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Preface

The African Phytosanitary Journal is a unique journal due to the scope of its coverage which is exclusively focused on sanitary and phytosanitary issues. The main aim of APJ is to provide a platform for dissemination of knowledge that is crucial in upholding standards set by International Plant Protection Convention (IPPC). These standards are aimed at protecting the world's plants, agricultural products and natural resources from plant pests. This involves a lot of research and innovations which the multitudes need to be aware of. African Phytosanitary Journal is emerging as one of the most authoritative journals covering phytosanitary issues. In APJ volume 4, we present seven articles; (i) Morphological and molecular characterization of the rust fungus, *Phakopsora apoda* causing leaf rust on *Brachiaria* grass in Rwanda, (ii) Reaction of potato cultivars to Potato Cyst Nematodes (*Globodera rostochiensis* and *G. pallida*) under greenhouse conditions in Kenya, (iii) Displacement of *Fusarium* species by atoxigenic *Aspergillus flavus* (Aflasafe KE01) application in maize fields in lower Eastern Kenya, (iv) Evaluation of pests affecting maize imported across Malawi borders, (v) Farmer perception, knowledge and management of the scale insect pest complex infesting crops and trees in Coastal Kenya (vi) Atoxigenic *Aspergillus flavus* (Aflasafe KE01) application reduces Fumonisin contamination in maize in lower Eastern Kenya, and (vii) Impacts of selected Climate Smart Agricultural Practices on African Indigenous Vegetables in Kenyan drylands.

The management is grateful to the editorial team and authors for their efforts and dedication towards the publication of this issue.

Prof. Theophilus M. Mutui, PhD

Managing Editor

Foreword

The African Phytosanitary Journal is a peer reviewed, open access journal which provides a platform for knowledge dissemination in the dynamic sanitary and phytosanitary sphere. The aim of this journal is to keep stakeholders informed on the current and emerging phytosanitary issues in Africa and beyond. Stakeholders targeted include; research institutions, National Plant Protection Organizations (NPPOs), institutions of higher learning and the industry. These stakeholders carry out research and come up with innovations or discoveries that are or contribute towards solutions to sanitary and phytosanitary issues and thus promote trade.

This issue of APJ focusses on invasive species (fungi, scale insects and nematodes) and potential biopesticides that may be used in pest management. Morphological and molecular characterization of pests, reaction of selected potato cultivars to Potato Cyst Nematodes, pest detection across borders, Climate Smart Agricultural Practices (CSAPs), a potential biopesticide (Aflasafe KE01) and the management of invasive scale insect pest are presented. This content is also available online at www.africanphytosanitaryjournal.org.

I would like to convey my sincere gratitude to the team that was involved in the publication of this issue. Many thanks to the editorial board, the reviewers and the authors.

Dr. Isaac Macharia

Editor in Chief

Scope of the journal

This journal has been developed to enrich knowledge and information in the following thematic areas:

- Pest surveillance
- Pest reporting
- Phytosanitary measures
- Pest Risk Analysis
- Pest identification and analysis
- Food safety
- Quarantine and Biosecurity
- Phytosanitary policy and regulation
- Phytosanitary treatment
- Emerging technologies
- Biological agents
- Pest Management
- Agricultural Chemistry
- Emerging phytosanitary issues
- Biosafety
- Phytosanitary issues on trade
- Other relevant phytosanitary issues

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Morphological and molecular characterization of the rust fungus, *Phakopsora apoda* causing leaf rust on *Brachiaria* grass in Rwanda

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Abstract

Brachiaria grass is one of the major forages that plays a key role in push pull technology. Different diseases including leaf rust caused by rust fungus, *Phakopsora apoda* have recently become an important challenge resulting in yield loss of *Brachiaria* grass in different countries including Rwanda. The objective of this study was to provide morphological and molecular characteristics of *Phakopsora apoda* causing *Brachiaria* leaf rust. Spores isolated from rusted *Brachiaria* leaves collected in five districts of Rwanda were analyzed. Samples were put in paper bags and left at room temperature for two days. Thereafter, spores were removed using brushes and put in eppendorf tubes, stored at 4^{o}C in darkness for further characterization. For morphological characteristics, colour and measurements of spores were recorded using Optika B-350 microscope with installed camera and calibrated micrometer. Multigene analysis using rust specific primers and large subunit (LSU) of nuclear ribosomal RNA was performed for molecular characterization. The results indicated that the colour of spores was yellowish or brownish while the shape was ellipsoidal or circular with average measurements of 25.1µm and 16.8µm for length and width respectively. Primers amplified the size of DNA between 1291bp and 1381bp and the size between 874bp to 882bp for rust specific primers and LSU respectively. The identity of the sequence was 96% with e-value of 0, sequence coverage of 60% for rust primer and the sequence identity was 94.97% to 95.37%, sequence coverage of 99% to 100% and e-value of 0 for LSU primer. All sequences recovered from five isolates of leaf rust matched to the genebank accession number MG461668.1 for both rust specific and LSU sequences. The results of this study provide useful information to develop effective options for leaf rust disease management in Rwanda.

Key words: Internal transcribed spacer, large subunit, nuclear ribosomal RNA, *Phakopsora apoda*, sequence, spore.

Introduction

Brachiaria grass is one of the nutritious forages originating from Africa which is also its centre of biodiversity. It belongs to the *Poaceae* family and is appreciated by many farmers in Sub-Saharan region due to its different attributes such as high biomass production with high nutrient content, drought tolerance and adaptation to infertile soils. Improvement of this genus was done outside Africa, especially in America and Australia (Maass *et al*., 2015). Seven species originating from Africa including *Brachiaria ruziziensis; Brachiaria arrecta, Brachiaria humidicola; Brachiaria dictioneura, Brachiaria decumbens, Brachiaria brizantha* and *Brachiaria mutica* are used as forages. The development of improved cultivars outside Africa led to different challenges and little attention has been given to biotic stress including leaf rust. With the increased promotion of improved *Brachiaria* cultivation in East African countries, there have been reports of occurrence of various diseases including leaf rust (Nzioki *et al*., 2016; Uzayisenga *et al*., 2020, 2021).

The wide distribution of leaf rust on *Brachiaria* grass was reported in Rwanda (Uzayisenga *et al*., 2020) and other causal agents of leaf rust on *Brachiaria* grass species such as *Puccinia levis var. panici sanguinalis* and *Uromyces setariae-italicae* are well documented (Lenné, 1990; Lenné and Trutmann, 1994). Several *Brachiaria* cultivars including CIAT 6369, Llanero, CIAT 679 and CIAT 16126 showed susceptible reaction to leaf rust in Central and South America (Lenné and Trutmann, 1994). Rust disease symptoms are characterised by yellowish to blackish pustules on leaves. It is not easy to recognise rust disease at the beginning of infection (Lenné, 1990).

