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Mycotoxic effects of entomopathogenic fungi of fall armyworm *(Spodoptera frugiperda* J.E. Smith) on poultry feed safety

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Abstract

This study was carried out to analyze mycotoxins of entomopathogenic fungi of fall armyworm (*Spodoptera frugiperda* J.E. Smith) and poultry feed safety. An experiment was set up to assess the types of mycotoxins produced by entomopathogenic fungal parasite of fall armyworm larvae and their subsequent effect on the safety of the larvae as a feed ingredient. Molecular characterization was done to estimate the diversity of entomopathogenic fungi on fall armyworm larvae specimens from the treatment plots. Sequenced data was analyzed and processed using Molecular Evolutionary Genetic Analysis 6.0 software. The results showed relative diversity of fall armyworm larvae with 11 species isolated belonging to *Aspergillus*, *Penicillium*, *Fusarium*, *Trichoderma*, *Bipolaris* and *Irpex* genus. Some of these are potential mycotoxin producing fungi. Although isolated fungi potentially produce Ochratoxin, Fumonisin, Zearalenone and Trichothecene mycotoxins, only aflatoxins were analyzed in this study. About 3.98 µg /kg of aflatoxin was observed using the ELISA total assay which is within the threshold toxicity levels set in Kenya for feed of about 20 µg /kg. While this is under the threshold set by Kenya, it is still enough to cause concern as the cumulative exposure of even low doses can have impacts. This study therefore concludes that, *Spodoptera frugiperda* can be potentially contaminated with aflatoxins and when formulating poultry diets, there is need to monitor production so that the quality is not compromised and feed safety is ensured. Further studies are recommended to determine how much produced aflatoxins are then transferred into the poultry products such as eggs and meat.

Keywords Entomopathogenic, Mycotoxins, Fungi, Poultry, Safety, Feed, Aflatoxins

Introduction

Fall armyworm (*S. frugiperda*) can cause significant economic losses in maize production (Yan et al. 2022), especially due to its strong dispersal ability of about 500 km before oviposition. Extensive destruction of crops by its larvae are a cause of global concern (Nwanze et al. 2021). As a pest, *S. frugiperda* can cause significant economic losses in the production of maize and other cereals (Idrees et al. 2022; Basimile et al. 2023). However, its larvae can be collected in infested fields where EPF are applied as biocontrol method and used as a protein-rich source of nutrition for poultry that also has low environmental impact as the larvae require low amounts of land and water. Striking a balance between the two is necessary for sustainability of both the crop and poultry sectors. However, this is not the case, because managing *S. frugiperda* as a pest of agricultural crops is getting more attention over its use as a potential feed source.

The FAW outbreak has also resulted in attempts being made to integrate *EPF* into diverse cropping

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systems for FAW management in different areas (Otim et al. 2021). These cropping systems contributed significantly in either reducing the pest attack and or enhancing natural enemies. Tillage system is one of attempted crop management practice that has been practiced. Findings in Zimbabwe and Zambia showed that maize farmed under minimum or no-tillage significantly reduced FAW infestation on maize (Matova et al. 2020). Intercropping by smallholder farmers in Sub-Saharan Africa is reported to reduce FAW pest populations and enhance the potential entomopathogens (McGrath et al. 2018; Babendreier et al. 2020). However, further studies are required to determine the mechanism by which crop management practices reduce damage caused by the FAW and enhances natural enemy diversity (Otim et al. 2021).

Efforts to control the pest have included chemical, cultural, botanical and biological methods (Kumela et al. 2019). Synthetic chemicals are the most commonly used method despite being hazardous to people and the environment (Idrees et al. 2021). Biological control is potentially the most economically viable, technically feasible, environmentally sustainable, and socially acceptable pest management strategy for fall armyworm (FAW) (Assefa and Ayalew 2019; Yan et al. 2021). The potential of entomopathogenic fungi as bio-control agents for managing fall armyworm in agricultural fields is receiving much attention due to their specificity (Uma Devi et al. 2008; Rajula et al. 2021).

At least 16 species of entomopathogens, including viruses, fungi, protozoa, bacteria, and nematodes, have been identified to be virulent to different species of FAW (Assefa and Ayalew 2019). Fungi species identified to be virulent against FAW larvae include *Metarhizium anispliae* (Idrees et al. 2023) *Beauveria bassiana* (Idrees et al. 2021; Yan et al. 2021) and *Nomuraea rileyi* among others. The entomopathogenic fungi also have potential to produce mycotoxins and antimicrobial compounds (Litwin et al. 2020) among other contaminants that can affect its suitability as potential poultry feed ingredient. Some of the mycotoxins potentially produced by the EPF such as aflatoxins can be potentially carried to poultry products thus posing a health hazard. This study focused on aflatoxins, potentially produced by EPF and a concern to feed and food safety. Given this background, controlling FAW larvae with entomopathogenic fungi (EPF) may impact other non-targeted production systems, in this case its potential as a feed supplement.

