabia (Gherbawy *et al.*, 2015).

In addition, in this study, the mean FUM level detected in potato samples was 740.14 μg/kg. Nevertheless, a relatively high level of FUM (5,531 μg/kg) was seen in a potato sample when compared with a safe limit of 4,000 μg/kg. In a past study conducted by El-Hassan *et al.* (2007) in Egypt, a concentration of 98 μg/kg of FUM was recorded in infected potato tubers collected. This was an acceptable level for human consumption. However, in another study, the level of FUM in sweet potatoes, another starchy tuber, was above the maximum tolerable limit (267.86 μg/kg) (CE, 2010; Amri and Lenoi, 2016). It is worth mentioning that there was no statistically significant difference in the FUM level for samples tested by ELISA and LC/MS-MS.

The mean values for the level of various mycotoxins in the different crops are presented in Table 3. It is to be noted that due to natural variability in the occurrence of mycotoxins in other samples, the values fell in a broad range, thus masking any statistically significant differences (Gibbs, 2013). Additionally, the average AOH and FUM concentrations indicated no significant differences

Table 3. The mean level of mycotoxin detected on the various vegetables tested over the study period.

	AFL	AOH	CIT	DON	FUM	OT	T-2/HT-2	ZEN
Garlic	1.3^{a}	—	5448.6^{a}	—	—	0.0045^{a}	—	—
Onion	2.0^{a}	—	455.5^{a}	15.5^{ab}	—	9.2^{a}	18.9^{ab}	40.6^{a}
Potato	—	1.0^{a}	—	4.3^{a}	740.1^{a}	—	98.7^{a}	0.3^{b}
Pumpkin	—	0.5^{a}	—	—	16.4^{a}	—	—	—
Tomato	—	1.2^{a}	—	18.8^{b}	533.3^{a}	—	12.8^{b}	1.6^{b}

*Different lowercase superscript letters in the same column for the same mycotoxin indicated significant differences among the different vegetables ($P < 0.05$)

($P > 0.05$) among the different crops analysed (potato, pumpkin and tomato). It is also worth mentioning that pumpkin samples tested for these two mycotoxins had a relatively lower level than the other two crops. This could be related to the fact that pumpkin crops have a very short supply chain in Mauritius and are not stored for an extended period. As a result, they are a low-risk commodity for mycotoxin contamination. Equally, pumpkin is the only crop which is not imported, and Mauritius is self-sufficient in the production of pumpkins (AMB, 2021).

Moreover, concerning T-2/HT-2, levels in potato samples were statistically higher than those of tomatoes ($P < 0.05$). As for DON, there was no significant difference ($P > 0.05$) in the level between potatoes and onions. The high level of DON and T-2/HT-2 in potatoes produced by *Fusarium* spp. could be related to the fact that potatoes are stored for an extended period in storage areas, which can be damp and humid. Moreover, since they are seasonal crops, potatoes and onions are imported from several countries to meet the local demand (FAREI, 2021; AMB, 2022). Similarly, in a past study, potato was reported to be more susceptible to infection by *Fusarium* spp. than other vegetables tested, such as chilli, pointed gourd, garlic and onion (Gupta *et al*., 2009). ZEN concentration in onion, potato and tomato samples showed a significant difference, with ZEN level in onions being statistically higher than in potatoes or tomatoes. In fact, similar to potato crops, onions are also stored longer in packhouses (FAREI, 2021; AMB, 2022), presenting ample opportunities for fungal growth and mycotoxin contamination.

High levels of mycotoxin in crops could be related to several factors, with climate and storage conditions during postharvest being the most important ones (Bryden, 2012). High temperatures and moisture are contributory factors for the growth of mycotoxigenic fungi (Lacetera *et al*., 2019). Mauritius, being a tropical island, unfortunately, faces highly humid conditions, which can enhance the growth of mycotoxigenic fungi on crops, thereby compromising the safety and quality of produce. (Fernández-Cruz *et al*., 2010). It is likely that climate change, characterised by higher air temperatures and erratic rainfall events in tropical regions, could exacerbate growth and mycotoxin production by certain species of pathogenic and mycotoxigenic fungi.

Moreover, mycotoxin contamination of crops can occur at any stage of production, including cultivation, harvesting, storage, processing, transport or retail (Rahmani *et al*., 2009; Dombrink-Kurtzman, 2008; Yang *et al*., 2014; Darwish *et al*., 2014; Afolabi *et al*., 2019). Hence, ensuring the safety of agricultural produce is more complex as it requires the concerted effort of all actors in the value chain.