Leaf rust reduces the quantity and quality of *Brachiaria* biomass. The biomass reduction is up to 100% and the reduction of crude proteins of *Brachiaria* leaves was reduced to 49% - 53%. The availability of other nutrients was affected due to leaf rust infection (Lenné and Trutman, 1994). Leaf rust management options include establishment of hedges, acceleration of *Brachiaria* growth by application of nitrogen fertilizers; use of rust-free

planting materials, planting at appropriate time since leaf rust is favoured by rainfall, use of mixed *Brachiaria* genotypes and avoiding burning and early harvesting of *Brachiaria g*rass ranging between four and eight weeks (Alvarez *et al*., 2014). The objective of this study was to provide morphological and molecular characteristics of *Phakospora apoda*, the causal agent of *Brachiaria* leaf rust in Rwanda.

Materials and methods

Collection of samples and isolation of leaf rust spores

Brachiaria grass with leaf rust symptoms were sampled from five different *Brachiaria* fields located in different districts (Table 1). A total of five sample in each district was collected, put in separate paper bags and carried to Plant pathology laboratory of Rwanda Agriculture and Animal Resources Development Board (RAB) in Southern Province of Rwanda for processing and analysis. Leaf samples kept in paper bags were put on table to dry at normal room temperature for two days. Thereafter, brushes were gently used to remove the rust spores from *Brachiaria* leaves and the spores were collected on aluminium foil, put in eppendorf tubes and stored in darkness at – 4 ºC for further characterization (Guo *et al*., 2016).

Table 1. Sampling location and coordinates of collected leaf rust samples

Isolate name	Cultivar	District	Altitude (m.a.s.l)	GPS coordinates	
BRRWR1	Mulato II	Bugesera	1455	E030°01'58.6''	S02°15'33.8''
HYRWR2	Mulato II	Huye	1685	E29°46'.56.9″	S02°28'54.8″
NBRWR3	Cayman	Nyamagabe	1839	E29°36'40.1″	S02°26'06.7″
NRRWR4	Piata	Nyagatare	1346	E030°18.304'	S01° 18.936'
RN RWR5	Mulato II	Rwamagana	1520	E030 22.662'	S01 53.310'

Morphological characterization of the causal agent of *Brachiaria* leaf rust

Morphological characteristics including the colour, shape and measurements of leaf rust spores associated with *Brachiaria* grass were visualised using the microscope Optika B – 350 at x40 magnification and the digital image of leaf rust spore was recorded using a camera installed on the microscope. Measurements of the size of leaf rust spores were taken using a calibrated micrometre.

Molecular characterization of the causal agent of *Brachiaria* leaf rust

Genomic DNA (gDNA) was extracted from leaf rust spores obtained from five different samples representing five *Phakopsora apoda* isolates (BSRWR1, HYRWR2, NBRWR3, NRRWR4 and RNRWR5). Qiagen DNeasy Plant Mini Kit was used for DNA extraction. The manufacturer protocol was used and leaf rust spores were mixed with sterilized carborundum (0.01%) for easy breakage of cells. The crushing was done in liquid nitrogen using both cooled and sterile mortar and pestle. Further steps of DNA extraction used 20 mg of fine ground powder of each of the five samples. For confirmation of causal pathogen, multi-gene analysis was used where rust primers and 28S large subunit of nuclear ribosomal RNA (LSU) were used for identification of the causal agent of *Brachiaria* leaf rust. The PCR reaction volume was set at 25µl (3µl of diluted gDNA containing 20 to 40 ng DNA, 12.5µl premix, 0.5µl each primer (forward and reverse) and 8.5µl of water for molecular biology), along with the negative control reaction without DNA template. Rust specific primers ITS1rustF10d (5′-TGAACCTGCAGAAGGATCATTA-3′) and rust1 (5′-GCTTACTGCCTTCCTCAATC-3′) and 28S large subunit of nuclear ribosomal RNA LR5/LROR TCCTGAGGGAAACTTCG/ ACCCGCTGAACTTAAGC were used for DNA amplification (Barnes and Szabo, 2007).

The PCR conditions were 4 minutes of initial denaturation at 94°C, followed by 35 cycles of 94°C for 45 seconds, 59.5°C for 45 seconds and 72°C for 45 seconds, with final extension at 72°C for 10 minutes and hold at 4°C and initial denaturation step at 95 °C for 3 minutes followed by 34 cycles of 30

seconds of denaturation at 95°C, 30 seconds of annealing temperature at 52°C, 1 minute of elongation at 72°C, final extension of 10 min at 72°C and hold at 4◦C at the end for rust and LSU primers respectively. The presence of targeted products was verified by loading 3µl of PCR product on 1.5% agarose gel for one hour at 70 volts.

Thereafter, PCR products were purified using QIAquick PCR Purification Kit (QIAGEN) following instructions of the manufacturer. Purified PCR products were sanger-sequenced at BecA -ILRI, Nairobi, Kenya using the respective primer sets used for PCR amplification. Raw DNA sequences were cleaned and consensus sequences were determined by aligning nucleotide sequences generated by forward and reverse primers. Consensus sequences were submitted to the National Centre for Biotechnology Information (NCBI) for homology search and identification of species using Basic Local Alignment Search Tools (BLAST) programme (Altschul *et al*., 1990).

Pathogenicity test

A total of five isolates of *Phakopsora apoda* with one isolate from each district (BSRWR1, HYRWR2, NBRWR3, NRRWR4 and RNRWR5) were tested for pathogenicity using one-month old seedlings of *Brachiaria hybrid* cv. Mulato and leaf rust spores suspended in water and tween 20. The spore concentration was 10^6 ml^{-1}. To facilitate the penetration of fungus into host tissue, 1% carborandum was added into the inoculum. Three seedlings per isolate were inoculated by hand rubbing and the development of leaf rust symptom was checked on daily basis for 30 days. Symptoms on inoculated plants were compared with those which occur under natural conditions. Seedlings for negative control were inoculated with the inoculum prepared in the same way but leaf rust spores were not added.

To maintain high humidity, inoculated plants were covered in polyethylene bags for two days (Figure 1). The pathogen was re-isolated from artificially inoculated leaves of *Brachiaria* hybrid cv. Mulato using the same procedure as described before and morphological characteristics of re-isolated spores were compared with the original isolates.

Figure 1. *Brachiaria* hybrid cv. Mulato seedlings covered in plastic bags after leaf rust inoculation

Data analysis

The CLC Genomics Workbench Version 8.0.3 software (https://digitalinsights.qiagen.com) was used to process data of DNA sequences from Sanger sequencing. Trimming was performed using quality scores of 0.05. The analyses did not consider all sequences with scores rating below 50%. Nucleotide sequences generated by the forward and reverse primers were checked, edited and consensus sequences were also generated using CLC Workbench version 8.0.3 software. Sequences were aligned using the same software and gaps were considered as missing data. To allocate identities to the test isolates, identification of species was done using BLAST programme of NCBI sequence database and comparison of the GenBank database was based on high similarity, coverage and identity.