A field factorial experiment was established for this research in Siaya County of Kenya because, it is one of the Counties that has been affected by FAW pest problem. In Siaya, maize is grown for subsistence, alongside pearl millet and sorghum. While droughts have affected most people, the effects are exacerbated by outbreak of pests such as fall armyworm and locusts.

This study's focus was to assess the type and levels of mycotoxins in EPF -infected FAW larvae, that could be used as a potential poultry feed ingredient. To determine the safety of FAW larvae-based feeds, mycotoxins caused by entomopathogenic fungi activity were identified. The assumption is that *S. frugiperda* -based diet like other insect-based diets is an environment-friendly source of protein with a more nutritious amino acid profile. In addition, it is also cost effective. This study therefore has a great potential for improved poultry production through enhanced food security.

Materials and methods

Study area

The study was conducted in Siaya County in Kenya. Siaya is one of the areas which have been affected by droughts. While droughts affect most people, the effects are exacerbated by outbreak of pests such as fall armyworm and locusts. This has further affected people's vulnerability to climate change by limiting their social and financial options for adaptation (Census 2019). This study aimed at assessing the occurrence of mycotoxin in native entomopathogenic fungi of fall armyworm and determining how much are mycotoxin levels of FAW larvae's EPF on poultry feed safety.

Experimental design

The study was conducted using a factorial experimental design. A 40 m by 30 m plot with subplots was set up with each plot measuring 6 m by 5 m (Fig. 1). Each plot was separated by 2 m and *Brachiaria CV Mulato* planted in the border space as recommended by the ICIPE's fall armyworm push pull technology (Midega et al. 2018). The barriers were to act as repellents to prevent FAW migration to adjacent plots. The plots were placed in a randomized complete block design (RCBD) (Fig. 1) to account for spatial effects in the plots, eliminating the selection bias and balancing the plots with respect to known and unknown confounding variables. Three crop management practices (Intercropping, weed manipulation and organic manure application) were used as factors in the field experiment at 2 levels and replicated 4 times, totalling to 24 sub plots as shown by Fig. 1. This research incorporated different crop management conditions under a field experiment so as to mimic the widely applied management options and determine the native EPF and its diversity. The Hybrid seed maize variety (DH 04) was used, being drought resistant and recommended by agronomists for Siaya County areas.

The FAW larvae physically infested to the plots at the vegetative stage about 2 weeks after germination