Unlike other food hazards, mycotoxin contamination of food is also a global “one-health” concern (Alshannaq and Yu, 2017) that can simultaneously impact human, animal and environmental health. In this study, many of the vegetable samples collected had minor signs of fungal spoilage. They were either sold at reduced prices as vegetables of inferior quality to street food vendors or were processed and transformed into other products. Since mycotoxins are generally heat stable, they cannot be destroyed easily during thermal processing or cooking (Liu *et al*., 2020). In addition, chronic exposure to low levels of mycotoxins, albeit frequently undetectable, is responsible for chronic carcinogenicity in humans (Alshannaq and Yu, 2017).

As for vegetables which are unfit for sale due to fungal spoilage or other, they are often discarded or used as animal feed. Studies have documented that mycotoxins accumulated in food animals are not only a health threat for animals but also represent a food safety and public health issue through ingestion of potentially contaminated animal-derived food products (Alshannaq and Yu, 2017; Duchenne *et al*., 2021).

Conclusions

The presence of mycotoxins in our strategic crops (garlic, onion, potato, pumpkin and tomato) represents a significant threat to the food safety and security of Mauritius. The relatively high concentrations of citrinin, fumonisin and ochratoxin detected in some vegetable samples tested urge for awareness and continued surveillance of these products in the future. Moreover, our findings indicated that extended storage of produce such as potatoes and onions can increase the risk of harbouring a high level of mycotoxin. Therefore, this research highlights the paramount importance of carrying out further mycotoxin testing in the fresh produce chain to ensure the safety and health of consumers. Future work will shed light on temperature and humidity conditions prevailing in storage houses conducive to mycotoxin contamination of vegetables.

References

Adegoke G.O. and Letuma P. 2013. Strategies for the Prevention and Reduction of Mycotoxins in Developing Countries. Ch. 5. In “Mycotoxin and Food Safety in Developing Countries”. H. A. Makun (Ed.), IntechOpen Limited, United Kingdom.

Afolabi C.G., Ezekiel C.N., Ogunbiyi A.E., Oluwadairo O.J., Sulyok M. and Krska R. 2019. Fungi and mycotoxins in cowpea (*Vigna unguiculata* L) on Nigerian markets. Food Addit. Contam. Part B Surveill. 13(1): 52–58. https://doi.org/10.1080/19393210.2019.1690590

Alshannaq A. and Yu J.H. 2017. Occurrence, Toxicity, and Analysis of Major Mycotoxins in Food. Int. J. Environ. Res. Public Health. 14(6): 632. https://doi.org/10.3390/ijerph14060632

Agricultural Marketing Board (AMB). 2021. Local ware potato specification. [Internet]. [retrieved on 2021 Sep 12]. Available from: https://ambmauritius.mu/portofolio/potato/

Agricultural Marketing Board (AMB). 2022. Vegetables and Spices. [Internet]. [retrieved on 2021 Sep 12]. Available from: https://ambmauritius.mu/products/

Amri E. and Lenoi S. 2016. Aflatoxin and Fumonisin Contamination of Sun-Dried Sweet Potato (*Ipomoea batatas* L.) Chips in Kahama District, Tanzania. Appl. Environ. Microbiol. 4(3): 55–62.

Barkai-Golan R. and Pasteur N. 2008. “Mycotoxins in Fruits and Vegetables” 1st ed. Burlington, The USA. https://doi.org/10.3920/WMJ2008.x018

Bryden W.L. 2012. Mycotoxin contamination of the feed supply chain: Implications for animal productivity and feed security. Anim. Feed Sci. Technol. 173: 134–158. https://doi.org/10.1016/j.anifeedsci.2011.12.014

Cinar A. and Onbaşı E. 2019. “Mycotoxins The Hidden Danger in Foods Mycotoxins and Food Safety” IntechOpen, Boston, United States. https://doi.org/10.5772/intechopen.89001

Darwish W.S., Ikenaka Y., Nakayama S.M.M. and Ishizuka M. 2014. An Overview on Mycotoxin Contamination of Foods in Africa. Toxicology. 76(6): 789–797. https://doi.org/10.1292/jvms.13-0563

De Medeiros F.H.V., Martins S.J., Zucchi T.D., de Melo I.S., Batista L.R. and da Cruz Machado J. 2012. Biological Control of Mycotoxin-Producing Molds. Cienc. e Agrotecnologia. 36(5): 483–497. https://doi.org/10.1590/S1413-70542012000500001

Dombrink-Kurtzman M.A. 2008. Economic Aspects of Mycotoxins in Fruits and Vegetables. Ch. 2. In “Mycotoxins in Fruits and Vegetables”. R. Barkai-Golan and N. Paster (Ed.), p. 27. Academic Press, Elsevier, UK. https://doi.org/10.1016/B978-0-12-374126-4.00002-4

Duchenne R., Ranghoo-Sanmukhiya V.M. and Neetoo H. 2021. Impact of Climate Change and Climate Variability on Food Safety and Occurrence of Foodborne Diseases. Ch. 24. In “Food Security and Safety”. O. O. Babalola (Ed.), p. 461–462. Springer Nature, Switzerland AG.