Results

Morphological characteristics of fungal species associated with *Brachiaria* leaf rust

The association of *Phakopsora apoda* with rust symptoms was identified through macroscopic and microscopic analysis. Observations indicated that pustules with yellowish or brownish colour were mostly found on adaxial surface of leaves. Spores were ellipsoidal or circle, yellowish and brownish with average length of 25.1µm and the average width of 16.8µm (Figure 2, Table 2).

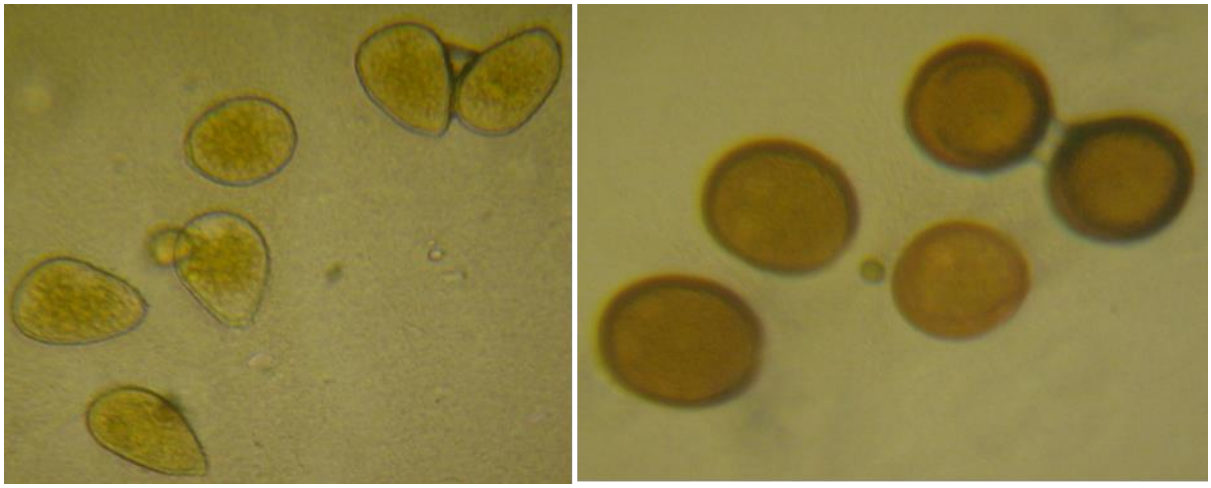

Figure 2. *Phakopsora apoda* spores isolated from rusted leaves of *Brachiaria* grass in Rwanda. (Photo taken using a camera installed on an OPTIKA B-350 microscope).

Table 2. Size of spores of *Phakopsora apoda* isolates

Isolate	Size of leaf rust spore (µm)	
	Length	Width
BRRWR1	23.8 ± 2.4^{ab}	16.7 ± 1.7^{a}
HYRWR2	23.3 ± 1.7^{ab}	15.8 ± 0.8^{a}
NBRWR3	29.2 ± 2.4^{a}	15.8 ± 2.0^{a}
NRRWR4	22.5 ± 1.7^{b}	18.3 ± 1.7^{a}
RN RWR5	26.7 ± 1.7^{ab}	17.5 ± 1.1^{a}
Mean	25.1 ± 0.9	16.8 ± 0.7

± is followed by standard error of the mean. Values with the same letters within the column are not statistically different at the probability level of 0.05.

Molecular identification of *Phakopsora apoda* isolates

For all five leaf rust isolates, rust specific primers amplified the size of DNA ranging between 1291bp and 1381bp while the DNA size varied between 874bp to 882bp when the amplification was done using LSU primers. The identity of the sequence was 96%, sequence coverage of 60% with e-value equal to zero for rust primers and the sequence identity was 94.97% to 95.37%, sequence coverage of 99% to 100% and e-value of 0 for LSU primers. All sequences recovered from five isolates of leaf rust matched the gene-bank accession number MG461668.1 for both rust specific and LSU sequences (Table 3).

Table 3. Five *Phakopsora apoda* isolates, rust primer and LSU sequence characteristics, homology search results

Primer	Isolate	Identified species	Sequence length (bp)	Sequence coverage (%)	e-value	Sequence identity (%)	Matching NCBI accession
Rust specific	BRRWR1	*P. apoda*	1370	60	0	96	MG461668.1
	HYRWR2	*P. apoda*	1381	60	0	96	MG461668.1
	NBRWR3	*P. apoda*	1374	60	0	96	MG461668.1
	NRRWR4	*P. apoda*	1291	60	0	96	MG461668.1
	RN RWR5	*P. apoda*	1378	60	0	96	MG461668.1
LSU	BRRWR1	*P. apoda*	882	100	0	95.37	MG461668.1
	HYRWR2	*P. apoda*	882	100	0	95.25	MG461668.1
	NBRWR3	*P. apoda*	868	100	0	94.72	MG461668.1
	NRRWR4	*P. apoda*	876	99	0	95.09	MG461668.1
	RN RWR5	*P. apoda*	874	99	0	94.97	MG461668.1

Pathogenicity of *Phakopsora apoda* isolates on susceptible *Brachiaria* seedlings

Results of the pathogenicity test revealed that all isolates produced a typical leaf rust symptom on leaves of *Brachiaria* hybrid cv. Mulato seedlings. Inoculated *Brachiaria* seedlings showed symptoms of leaf rust 10-18 days after inoculation for all isolates whereas leaves of seedlings used for negative controls did not show any disease symptoms (Figure 3). All five isolates were pathogenic to *Brachiaria* hybrid cv. Mulato and caused similar symptoms as the ones observed following natural infection. Symptomatic leaves from the inoculation experiment were used to re-isolate the causal agent.

Figure 3: Pathogenicity of *Phakopsora apoda* isolates from different regions of Rwanda on *Brachiaria* Hybrid cv. Mulato. 1: BSRWR1 (Bugesera isolate); 2: HYRWR2 (Huye isolate); 3: NBRWR3 (Nyamagabe isolate), 4: NBRWR3 (Nyagatare isolate), 5: RNRWR5 (Rwamagana isolate), 6: Negative control, 7: Leaf rust symptoms caused by *Phakopsora apoda* on naturally infected leaves of *Brachiaria* hybrid cv. Mulato.