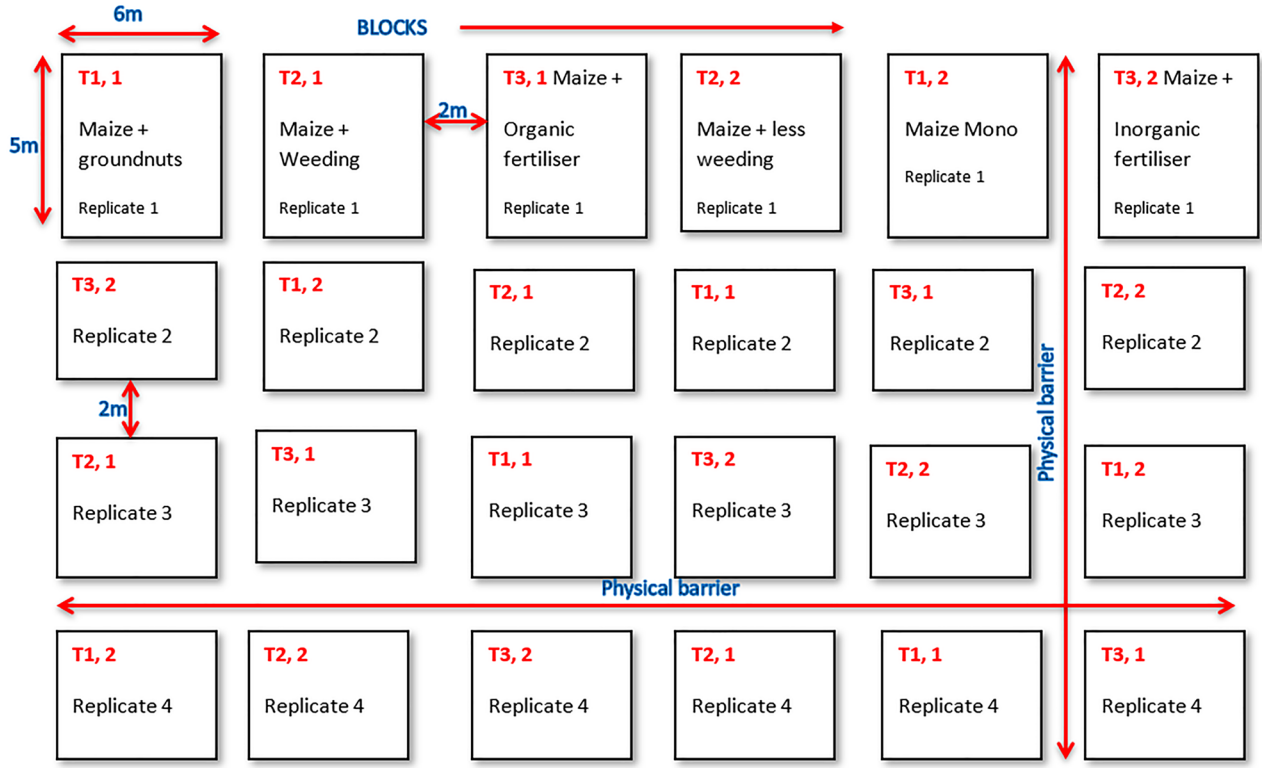

Fig. 1 Field experimental design setup

of the maize crop. The FAW larvae were then collected at week 3, 4 and 5 after germination of the maize for analysis, around the late 2nd instar and early 3rd instar of the larvae development. Each sample collected was labelled as per plot and kept in the microbiology laboratory for further analysis. Abundance of EPF of FAW was then assessed against each maize management practice through morphological and molecular analysis.

Field experimental design setup

Key:

- **T1**: Intercropping vs Monocropping ((McGrath et al. 2018; Babendreier et al. 2020)
- **T2**: Weed manipulation (Kansiime et al. 2019)
- **T3**: Organic fertiliser vs inorganic fertiliser (Otim et al. 2021; Jabbour and Barbercheck 2009)
- **T1,1:** Treatment 1 replicate 1
- **Physical barrier**: *Brachiaria CV Mulato*

Sampling and data collection

Scouting

The specimen collection was done during the vegetative growth stage of the maize. This is because spot infestations and hotspots in the field can be seen at this stage. Previous day rainfall was recorded because heavy rain showers may kill the 1st, 2nd and 3rd instar larvae causing a confounding effect on entomopathogenic activity (FAO, 2018).

Scouting of the field was done through serpentine visual assessment and sampling at five different locations within each sub-plot as shown in Fig. 2 so as to observe any signs of FAW infestation. At each of these locations 10–20 plants were assessed for signs of FAW damage as

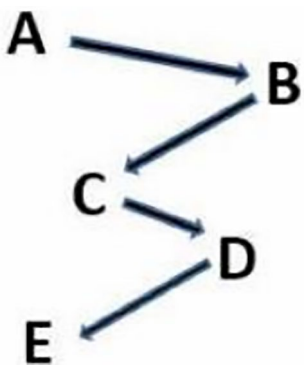

Fig. 2 Sample scouting within each maize sub-plot

recommended by FAO (2018). Signs of infested whorls including fresh window panes, FAW larvae, fresh faecal matter (frass), and fresh whorl-feeding-damage were observed so as to determine the timing of sample collection.

Collection of FAW larvae specimens

Once the FAW infestation was observed in the plots, All the larvae were then collected from all the 24 established plots at the late 2nd and early 3rd instar for laboratory assessments. The specimens were kept at JOOUST Microbiology laboratory for further analysis.

Sample processing

FAW samples collected were labelled according to their specific sub-plot. Live samples were processed according to Kurdi et al. 2021 where larvae were first frozen for 5 min to immobilize them. Five grams of larvae sample was weighed and homogenized in 5 ml 0.9% sterile saline solution using mortar and pestle. The samples were serially diluted in sterile saline solution up to 10^{-5} dilution and used for isolation of fungi.

Isolation of fungi from the FAW larvae specimens

Fungi species were isolated on potato dextrose agar (PDA) (60×10 mm) lined with filter paper moistened with sterile distilled water for the fungus to sporulate on the insect (Ruiz-Nájera et al. 2013). For fungal isolation, PDA was mixed with streptomycin to inhibit the growth of bacteria. One millilitre from the last three dilutions was mixed separately with media on culture plates and left to set while sterile water was used as control in triplicates. Plates were sealed with parafilm at 28 ℃ for 72 h for isolation of fungi. All plates were arranged in a completely randomized design (Ruiz-Nájera et al. 2013; Jaber et al. 2016). In terms of the isolated fungi, of the 24 samplesof FAW collected from the sub-plots, only 19 entries had fungi.