El-Hassan K.I., El-Saman M.G., Mosa A.A. and Mostafa M.H. 2007. Variation among *Fusarium* spp. the Causal of Potato Tuber Dry Rot in their Pathogenicity and Mycotoxins Production. Egypt J. Phytopathol. 35(2): 53–68.

Eskola M., Kos G., Elliott C.T., Hajslova J., Mayar S., and Krska R. 2019. Worldwide contamination of food crops with mycotoxins: Validity of the widely cited ‘FAO estimate’ of 25%. Crit. Rev. Food Sci. Nutr. 1–17.

European Commission (EC). 2006. Commission Recommendation 2006/576/EC of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding. Off. J. L. 229:7–9.

Commission Regulation (EU). 2006. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Off. J. L. 364: 5–24.

Commission Regulation (EU). 2010. No 165/2010 of 26/02/2010. Official Journal of the European Union. (50/8): 8–12.

Commission Recommendation (EU). 2013. No 2013/165/EU of 27 March 2013 on the presence of T-2 and HT-2 toxin in cereals and cereal products. Off. J. L. 91: 12–15.

Fernández-Cruz M.L., Marcia L.M. and Tadeo J.L. 2010. Mycotoxins in fruits and their processed products: Analysis, occurrence and health implications. J. Adv. Res. 1:113–122. https://doi.org/10.1016/j.jare.2010.03.002

Food and Agricultural Research and Extension Institute (FAREI). 2021. Le Guide Agricole. [Internet]. [retrieved on 2022 May 05]. Available from: http://farei.mu/apmis/publications/guide/guide-interactif/main.htm

Food Regulations. 1999. GN 173/1999. Regulations made by the ministry under Section 18 of the Food Act 1998.

Gherbawy Y., Elhariry H., Kocsube S., Bahobial A., El Deeb B., Alralhi A., Varga J. and Vagvolgyi C. 2015. Molecular Characterization of Black *Aspergillus* Species from Onion and Their Potential for Ochratoxin A and Fumonisin B2 Production. Foodborne Pathog. Dis. 0(0): 1–10.

Gibbs N.M. 2013. Errors in the interpretation of ‘no statistically significant difference. Anaesth Intensive Care. 41:151–153. https://doi.org/10.1177/0310057X1304100203

Guo C., Liu Y., Jiang Y., Li R., Pang M., Liu Y. and Dong J. 2016. *Fusarium* species identification and fumonisin production in maize kernels from Shandong Province, China, from 2012 to 2014. Food Addit Contam Part B Surveill. 9(3):203–209. https://doi.org/10.1080/19393210.2016.1175515

Gupta S., Aggarwal R. and Ahluwalia P. 2009. Assessing the mycotoxin producing potential of pathogens causing vegetable rots. Indian Phytopathol. 62(2):137–143.

Jésus A.A., Beaulys F. and Adolphe Z. 2020. Antifungal activities of endophytic fungi isolated from plantain tissues on *Fusarium solani* a potential pathogen. Nat. Microbiol. 1(3):96–106.

Jeswel P. and Kumar D. 2016. Mycotoxins and their Producing Fungi from Spices of Bihar (India). Int. J. Biotech. Biomed. Sci. 2(2):174–177.

Ji X., Deng T., Xiao Y., Jin C., Lyu W., Wu Z., Wang W., Wang X., He Q. and Yang H. 2023. Emerging *Alternaria* and *Fusarium* mycotoxins in tomatoes and derived tomato products from the China market: Occurrence, methods of determination, and risk evaluation. Food Control. 145:109464. https://doi.org/10.1016/j.foodcont.2022.109464

Kifer D., Sulyok M., Jaksic D., Krska, R. and Klaric M.S. 2021. Fungi and their metabolites in grain from individual households in Croatia. Food Addit Contam Part B Surveill. 14(2):98–109.