Discussion

The isolation and identification of microorganism causing leaf rust of *Brachiaria* grass indicated that leaf rust was associated with *Phakopsora apoda*. Morphological characteristics of spores of *Phakopsora apoda* were ellipsoidal or circle, yellowish and brownish with average of 25.1µm and 16.8µm in length and width respectively. Morphological characteristics of the causal agent of *Brachiaria* leaf rust found in this study are in the range of the findings by other authors (Cherunya, *et.al.*, 2019)

Identification of leaf rust pathogen isolates using molecular techniques matched *Phakopsora apoda* sequence on top in the database of NCBI query cover of 60%. The low percent query cover found in this study might be attributed to rust fungi sequences from sequences that are unique and available in genebank. *Phakopsora apoda* has been proven to cause rust disease infecting Kikuyu grass (Gardner, 1984; Adendorff and Rijkenberg, 1995; Adendorff, 2014). Lenné (1990) indicated *Uromyces setariae-italicae* and *Puccinia levis* var. *panici-sanguinalis* as causal agents of rust disease affecting *Brachiaria* grass.

Likewise, analysis of sequences generated from rust and LSU primers confirmed *Phakopsora apoda* as the causal agent of *Brachiaria* grass leaf rust. The current study provides new etiological information about *Phakopsora apoda*, revealing these species for the first time as the causal agent of leaf rust on *Brachiaria* grass in Rwanda.

Conclusions and recommendations

Referring to the morphological features, rust and LSU sequence analysis and pathogenicity test of five isolates of leaf rust confirmed *Phakopsora apoda* as the causal agent of leaf rust of *Brachiaria* grass in Rwanda. All leaf rust isolates caused rust symptoms on *Brachiaria* hybrid cv. Mulato and had similar characteristics with re-isolates. Information generated in this study is highly important for development of leaf rust management strategies and will contribute to future studies on various aspects of *Phakopsora apoda* in order to protect *Brachiaria* grass.

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Reaction of potato cultivars to Potato Cyst Nematodes (*Globodera rostochiensis* and *G. pallida*) under greenhouse conditions in Kenya

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Abstract

Several measures have been recommended in the control of potato cyst nematodes (PCN) with resistant potato cultivars being considered as the most practical and affordable for smallholder potato farmers in Kenya. However, the level of resistance in locally grown potato varieties is yet to be established. The aim of this study was to screen Kenyan potato cultivars against PCN under greenhouse conditions. Eleven potato cultivars namely Shangi, Dutch Robijn, Sherekea, Nyota, Roseline tana, Tigoni, Unica, Asante, Chulu, Kenya Mpya and Arka were screened with Desiree (susceptible variety), Manitou (resistant cultivar) as controls. For each potato cultivar, there were two sets of plants with the first set being inoculated with 50 cysts, while the second batch was nematode-free. The treatments were arranged in a completely randomized design with three replications. A scale of 1-9, with 9 indicating the highest level of resistance was used in the assessment of disease severity. Nematode infestation caused a reduction in root mass across the 11 cultivars from 20 to 100% compared to uninoculated controls. Reproductive index of PCN viable eggs across the 11 cultivars was <1 compared to control (Desiree). Potato cultivars Shangi, Tigoni, Dutch, Chulu, Asante, Unica, Arka, Kenya Mpya, and Roseline Tana had a severity score of 1-3 (>95%), hence were considered to be susceptible to PCN. The cultivars Sherekea and Nyota had a severity score of 4-6 (<25%) and hence were considered partially resistant to PCN. The findings of this study provides a basis of integrating partially resistant potato cultivars into PCN management in smallholder farms.

Keywords: Cyst viable eggs, reproductive index, resistance, susceptible, smallholder farmers, severity score.

Introduction

Potato (*Solanum tuberosum* L.) is the world's third most important non-cereal food crop after wheat and rice (FAO, 2013). In Kenya, potato is the second most important food crop after maize and is highly commercialized across its value chain. About 2–3 million tonnes of potatoes worth Ksh. 40 to 50 billion (US$ 345–431 million) are produced each year, engaging millions of Kenyans directly and indirectly (MoAL&F 2016). In the tropics, potato cultivation faces a number of challenges including lack of certified seeds, poor soil fertility and infestations by various pests and diseases (Gildemacher *et al.,* 2009; Muthoni *et al*., 2013).

Potato Cyst Nematodes (*Globodera rostochiensis* and *G. pallida*) (PCN) cause 9% loss in potato yield worldwide (Turner and Subbotin, 2013). In Europe, PCN is the second most economically important pest of potato after late blight, with an estimated economic yield loss of £26 million annually and chemical nematicide use estimated at £20 million annually (Twining *et al*., 2009; NSP, 2017). It has been established that yield losses associated with PCN varies due to environmental conditions, varieties grown and levels of nematode infestation with every 20 viable eggs/g of soil causing a loss of 2.75 t/ha in potato yield (EPPO, 2013).

Potato cyst nematodes are classified as restricted quarantine pests in over 100 countries in the world (EPPO, 2009). The pest status under subtropical and tropical conditions in Africa is yet to be established. In potato growing areas in Kenya, PCN have caused qualitative and quantitative losses, with infested plants having low root mass development, stunted growth and leaf chlorosis with a potential yield loss of up to 80% when the pest is not controlled (Mwangi *et al*., 2015; Mburu *et al*., 2018). In predicting yield losses, different modeling parameters focusing on the level of initial PCN population at the start of planting season, type of soil, nematicide use, rotation period and use of susceptible/resistant cultivars have been used (Trudgill *et al.,* 2014).

Management of potato cyst nematodes through crop rotation is challenging since they are known to survive in soil for a long time with viable eggs surviving for decades in the absence of

suitable host. Crop rotation must therefore be integrated with other methods to lower PCN population density in the soil (Christoforou *et al.,* 2014). Use of trap cropping has been shown to lower the population density of PCN by inducing hatching without the formation of new cysts (Mimee *et al.,* 2015). This method requires an adequate understanding of the nematode's life cycle. Tolerant traits of plant varieties are an ideal strategy to reduce yield losses resulting from pests and diseases (Peng and Moens, 2002; Cook and Starr, 2006). Among all the methods used to lower PCN population density, the use of resistant varieties has proved to be most successful in managing plant-parasitic nematodes in various parts of the world (Cook and Starr, 2006).

Plant resistance to plant-parasitic nematodes is an important aspect in management of PCN given the concerns over environmental hazards caused by continuous use of chemical pesticides (Peng and Moens, 2002). Over 1,200 wild potato species from the Commonwealth Potato Collection gene bank have been screened for resistance to PCN and the first PCN resistance locus (*H1*) from *S. tuberosum* ssp. *andigena*, offering complete resistance to *G. rostochiensis* pathotypes Ro1 and Ro4 in potatoes has been identified (Whitworth *et al.,* 2018). Growing of resistant potato cultivars on farms with high PCN population densities has led to the realization of yield benefits with a significant reduction in nematode population densities (Lane and Trudgill, 1999). Subsequent studies have shown that population densities of *G. rostochiensis* can be reduced by up to 95% for each season that a resistant cultivar is grown depending on initial inoculum density, whereas when susceptible cultivars are grown in an infested field, the nematode population densities increase by 2–35 times (Brodie 1996). This study was therefore conducted to determine the reaction of Kenyan potato cultivars to PCN under greenhouse conditions.