Sub culturing and purification of fungi isolates

All fungal isolates from the FAW larvae specimens were purified through the single spore isolation technique (Acharya and Hare 2022). This technique was carried out by streaking the mycelia on water agar in a zigzag manner. Once germinated, a single conidium was transferred onto potato dextrose agar (PDA) plates for further studies. For preservation purposes, the single conidial isolates were maintained in distilled water with mild agar broth. Purification of fungal isolates was carried out at least twice on PDA containing 0.02% (w/v) chloramphenicol. The cultures from this study were stored in the fridge at 4 ℃ for later use in the microbiology laboratory at JOOUST.

Molecular characterization

Genomic DNA extraction

Pure fungal cultures derived from the single spores incubated in PDA were used. Genomic DNA was extracted using the Zymo Research DNA Mini $Prep_{TM}$ kit according to the manufacturer's specifications (Zymo Research Corp, South Africa). The concentration and purity of extracted DNA was estimated using a $Nanodrop_{TM}$ Lite Spectrophotometer (Thermo Scientific Inc, USA) at 260–280 nm and by horizontal gel electrophoresis (Thistle Scientific Ltd, USA) on a 0.8% (w/v) agarose gel at 100 V for 30 min and visualized under UV after staining with Gel Red_{TM} (Thermo Scientific, USA) (Emitaro et al. 2017).

PCR amplification and sequencing

Universal primers ITS1 5′ TCCGTAGGTGAACCTGCG G 3′ and ITS4 5′ TCCTCCGCTTATTGATATGC 3′ (Sigma Aldrich) were used to amplify the fungal intergenic spacer ITS1 and ITS4 region (Chowdhary and Kaushik 2015; Ofek-Lalzar et al. 2016). Bioneer Accu Power® PCR Premix (BioneerInc, USA) was used to perform PCR. To each 20 µl Bioneer reaction tube, 2 µl DNA, 2 µl Taq buffer, 1.4 µl Mgcl2, dNTPs 0.4 µl, Primers 2 µl, Taq DNA Polymerase 0.4 µl, Nuclease free water 11.8 µl was added. Amplification was performed in a programmable Master thermocycler (C1000-Bio Rad, USA). The PCR conditions included denaturation, annealing, initial and final extension at temperatures of 94 ℃ for 30 s, 55 ℃ for 1 min, 72 ℃ for 2 min respectively before cooling off at 15 ℃. PCR products were separated by horizontal gel electrophoresis on 1.5% (w/v) agarose gel at 100 V for 45 min and visualized under UV after staining with 2 µl Gel RedTM (Thermo Scientific).

The quality of amplified PCR products recovered was assessed in horizontal gel electrophoresis on 1.5% (w/v) agarose gel at 100 V for 45 min and visualized under UV after staining with 2 µl Gel RedTM (Thermo Scientific). The PCR products were then sent to Macrogen Europe B.V. (Meibergdreef 311105 AZ, Amsterdam, Netherlands) for purification and sequencing. Forward and reverse sequences were assembled and trimmed on Geneious Prime® 2020.0.4 and submitted to NCBI BLAST (www.ncbi.blast.lnm.nih.gov) to obtain the accession numbers of the isolates and similarity search.

Mycotoxin analysis

Sample preparation

Target mycotoxin was aflatoxin, which is a concern to feed and food safety, is potentially produced by EPF and is a family of toxins produced by fungi found on agricultural crops such as maize. The HELICA Total Aflatoxin Assay method was used, this is a competitive enzyme-linked immunoassay (ELISA) intended

for the quantitative detection of food and feed samples (Nishimwe et al. 2019). These aforementioned aflatoxins are a subset of mycotoxins formed by fungi most commonly from *Aspergillus* genus. The mycotoxin analysis was carried out at Egerton University Microbiology laboratory.

Extraction procedure

Extraction was done according to the HELICA manual instructions with the following steps. FAW larvae were divided into 2 equal parts measuring 20 *g* and ground to the particle size of fine instant coffee (95% passes through a 20' mesh screen). Secondly, an extraction solution was prepared with 70% methanol by adding 30 ml of distilled or deionized water to 70 mlL of methanol for each sample to be tested. Then, the 100 ml of the Extraction Solvent was added to the 20 *g* portion of the FAW sample with the sample to extraction solvent 1:5 (w/v). The particulate matter was then mixed by shaking in a sealed container for 2 min and allowed to settle. 10 ml of the extract was filtered through Whatman number 1 filter paper and the filtrate was collected.

Assay procedure

All the reagents were brought to room temperature before use. The PBS-Tween was reconstituted into a packet by washing out the contents with a gentle stream of distilled water into a 1-Liter container. One mixing well was placed in a microwell holder for each Standard and Sample to be tested with an equal number of Antibody Coated Microliter Wells in another microwell holder. 200 µl of the aflatoxin-HRP Conjugate was dispensed into each mixing well. Using a new pipette tip for each, 100 µl of each Standard and Sample was added to appropriate mixing well containing Conjugate. Furthermore, it was mixed by priming the pipettor at least 3 times and the location of each Standard and Sample was determined throughout test.