Lacetera N. 2019. Impact of climate change on animal health and welfare. Anim. Front. 9(1): 26–31. https://doi.org/10.1093/af/vfy030

Liu Y., Yamdeu J.H.G., Gong Y.Y. and Orfila C. 2020. A review of postharvest approaches to reduce fungal and mycotoxin contamination of foods. Compr. Rev. Food Sci. Food Saf. 1–40.

Luo S., Du H., Kebede H., Liu Y. and Xing F. 2021. Contamination status of major mycotoxins in agricultural product and food stuff in Europe. Food Control. 125:108120.

Madarbokus S. and Ranghoo-Sanmukhiya V.M. 2012. Identification of Genetic Diversity among Papaya Varieties in Mauritius using Morphological and Molecular Markers. Int. J. Life Sci. Biotechnol. Pharma. Res. 1(4).

Meena M., Swapnil P. and Upadhyay R.S. 2017. Isolation, characterization and toxicological potential of *Alternaria*-mycotoxins (TeA, AOH and AME) in different *Alternaria* species from various regions of India. Sci. Rep. 7:8777.

Murugan L., Krishnan N., Venkataravanappa V., Saha S., Mishra A.K., Sharma B.K. and Rai A.B. 2020. Molecular characterization and race identification of *Fusarium oxysporum* f. sp. *lycopersici* infecting tomato in India. Biotechnol. 10:486. https://doi.org/10.1007/s13205-020-02475-z

Nirmaladevi D., Venkataramana M., Srivastava R.K., Uppalapati S.R., Gupta V.K., Yli Mattila T., Clement Tsui K.M., Srinivas C., Niranjana S.R. and Chandra N.S. 2016. Molecular phylogeny, pathogenicity and toxigenicity of *Fusarium oxysporum* f. sp. *lycopersici.* Sci. Rep. 6:21367. https://doi.org/10.1038/srep21367

Palou L. 2014. *Penicillium digitatum*, *Penicillium italicum* (Green Mold, Blue Mold). Ch. 2. In "Postharvest Decay Control Strategies". S. Bautista-Baños (Ed.), p. 45. Academic Press, Elsevier, UK. https://doi.org/10.1016/B978-0-12-411552-1.00002-8

Plascencia-Jatomea M., Yépiz-Gómez M.S., Velez-Haro J.M. 2014. *Aspergillus* spp. (Black Mould). Ch. 8. In "Postharvest Decay Control Strategies". S. Bautista-Baños (Ed.), p. 267. Academic Press, Elsevier, UK.

Rahmani A., Jinap S. and Soleimany F. 2009. Qualitative and Quantitative Analysis of Mycotoxins. Compr. Rev. Food Sci. Food Saf. 8: 202–251. https://doi.org/10.1111/j.1541-4337.2009.00079.x

Ristaino J.B., Madritch M., Trout C.L. and Parra G. 1998. PCR amplification of ribosomal DNA for species identification in the plant pathogen genus *Phytophthora*. Appl. Environ. Microbiol. 64(3):948–954. https://doi.org/10.1128/AEM.64.3.948-954.1998

Şengül Ü., Yalçın E., Şengül B. and Çavuşoğlu K. 2016. Investigation of aflatoxin contamination in maize flour consumed in Giresun, Turkey. Qual. Assur. Saf. Crop. Foods. 8(3):385–391.

Stefańczyk E., Sobkowiak S., Brylińska M. and Śliwka J. 2016. Diversity of *Fusarium* spp. associated with dry rot of potato tubers in Poland. Eur. J. Plant Pathol. 145:871–884. https://doi.org/10.1007/s10658-016-0875-0

Swamy S.D., Mahadevakumar S., Hemareddy H.B., Amruthesh K.N., Mamatha S., Kunjeti S.G., Swapnil R., Vasantha Kumar T. and Lakshmidevi N. 2020. First report of *Fusarium equiseti*–the incitant of post flowering stalkrot of maize (*Zea mays* L.) in India. Crop Prot. 129:105035. https://doi.org/10.1016/j.cropro.2019.105035

Takooree S.D., Neetoo H., Ranghoo-Sanmukhiya M., van der Waals J., Vojvodić M. and Bulajić A. 2021. First report of charcoal rot caused by *Macrophomina phaseolina* on potato tubers in Mauritius. Plant Dis. 105(9):2721. https://doi.org/10.1094/PDIS-02-21-0258-PDN

Takooree S.D., Neetoo H., Ranghoo-Sanmukhiya V.M., Vally V., Bulajić A., van der Waals J. 2022. A comparison of methods for the detection of *Phytophthora infestans* on potatoes in Mauritius. J. Agric. Sci. (Belgr.). 67(2):203–217. https://doi.org/10.2298/JAS2202203T