Materials and methods

Experimental site and cultivar selection

A greenhouse experiment was conducted from March-May and June-August 2021 for seasons one and two respectively, at the University of

Nairobi-Upper Kabete campus (1° 15´S and 36° 44´E) at an elevation of 1,820 m above sea level. Eleven potato cultivars commonly grown by Kenyan farmers; Dutch Robijn, Shangi, Roslin Tana, Tigoni, Asante, Sherekea, Nyota, Kenya Mpya, Unica, Chulu and Arka were selected for the study. Potato cultivars Desiree (susceptible) and Manitou (resistant) were included as controls. Certified potato tubers were obtained from Kenya Agricultural and Livestock Research Organization – Tigoni.

Preparation of PCN inoculum

Potato cyst nematode inoculum was extracted from naturally infested fields in Nyandarua county. The Fenwick can method (Fenwick, 1940), was used to extract PCN cysts from infested soil. Viability of eggs within the cysts was verified by soaking a random sample of 10 cysts per soil sample in 0.001% Nile blue stain (*v/v*) for 48 hours (Faggian *et al.*, 2012). Dead eggs or juveniles stained blue while the live ones did not stain after opening the cyst. Samples with at least 50% of either live eggs or juveniles were used as sources of inoculum. Each eppendorf tube was used to conserve 50 cysts until needed.

Set-up of greenhouse experiment

Each of the eleven chitted potato cultivars in two batches (inoculated and nematode-free) was sown in three plastic pots containing 400g of soil and sand (steam-sterilized at 180°C for 30 minutes) mixed at a ratio of 3:1 (*v/v*). This was replicated three times. Two weeks later, 50 cysts of PCN having about 100 eggs/ cyst were introduced into each pot as described by Whitworth *et al.*, 2018, while the controls pots were nematode-free (non-inoculated). Treatments were arranged in a completely randomized design. Plants were watered at intervals of four days.

Data collection

Data on root mass, cyst count and egg viability from each potato cultivar were collected 70 days after planting. Final population (P_f) densities of cyst and egg viability per 3 cysts of each treatment were evaluated, following cyst extraction, picking and counting under a dissecting microscope at 40x magnification.

Data analysis

Data on root mass were compared using *t*-test. Potato cyst nematodes and

egg reproduction index was expressed as RI=Pf /Pi (Van Den Berg and Rossing, 2005). Susceptibility rating of the eleven potato cultivars to PCN were determined by calculating, PF (final cyst population of the test variety)/ PF (final cyst population of standard susceptible control variety i.e. Desiree) × 100, while severity score was divided into 9 susceptibility groups i.e. 1 (>100), 2 (50.1–100%), 3 (25.1–50%), 4 (15.1–25%), 5 (10.1–15%), 6 (5.1–10%), 7 (3.1–5%), 8 (1.1–3%), 9 (≤1%), with 1-3 being susceptible, 4-6 partially resistant and 7-9 resistant (EPPO, 2006). Data on cyst and egg counts were normalized using square root transformation before ANOVA was performed (Gomez, 1984). Thereafter, ANOVA was done using GenStat (15^{th} edition) and means of cysts and egg counts in potato cultivars were compared and separated using Tukey's least significant difference (LSD) test ($p<0.05$).

Results

Effect of PCN infections on root mass of different potato cultivars

Potato cyst nematode infestation caused a decrease in root mass of the 11 potato cultivars compared to their controls 70 days after planting. There was a significant difference ($p \leq 0.05$) in root mass of nematode-infested plants compared to non-inoculated controls (Table 1). Cultivar Arka had significantly reduced root mass between inoculated and non-inoculated treatment and was comparable to Desiree (susceptible cultivar). Amongst the 11 cultivars tested, Arka, Tigoni, and Unica had a significant reduction in root mass by >100%. Cultivars Sherekea and Chulu had the least reduced root mass by 2.5 and 3.3 % respectively. Root mass reduction ranged from 20% to 75% in other potato cultivars (Table 1)

Table 1. Effect of PCN infection on root mass of different potato cultivars

Potato	Season one				Season two			
cultivar	Inoculated	Non-inoculated	*p*-value	% Decrease in root mass from uninfected	Inoculated	Non-inoculated	*p*-value	%Decrease in root mass from uninfected
Shangi	6.5	10.6	0.1	62	38.2	67.1	0.9	75
Dutch Robijn	7.2	13.4	0.2	87	51.0	80.4	0.3	58
Sherekea	4.6	4.8	0.9	2	31.6	32.7	0.9	3
Desiree	2.7	4.1	0.05*	48	19.5	32.4	0.5	66
Nyota	3.6	4.6	0.4	28	20.4	23.1	0.6	14
Roseline Tana	4.2	5.4	0.14	28	20.9	23.9	0.3	14
Tigoni	5.2	12.0	0.06	132	16.6	37.4	0.09	125
Unica	6.7	14.2	0.16	111	11.8	22.9	0.8	93
Asante	3.7	6.3	0.24	71	24.4	39.1	0.1	60
Chulu	10.5	11.1	0.82	6	39.7	40.0	0.2	0.6
Kenya Mpya	3.4	4.5	0.08	34	83.2	98.0	0.5	18
Arka	1.7	4.1	0.04*	142	6.1	17.2	0.7	183
Manitou	4.1	5.6	0.3	35	21.9	25.7	0.6	17
SE	2.9	5.3			25.0	32.2		
L. S. D	5.1	9.5			44.5	57.3		
CV%	111.6	131.7			153.7	145.5		

Means of root mass in inoculated and non-inoculated plants across the eleven potato cultivars are significantly different *($p \leq 0.05$)
CV = Coefficient of Variation, LSD = Least Significant Difference, SE = Standard Error

Population density of PCN on different potato cultivars

The final PCN population density was significantly different ($p \leq 0.05$) among the cultivars in both seasons one and two. The final cyst population in cultivars Manitou (resistant) and Nyota had significantly lowered by 14-67% compared to controls, while an increase by up to 300% in cultivars Sherekea, Shangi, Tigoni, Dutch Robijn, Chulu, Asante, Unica, Arka, Kenya Mpya and Roseline Tana was observed compared to controls. The lowest PCN reproductive index of RI=0.6 and RI=0.53 were observed in cultivars Nyota and Manitou, followed by Sherekea with the reproductive index of RI=1.18 compared to Desiree (Table 2). Cultivars (Shangi, Tigoni, Dutch Robijn, Chulu, Asante, Unica, Arka, Kenya Mpya, Roseline Tana) had PCN with reproductive index of >1.The severity score of cultivars Shangi, Tigoni, Dutch, Chulu, Asante, Unica, Arka, Kenya Mpya, and Roseline Tana was 1-3 and were hence considered susceptible to PCN. Sherekea and Nyota had a severity score of 4-6 and were hence considered partially resistant to PCN (Table 3).