Then, 100 µl of content from each mixing well was transferred to a corresponding Antibody Coated Microtiter Well using a new pipette tip for each and incubated at room temperature for 15 min. The contents from microwells were poured into a discard basin and microwells washed by filling each PBS-Tween wash buffer, then decanting the buffer into a discard basin. This wash was repeated for a total of 5 washes.

Afterwards, the microwells were tapped face down on a layer of absorbent towels to remove residual buffer. 120 µl/well of substrate reagent was measured and placed in a separate container. 100 µl was then added to each microwell, incubated at room temperature for 5 min and covered to avoid direct light. 120 µl/well of Stop Solution was then measured and placed in a separate container. 100 µl was finally added in the same sequence and at the same pace as the Substrate Reagent was added. The optical density (OD) of each microwell was read with a microliter plate reader using a 450 nm filter and recorded (Procedure done according to HELICA manual instructions).

Results

Molecular characterization of FAW larval specimen

The molecular weight of the genomic DNA of fungal isolates was confirmed on 0.8% agarose stained with SYBR green. Intense bands of the fungal DNA (Fig. 3) had equal molecular weight of 700 bp along with the 100 bp DNA ladder. Figure 3 shows Gel image of ITS rRNA amplified amplicon. Fungal isolate rDNA amplified at ITS rRNA region and run on agarose gel are labelled 1-19. Amplified rDNA from all the fungal isolates had almost the same molecular weight of 700 bp and aligned at 700 bp portion of the DNA ladder as clear bands.

On sequencing ITS rDNA gene for fungi, sequences of the isolates were different in the arrangement of the nucleotide base pairs. Fungal sequences submitted to National Centre of Biotechnology Information (NCBI) for similarity search showed ˃99% match identity to those already deposited in the Genebank database. The isolate sequences were deposited to NCBI GeneBank and given accession numbers ranging from ON245046.1 to ON245064.1. Based on Basic Alignment Search Tool (BLAST) searches, fungal isolates were found to belong to five orders with the dominant order being Pleosporales. Nutritionally, the Pleosporales order includes saprotrophs, parasites and pathogens and is also known to be septate. The species in this order produce 71 known mycotoxins and phytotoxins. Overall, 11 species were found in this study with more *Trichoderma* species (16%, n = 3) (Table 1). Other species which were common are; *Curvularia*, *Bipolaris*, *Pithomyces* (11%, n = 2) and least species (*Aspergillus* and *Talaromyces*) (5.2%, n = 1).

Evolutionary relationships of fungal isolates

The evolutionary profile of the fungal endophytes isolated from FAW larval specimen conducted in Molecular Evolutionary Genetic Analysis (MEGA 6.0) software clustered the isolates into three (Matova et al. 2020) clades (Fig. 4). Clade one comprised of orders Eurotiales and Hypocreales, clade two had one order Pleosporales while clade three had two orders Polyporales and Agaricales.

Out of the 19 sequences analyzed, 10 clustered in Clade I with 100% bootstrap support. Clade II had six isolates in the order Pleosporales divided into two with 100% bootstrap support. Clade III had three isolates belonging to the orders Polyporales and Agaricales with 100% bootstrap support (Fig. 4). Evolutionarily, members of