Tamura K., Stecher G., Peterson D., Filipski A. and Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Mol. Biol. Evol. 30:2725–2729. https://doi.org/10.1093/molbev/mst197

Thompson J.D., Higgins D.G. and Gibson T.J. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22:4673–4680. https://doi.org/10.1093/nar/22.22.4673

Thrane U. 1999. *Fusarium*. Academic Press. https://doi.org/10.1006/rwfm.1999.0725

Troncoso-Rojas R. and Tiznado-Hernández M.E. 2014. *Alternaria alternata* (Black Rot, Black Spot). Postharvest Decay. 147–187. https://doi.org/10.1016/B978-0-12-411552-1.00005-3

Van de Perre E., Deschuyffeleer N., Jacxsens L., Vekeman F., van der Hauwaert W., Asam S., Rychlik M., Devlieghere F. and de Meulenaer B. 2013. Screening of moulds and mycotoxins in tomatoes, bell peppers, onions, soft red fruits and derived tomato products. Food Control. 37(1):165–170. https://doi.org/10.1016/j.foodcont.2013.09.034

Van Egmond H.P. and Jonker M.A. 2008. Worldwide Regulations on Aflatoxins-The Situation in 2002. J. Toxicol. Toxin Rev. 23(2–3), 273–293.

WHO. 2018. Mycotoxins. [Internet]. [retrieved on 2023 Sep 08]. Available from: https://www.who.int/news-room/fact-sheets/detail/mycotoxins

Woudenberg J.H.C., Groenewald J.Z., Binder M. and Crous P.W. 2013. *Alternaria* redefined. Stud. Mycol. 75:171–212.

Yang J., Li Y., Jiang Y., Duan X., Qu H., Yang B., Chen F. and Sivakumar D. 2014. Natural Occurrence, Analysis, and Prevention of Mycotoxins in Fruits and their Processed Products. Crit. Rev. Food Sci. Nutr. 54(1): 64–83. https://doi.org/10.1080/10408398.2011.569860

Youssef N.H. and Sabra M.A. 2022. Impact of *Bacillus thuringiensis* on inhibiting certain *Alternaria alternata's* mycotoxins isolated from infected potatoes. Malays. J. Microbiol. 18(2):163–169.

Zohri A.A., Saber S.M and Abdel-Gawad K.M. 1992. Fungal Flora and Mycotoxins Associated with Onion (*Allium cepa* L.) in Egypt. Korean Mycol. 20:302–308.

Supplementary

Table S1. **List of mycotoxigenic fungal strains used as reference sequences with their accession number.**

Mycotoxigenic fungal species used as reference strains	Accession Number	References
Fusarium oxysporum	KP719138	Stefańczyk *et al.*, 2016
	KC478622	Murugan *et al.*, 2020
Fusarium equiseti	MK290391	Swamy *et al.*, 2020
	KP264661	Stefańczyk *et al.*, 2016
Fusarium graminearum	KP292811	Stefańczyk *et al.*, 2016
Alternaria solani	KX139151	Meena *et al.*, 2017
Alternaria alternata	KX118413	Meena *et al.*, 2017
Outgroup	**Accession Number**	
Fusarium nygamai	AY898252	Jésus *et al.*, 2020
Alternaria gypsophilae	KC584199	Woudenberg *et al.*, 2013

Table S2. **Details pertaining to the isolates obtained from the four investigated crops (potato, tomato, onion and pumpkin).**

Sampling Location	Host	Code	Identity of fungal isolates	Accession number
Medine	Potato	P2	*Fusarium oxysporum*	ON738590
Vacoas	Potato	P124	*Fusarium oxysporum*	ON738660
Plaine Sophie	Potato	P198	*Fusarium oxysporum*	ON738604
Moka	Onion	G11	*Fusarium oxysporum*	ON738593
Cluny	Tomato	T1	*Fusarium oxysporum*	OM980720
Moka	Tomato	BO3	*Fusarium equiseti*	ON738587
Moka	Pumpkin	FV1	*Fusarium equiseti*	ON738582
Cluny	Pumpkin	GF1	*Fusarium graminearum*	ON738580
Belle Vue	Potato	P140	*Alternaria alternata*	ON738597
Moka	Potato	P170	*Alternaria alternata*	ON738659
Medine	Tomato	ME11	*Alternaria alternata*	OM980727
Medine	Tomato	G4	*Alternaria solani*	OM980732