Table 2. Cyst numbers and reproductive index (RI) of PCN in different potato cultivars

Potato cultivars	Season one			Season two		
	Final cyst count (Pf)/pot	RI (Pf/Pi)	% Change in cyst count from initial population (Pi)	Final cyst count (Pf)/pot	RI (Pf/Pi)	% Change in cyst count from initial population (Pi)
Desiree	241.0e	4.82e	382	170.7e	3.41e	241
Shangi	172.7cde	3.45cde	245	122.3de	2.45de	145
Tigoni	200.0de	4.00de	300	98.0cd	1.96cd	96
Dutch Robijn	158.3bcd	3.17bcd	216	87.7cd	1.75cd	75
Chulu	151.7bcd	3.03bcd	203	85.0cd	1.70cd	70
Asante	109.0abc	2.18abc	118	93.0cd	1.86cd	86
Unica	95.3abc	1.91abc	91	70.7bcd	1.41bcd	41
Arka	107.7abc	2.15abc	115	66.0abc	1.32abc	32
Kenya Mpya	95.3abc	1.91abc	91	64.0abc	1.28abc	28
Roseline Tana	69.0ab	1.38ab	38	53.3abc	1.07abc	6
Manitou	34.7a	0.69a	-31	18.7ab	0.37ab	-63
Sherekea	70.7ab	1.41ab	41	47.0abc	0.94abc	-6
Nyota	43.0a	0.86a	-14	16.3a	0.33a	-67
S.E.M	34.5	0.69		18.0	0.36	
L. S. D	100.2	2.00		15.3	0.31	
CV (%)	50.1	50.1		40.8	40.8	

Means in the same column with a different letter(s) are significantly different ($p \leq 0.05$). CV (%) = Coefficient of Variation, LSD=Least Significant Difference, Initial Population (Pi) = 50 cysts, reproductive index (RI = Pf/Pi).

Table 3. Susceptibility rating of potato cultivars to PCN

Potato cultivars	Season one		Season two	
	Severity score	% Susceptibility score	Severity score	% Susceptibility score
Desiree	2	100	2	100
Shangi	2	72	2	72
Tigoni	2	57	2	83
Dutch Robijn	2	51	2	66
Chulu	2	50	2	63
Asante	2	54	3	45
Unica	3	41	3	40
Arka	3	39	3	45
Kenya Mpya	3	37	3	40
Roseline Tana	3	31	3	29
Manito	4	11	4	14
Sherekea	4	25	4	24
Nyota	4	10	4	18

Severity score; 1-3 susceptible, 4-6 partially resistant and 7-9 resistant.

Reproductive index of PCN viable eggs on different potato cultivars

There was no significant difference ($p \leq 0.05$) in viable eggs across the varieties tested in seasons one and two. All the 11 cultivars had a reproductive index of less than 1 compared to Desiree which was the susceptible control (Table 4).

Table 4. Reproductive index of PCN viable eggs on different potato cultivars

Potato cultivars	Season one		Season two	
	Final egg count (Pf)/cyst	RI (Pf/Pi)	Final egg count (Pf)/cyst	RI (Pf/Pi)
Chulu	35.0 a	0.35 a	55.0 abc	0.55
Shangi	90.7 ab	0.9 ab	98.7 cd	0.99
Unica	68.7 ab	0.69 ab	74.0 abc	0.74
Nyota	24.7 a	0.25 a	89.0 bcd	0.89
Dutch Robijn	30.3 a	0.30 a	34.3 ab	0.34
Kenya Mpya	64.7 ab	0.65 ab	89.0 bcd	0.89
Tigoni	48.3 ab	0.48 ab	28.7 a	0.29
Sherekea	32.3 a	0.32 a	84.3 abc	0.84
Roseline Tana	30.0 a	0.30 a	48.3 abc	0.48
Manitou	52.3 ab	0.52 ab	67.7 abc	0.68
Desiree	114.3 b	1.14 b	139.7 d	1.4
Arka	70.0 ab	0.70 ab	84.7 abc	0.85
Asante	84.7 ab	0.85 ab	69.7 abc	0.7
S.E.M	24.38	0.24	19.51	2.0
L. S. D	70.89	0.71	56.71	0.57
CV (%)	63.9	63.9	45.6	45.6

Means in the same column with a different letter(s) are significantly different ($p \leq 0.05$). CV (%) = Coefficient of Variation, LSD = Least Significant Difference, Reproductive index (RI) = Pf/Pi, Pi- initial population (100 viable eggs).

Discussion

Potato cyst nematodes had a significant effect on root mass of different potato cultivars during their growth. Potato cultivar Arka had significantly lower root mass followed by Tigoni and Unica amongst the eleven potato cultivars. PCN J2 penetrated and developed in the root systems of potato cultivars Arka, Tigoni and Unica forming feeding tubes that limited the absorption of water and nutrients (Urek *et al.* 2008; Sudha *et al.* 2017). Cultivars Chulu and Sherekea had higher root density due to ability to produce extra roots when infested with PCN hence increasing their efficiency in nutrient and water uptake (Mei *et al.*, 2015). These findings are reflected by Thorpe *et al.*, (2014) who noted that host-nematode defense response to resistance and susceptible potato cultivars in the root system at early stage are affected by PCN effectors, which are important in syncytia formation, hence suppressing defense response in susceptible cultivars, unlike resistant cultivars.

There was a significant difference in final PCN population density among the cultivars in seasons one and two.

Difference in final cyst population on susceptible potato cultivars is dependent on different PCN species and pathotypes, as same pathotypes have different population densities across susceptible cultivars (Sudha *et al.*, 2016). On the other hand, increase in the number of cysts and viable eggs in susceptible potato varieties was because of their sensitivity towards PCN (Urek *et al.*, 2008, Hajji-Hedfi, 2017, Mezerket *et al.*, 2018). Decrease in number of cysts and viable eggs in resistant potato cultivars Nyota and Sherekea was due to presence of H1 resistance gene which prevents the nematode from molting to adult stage (Simko *et al.,* 2007).