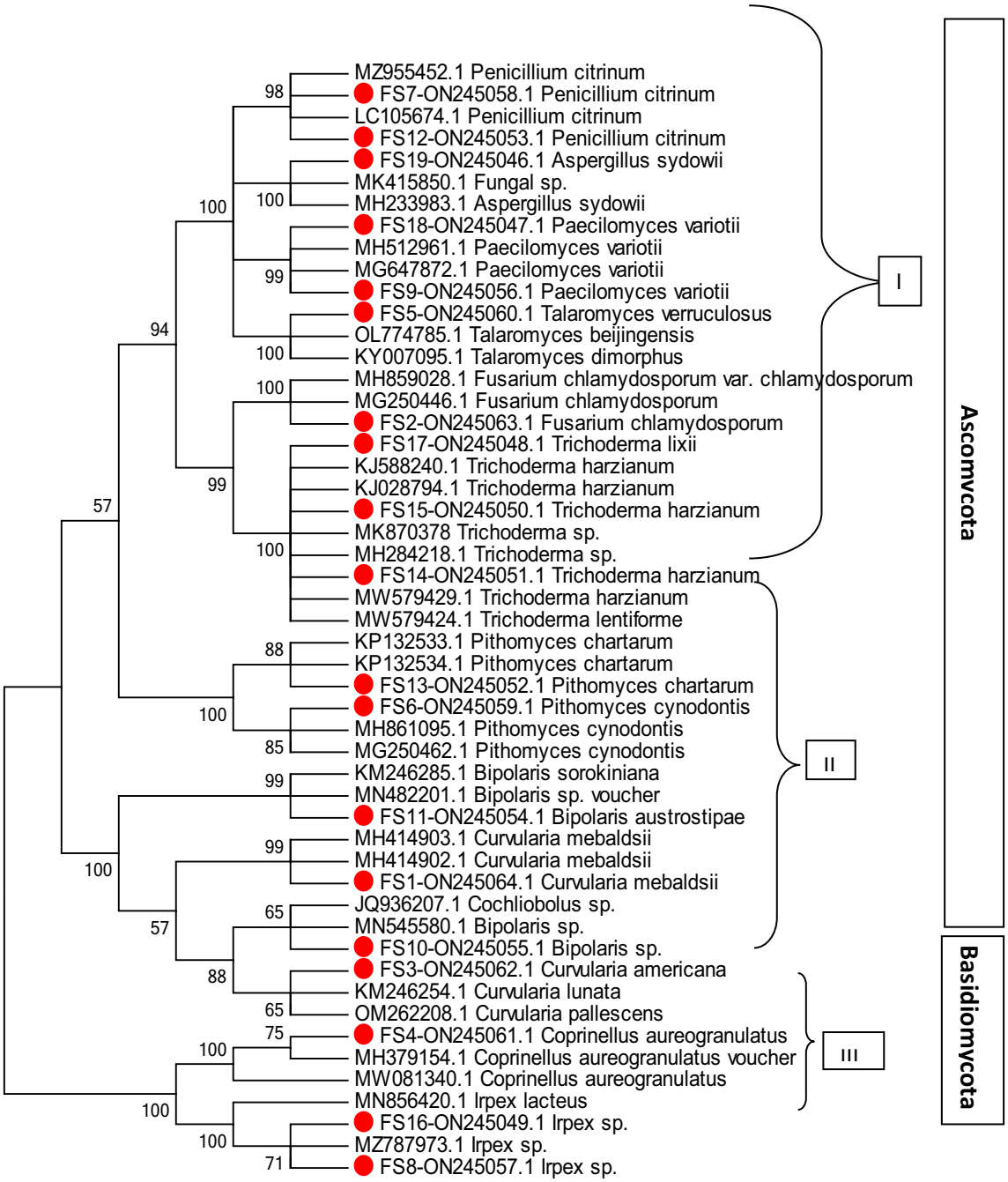

Fig. 3 Gel image of ITS rRNA amplified amplicon

order Eurotiale, Hypocreales and Pleosporales belong to the phylum Ascomycota while members of the order Polyporales and Agaricales belong to the phylum Basidiomycota. Majority of the isolates were from phylum Ascomycota while a few were from phylum Zygomycota. Ascomycota is important for this study because they are responsible for most of pathogenic fungal diseases and represent the group with largest number of fungi species. Species of microscopic filamentous fungi belonging to several genera in the phylum Ascomycota, primarily *Fusarium*, *Aspergillus* and *Penicillium* often produce mycotoxins which are of concern to food or feed safety.

The phylogenetic tree was based on ITS rDNA gene sequence. The ones marked in red circles represent fungal isolates obtained from this study. Clade I-III shows how the sequences of the fungal isolate clustered in

Table 1 Fungal Species Isolated

Species	Number (n)	%
Curvularia	2	11%
Bipolaris sp.	2	11%
Pithomyces	2	11%
Fusarium	1	5.2%
Trichoderma	3	16%
Talaromyces	1	5.2%
Penicillium	2	11%
Paecilomyces	2	11%
Aspergillus	1	5.2%
Irpex sp.	2	11%
Coprinellus	1	5.2%

Table 2 ELISA total aflatoxin detected

Standard	Optical Density	1/Optical density	Quantity
0	1.101	0.91	0
1	1.232	0.81	1
/2.5	1.063	0.94	2.5
5	0.772	1.30	5
10	0.492	2.03	10
20	0.307	3.26	20
A1	0.74	1.35	4.47
B1	0.818	1.22	3.51
Total quantity			7.97
Average			**3.99**

relation to their similarity. Fungi clustering in the same clade have highly similar sequences and are closely related.

Aflatoxin analysis from fungal isolates

The mycotoxin was assessed using the ELISA total aflatoxin assay. Table 2: ELISA total aflatoxin detected Table 2 depicts the standard performed against the optical density values recorded. An increase in the standard concentration improved the recovery percentage across aflatoxins due to fluctuations in optical density (OD) values at the upper limit of detection (Table 2). The samples had a average total quantity aflatoxin of 3.98 µg /kg.

Although aflatoxins were targeted in this study, there could be concurrent contamination by more than one mycotoxin. Some potentially produced mycotoxins which can be found in the isolated fungi species highlighted in Table 3 below.