Severity score of potato cultivars Shangi, Tigoni, Dutch, Chulu, Asante, Unica, Arka, Kenya Mpya, and Roseline Tana showed that they were susceptible to PCN, while potato cultivars Sherekea and Nyota were partially resistant/tolerant to PCN. Resistance of potato cultivars to *G. rostochiensis* is attributed to several genes, which confer partial (Gro1.2, Gro1.3, Gro1.4, Grp1) or complete (H1, Gro1, GroVI) resistance (Finkers-Tomczak *et al.* 2011). The H1 gene confers resistance to pathotypes Ro1 and Ro4 of *G. rostochiensis,* which inhibits the multiplication of PCN juveniles to develop into females (Simko *et al.,* 2007, Finkers-Tomczak *et al.*, 2011). These findings corroborate with Sudha *et al.*, (2016), who showed that cultivar Kufri Swarna was resistant to PCN pathotype Ro1, 4, Pa2, 3 Pa2/3 but susceptible when tested with pathotype Ro5. Nematode reproduction levels on plant tissues are used to measure resistance while damage levels are used to quantify tolerance (Bethke *et al.*, 2017).

Conclusion

From this study, potato cultivars Sherekea and Nyota are partially resistant to PCN and have the potential to reduce PCN under greenhouse conditions. However further studies need to be conducted to assess reactions of locally available potato cultivars in controlling PCN under different field conditions in Kenya.

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Displacement of toxigenic *Fusarium* species by atoxigenic *Aspergillus flavus* (Aflasafe KE01) application in maize fields in lower Eastern Kenya

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Abstract

Maize is grown in areas with environmental conditions that are ideal for growth of most cereal pathogens such as *Fusarium* fungi. However, there is lack of effective fungicides to control fumonisin producing *Fusarium.* This study was conducted to determine the efficacy of atoxigenic *Aspergillus flavus* (Aflasafe KE01) on the population of *Fusarium* species in maize fields. The study was carried out in four sub counties in lower Eastern Kenya (Kaiti, Kathiani, Nzambani and Wote). Twenty-four maize fields were selected in each sub county where 12 fields were treated with Aflasafe KE01, while 12 fields comprised the untreated controls. Aflasafe KE01 was applied by hand broadcasting in the maize fields two to three weeks before tasselling of maize. Maize grain samples were collected from each field at harvest and ground using a Bunn coffee mill grinder. *Fusarium* species were isolated from the ground maize using pour plate method following serial dilution on low strength potato dextrose agar (PDA)and Spezieller Nahrstoffarmer. The results showed that application of Aflasafe KE01 effectively displaced toxigenic *Fusarium* species in maize fields. Maize samples from Aflasafe KE01 treated maize fields recorded significantly lower incidence (41%) of the *Fusarium* species compared to untreated maize fields (60%) ($p \leq 0.01$). These results indicate that Aflasafe KE01 is a potential biopesticide for the biocontrol of *Fusarium* species in maize.

Key words: Aflasafe KE01, atoxigenic *Aspergillus* sp, *Fusarium* species, maize.

Introduction

Maize (*Zea mays* L.) is an important crop to majority of Kenyan population and is grown in different agro-ecological zones characterized with varying temperature and rainfall (Ureta *et al.*, 2013). Over 90% of the Kenyan population depends on maize for food (Kirimi *et al.*, 2011; USDA, 2016). Over 38% of farmers in Kenya grow maize (FAO, 2016) and of this, small-scale farmers produce about 70% of the overall production. However, they retain up to about 58% of their total production for household consumption (Olwande *et al.*, 2015).

Maize is prone to degradation by mycotoxigenic fungi such as *Aspergillus* species*, Penicillium* species and *Fusarium* species which are always present in soils (Kumar *et al.*, 2016; Koskei *et al.*, 2020). Maize is grown in areas with environmental conditions that are ideal for growth of most cereal pathogens such as *Fusarium* fungi most of which grow in temperatures around 25–35^{o}C and high humidity (Aldars-garcía *et al.*, 2018). Mycotoxins are produced by fungi in products such as maize, wheat and groundnuts which are susceptible to mould infection (Wagacha & Muthomi, 2008; Cinar & Onbasi, 2019).

Fusarium verticillioides and *Moniliforme* have been identified in Kenya and are predominant in Makueni, Nandi, and western Kenya (Mutiga *et al.*, 2015; Kang'ethe *et al.,* 2017). These regions also record high incidences of *Aspergillus, Fusarium* and *Penicillium* species (Bii *et al.*, 2012; Kilonzi *et al.*, 2014). A timely fungicide application reduces infection and mycotoxin contamination. However, there is lack of effective fungicides to control fumonisin-producing *Fusarium*. According to a survey done in Eastern and western Kenya, *Fusarium* species are common contaminants of maize in these areas (Koskei *et al.*, 2020). The climatic conditions in lower Eastern region of Kenya are ideal for growth of the afore-mentioned pathogens. This has necessitated need to identify appropriate methods of controlling mycotoxins in maize and other crops. To effectively prevent or minimize future mycotoxin contamination and reduce long term exposure to mycotoxins such as aflatoxins, proper methods of controlling and reducing mycotoxin contamination are required.

This has necessitated research for alternative ways of mycotoxin management. Some of these mycotoxins can be controlled biologically through competitive exclusion methods. This is made possible by the presence of two distinct *A. flavus* populations: the toxigenic strains and the atoxigenic strains. Atoxigenic strains of *A. flavus* can effectively eradicate the highly toxigenic strains thereby reducing aflatoxin contamination. The objective of this study was to assess the effectiveness of atoxigenic *A. flavus* (Aflasafe KE01) in the displacement of *Fusarium verticilliloides* and fumonisins. This will contribute towards better management of *Fusarium* species in order to reduce maize degradation and mycotoxin contamination.

Materials and methods

Determination of population of *Fusarium* species in soil and maize grains in atoxigenic *Aspergillus flavus* (Aflasafe KE01) treated and untreated maize fields

Description of the study area

The study was conducted in Nzambani sub-county (Kitui County), Kathiani sub-county (Machakos County) and Wote and Kaiti sub-counties (Makueni County) in lower Eastern parts of Kenya (latitude between 4^{o}N to 4^{o}S, longitude 34^{o} to 41^{o}E). These regions receive an average rainfall of between 150 mm to 650 mm p.a. On average, Machakos and Kitui counties receive 500 to 700mm p.a and 500 to 1050 mm p.a. respectively. The soils in these areas are sandy to loamy sand texture with low organic matter contents, low water retaining capacity and low plant nutrients thus making is susceptible to erosion (Gachimbi *et al.*, 2007). Makueni county has several agro-ecological zones (AEZs) with altitudes ranging from 790-1770masl and receives about 600-1050mm of average annual rainfall (Jaetzold *et al.*, 2010).