Discussion

Abundance of EPF of FAW species

For the precise identification of entomopathogenic fungi, a DNA-based molecular characterisation is an effective approach (Idrees et al. 2021; Minarni et al. 2021; Butt et al. 2016), so regarding this study, isolates were genetically characterized using rDNA-ITS regions. The EPF isolates found in this study had an rDNA sequence similarity match of more than 99% to the reference species (BLAST), meaning that the isolates had a high phylogenetic relationship and were from the same species. These results are consistent with the findings of Ha Bich and Nguyen 2021 who stated that ribosomal DNA sequences are used to determine the phylogenetic relationships of organisms to taxa species. Some studies on molecular characterisation have shown that; based on the similarity value, 100% means that the isolates are the same strain,

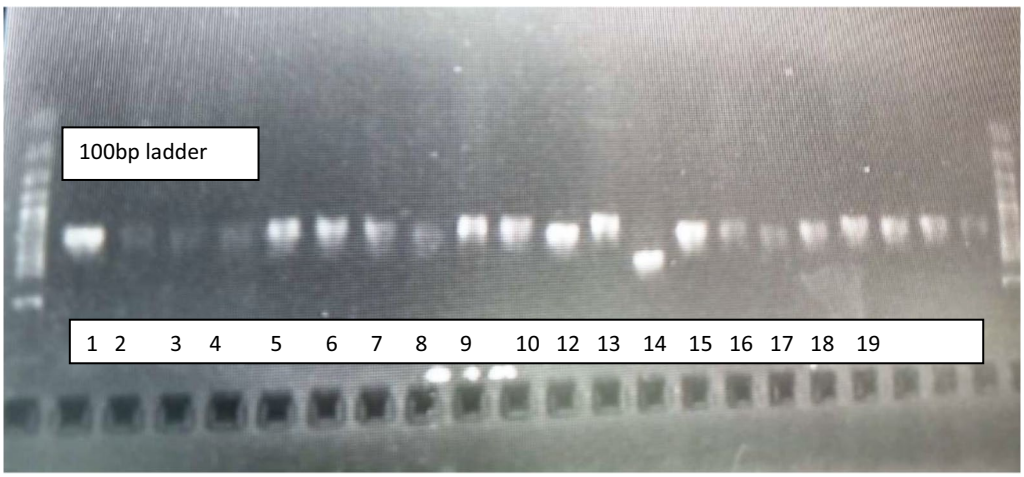

Fig. 4 Neighbour joining phylogenetic tree of fungal isolates of FAW larvae

Table 3 Potential Mycotoxins by other isolated fungi species

Potential mycotoxin	Fungi species
Ochratoxin	*Aspergillus* spp *Penicillium* spp
Fumonisin	*Fusarium* spp
Zearalenone	*Fusarium* spp
Trichothecene	*Fusarium* spp

99% indicates that the isolates are the same species, and 89–99% implies they are the same genus (Minarni et al. 2021; Henry et al. 2000).

Out of the 11 species of the EPF of FAW isolated in this study, there were only 2 identified up to genus level (Irpex sp and Bipolaris sp) with a similarity value of 99%. In terms of pathogenicity, 8 of the 11 species are known to be pathogenic against the larvae of *S. frugiperda*, the most abundant being *Trichoderma harzianum* and *Penicillium citrinum*. The *Curvularia* genus were also fairly common (Table 1) in this study. The diversity could be because of increased plant diversity as incorporated in the experimental design (Fig. 1) which may have created opportunities for species niche differentiation. However, this is contrary to a study by Idrees et al. (2021) who identified only 6 species out of the 20 isolates with *Beauveria bassiana* and *Curvularia lunata* being the most pathogenic against *S. frugiperda*. Though the isolates were not from maize, *Curvularia* genus isolated in different crops such as red chilli in Indonesia (Herlinda et al. 2021) showed more pathogenicity levels. A study on maize by Ramos et al. 2020 also concurred that *Curvularia lunata* effectively killed the larvae of *S. frugiperda* with fungi infection. Thus, the *Curvularia* genus stands out as being more virulent in terms of pathogenicity against *S. frugiperda* and the difference of this study findings may be due to variation in the geographic location (Ramos et al. 2020).

Different studies have showed that some EPF species produces mycotoxins (Litwin et al. 2020; Proctor et al. 2018). Contamination of poultry feed with aflatoxins is a major concern in poultry industry and results in economic loss while directly affecting consumers (Ochieng et al. 2021). This study targeted aflatoxins, which are mycotoxin fungal metabolites mainly belonging to the genera *Aspergillus*, *Alternaria*, *Fusarium*, *Cladosporium*, *Claviceps*, and *Penicillium* species (Ochieng et al. 2021) that contaminate poultry feed. The average aflatoxin quantities (Table 2) observed in this study could be attributed to the fact that, *Aspergillus sydowii* and *Penicillium citrinum* species were isolated from the FAW larvae as these are known to be aflatoxin producing fungi. (Mahato et al. 2019) also postulated that *Aspergillus* spp are the main aflatoxin producing fungi which have severely contaminated feed supplies of animals, resulting in health hazards due to its carcinogenic nature (Kumar et al. 2017).

Nevertheless, the quantities of aflatoxins were not above average in terms of the required amounts as the Kenyan Bureau of standards regulates total aflatoxin at 20 µg/kg (ISO 16050; Kenya Beaureau of Standards. 2013). At regional level, the East African Community has regulatory limits for poultry feeds and feed ingredients at 50 µg/kg, whilst countries such as Zimbabwe and Tanzania regulate at 10 µg/kg. Other countries only focus on aflatoxin AB1; these include the EU 20 µg/kg and USA 20 µg/kg (Ochieng et al. 2021). Even though the quantities were within the permissible limits, some studies still argue that aflatoxins are highly toxic such that even low concentrations are hazardous for livestock feed (Mahato et al. 2019).

The difference of this study findings can be largely attributed to the source of isolation which is the FAW larvae itself and the geographic location. Few studies have targeted EPF of FAW larvae but focused on poultry feed and other feed ingredients. As such, levels above the EU regulatory limit of 20 µg/kg are reported mainly in countries in the tropical regions, including Nigeria (Akinmusire et al. 2019; Ezekiel et al. 2012), Ghana (Aboagye-Nuamah et al. 2021), Kenya (Kemboi et al. 2020), and Uganda (Nakavuma et al. 2020). Aflatoxins were recorded in low levels of around 0.3 µg/kg to 0.7 µg/kg from poultry feeds from South Africa (Mokubedi et al. 2019), possibly due to differences in climatic conditions that affect the ecological niche of parent fungi and aflatoxins accumulation. Genetic and biochemical changes can occur in toxin biosynthetic pathways as fungi may adapt to different environmental conditions (Proctor et al. 2018).

However, the research is likely to have an underestimation since not all known mycotoxins are tested in this study and the mycotoxin analysis methods employed are not capable of detecting all mycotoxins of interest. Mycotoxins such as fuminosins, trichothecenes and zearalenone are also common contaminants of poultry feed besides aflatoxins (Ezekiel et al. 2012). Several studies also reported contamination of poultry feeds and feed ingredients by more than one mycotoxin (Ezekiel et al. 2012; Aboagye-Nuamah et al. 2021; Kemboi et al. 2020; Ochieng et al. 2021) Hence, the species isolated in this study such as mentioned in Table 1 can be prospective sources of mycotoxins which could have been detected. Therefore, FAW controlled by EPF may be safe from aflatoxins for use as poultry feed, with caution on handling to avoid further 'post-harvest' accumulation of aflatoxins.

Conclusions

This study concludes that, *S. frugiperda* can be potentially contaminated by the mycotoxin producing fungi. Even though in this study the aflatoxins were below threshold limits of many countries including Kenya, when formulating poultry diets, there is need to monitor production and ensure the quality is not compromised and feed safety is guaranteed. In addition, the study concludes that concurrent contamination by more than one mycotoxin occurs including concealed mycotoxins whose data were not included in the present study. Given this background, recommendations for future research include mycotoxins analysis on whole spectrum in the sample and also the toxicological effects and to determine how much aflatoxins are transferred into poultry products such as eggs and meat.

Abbreviations

BLAST	Basic Alignment Search Tool
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked Immunoassay
EPF	Entomopathogenic fungi
FAW	Fall army worm
ITS	Internal transcribed spacer
JOOUST	Jaramogi Oginga Odinga University of Science and Technology
MEGA	Molecular Evolutionary Genetic Analysis
NCBI	National Centre of Biotechnology Information
OD	Optical density
PCR	Polymerase Chain Reaction
PDA	Potato dextrose agar
RCBD	Randomized complete block design

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Author contributions

SS- Set up the field experiment, collected data and analysed it. ANM- Guidance in design of the project, analysis and interpretation of the results. COO-Guidance in design of the project, analysis and interpretation of the results.

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Availability of data and materials

Not applicable.

Declarations

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Ethics approval and consent to participate

This research did not involve human participants, hence no consent to participate in the study was obtained. However, Ethics approval for the study including handling and transportation of the biological specimens was obtained from Jaramogi Oginga Odinga University of Science and Technology's Ethics review office under approval number ERC 29/05/22-12. Additionally, the research was also licensed by the National Commission for Science, Technology and Innovation (NACOSTI) of Kenya under license number NACOSTI/P/22/20646.

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