Experimental design and application of atoxigenic *Aspergillus flavus* (Aflasafe KE01)

Selection of the study sites was purposive and based on areas where maize is commonly cultivated, have a history of aflatoxin contamination and have low risk of maize crop failure (Muthomi *et al.*, 2009). The farms were selected randomly within each sub county with each farm having a minimum of 2 acres except where it

was not possible to get such farms in a particular sub county.

The experiment was conducted on maize planted by the farmers who consented to take part in the study. In each of the four sub counties, 24 maize fields were selected; 12 fields were treated with Aflasafe KE01 while the other 12 were control fields. Within each area, control fields were a maximum of 100m from the treated fields. Aflasafe KE01 was obtained from the International Institute of Tropical Agriculture (IITA). Six of the individual farmers' fields were treated with Aflasafe KE01 at an application rate of 5 kg/Ha while the other six was treated with 10 kg/Ha. Aflasafe KE01 was broadcast by hand in the selected fields 2-3 weeks prior to tasselling of maize. The experiment was carried out in one maize cropping season across the four sub counties. Data collected from the experiment included the population of *Fusarium* species in the soil and grain samples and the fumonisin levels in the maize grains.

Collection of soil and maize cob samples

Soil samples and maize cobs were sampled from experimental maize fields to determine incidence of *Fusarium* species in the soil and maize grains. Additionally, maize cobs sampled were used to quantify the amount of fumonisin present in the grains. Samples of soil were collected from the farms to a maximum depth of 2cm using a spoon 2 to 3 weeks before flowering of maize just before application of Aflasafe KE01 and from control fields. A minimum of eight maize cobs were sampled at harvest from each farm. The cobs were randomly picked from the farm following a zigzag pattern and put in bags. The bags were properly labeled with farmer details and sampling date and transported in a cool box to the laboratory for analysis within 48 hours after collection.

Soil lumps were crushed gently using a hammer and plant debris removed by hand. The soil was then sieved through sieve number 20, standard testing sieves (0.833mm opening) and air dried at 23±3°C. The maize cobs were sun dried on plastic canvas to avoid contact with the ground. Thereafter, dry maize was manually shelled by hand and dried in an oven at 45°C for two days and then crushed to fine powder with a

Bunn coffee mill grinder (Bunn omatic Corporation, Spring Field Illnois, USA). The ground maize sample was thoroughly mixed and used for isolation and identification of mycotoxigenic fungal species. The samples were stored in a refrigerator at 4 °C.

Isolation of *Fusarium* species from soil and maize kernels

Species of *Fusarium* were isolated from the ground maize and soil samples using serial dilution method and plating on low strength potato dextrose agar (PDA) amended with mineral salts and antimicrobial agents as described by Muthomi (2001). One gram of each soil and ground maize samples was weighed and emptied into 10ml of sterile distilled water in a 40ml glass vial. The vials were placed on vortex mixer (Velp Scientifica, Europe) for three minutes at 1750 rpm. One milliliter of the mixture was drawn using a micropipette and mixed with 9ml to form the first dilution of 10^{-1}. One milliliter of the first dilution was measured and dissolved in 9ml of sterile distilled water to form the second dilution of 10^{-2}. This was done up to the third dilution of 10^{-3}. One milliliter aliquot of the second and third dilutions (10^{-2} and 10^{-3}) of soil and ground maize samples was plated on the low strength PDA in triplicate. The plates were incubated at room temperature (23±3°C) for 5-7 days during which the soil and maize samples showing growth of a fungus was recorded and the fungus identified. *Fusarium* isolates from low strength PDA were sub-cultured on PDA and incubated at 25°C for 14 days. *Fusarium* species growing on PDA were identified based on their cultural characteristics.

Identification of *Fusarium* species

Different *Fusarium* species growing on low strength PDA were identified based on the cultural, morphological and biological characteristics such as septation and sporophores, spore shape, pigmentation, mycelia color and colony pigmentation. *Fusarium* species showing similar characteristics were given a specific code and sub-cultured on PDA and Spezieller Nahrstoffarmer Agar (SNA). The SNA medium was prepared as described by Nirenberg (1981) and autoclaved for 20 minutes at 121°C at a pressure of 15 p.s.i. Approximately 20ml was dispensed in 9cm petri dishes. *Fusarium* species sub-cultured on SNA were incubated under

UV light to facilitate sporulation. *Fusarium* species growing on PDA and SNA were identified following cultural (pigmentation), morphological (mycelia color and colony pigmentation) and biological (septation and sporophores, spore shape) characteristics. Morphological characteristics of various *Fusarium* isolates were used in identification to species level based on manuals by Nelson *et al.* (1983) and Leslie & Summerell (2006). The formula by Gonzalez *et al*. (1995) was used in determining the relative isolation frequency of each *Fusarium* genus.

$$\text{Frequency}(\%) = \frac{\text{Number of samples in which a species occurred}}{\text{Total number of samples}} \text{x } 100$$

Microscopic identification of *Fusarium* species on SNA was based on macro-conidial shape, septation, widest part of macro-conidia, length of apical cell, relative abundance of microconidia in aerial mycelium, micro-conidia in chains or heads, microconidial shape and conidiophores in aerial mycelium (Seifert and Gräfenhan, 2012). The number of colonies growing in each serial dilution was counted and from this the colony forming unit per gram (CFU/g) of soil or maize samples was determined using the following formula:

$$\text{CFU/g} = \frac{\text{Number of } \textit{Fusarium} \text{ colonies/ml plated}}{\text{Total dilution factor}}$$

Data analysis

Data on *Fusarium* species population and percent incidence level was subjected to analysis of variance (ANOVA) to determine significant difference in incidence of *Fusarium* species in Aflasafe KE01 treated and untreated maize fields. Differences between treated and untreated fields were separated using Fishers protected LSD ($p \leq 0.05$). GenStat 15th edition analysis software was used for the analysis.

Results

Diversity of *Fusarium* species in soil and maize grains in atoxigenic *Aspergillus flavus* (Aflasafe KE01) treated and untreated maize fields

The main *Fusarium* species isolated from soil and maize samples in lower Eastern Kenya were *F. verticillioides*, *F. proliferatum, F. chlamydosporum, F. merismoides, F. semitectum* and *F. oxysporum*. *Fusarium verticillioides*

produced white aerial mycelium which was tinted with purple colour and the underside was dark purple on PDA while their microconidia were formed in chains. *Fusarium oxysporum* had a white aerial mycelium and sometimes had purple tinge on PDA. The basal part was creamy to tan orange in color while the underside was dark purple. It produced chlamydospores and microconidia borne on false head (Figure 1). *Fusaruim verticillioides* had an aerial white mycelium on PDA. It grew rapidly and was tinged with purple. When sporodochia was present it was tan to orange in colour. The undersurface was dark purple in colour. Its microconidia were formed in chains on monophialides. *Fusarium avenaceum* had scarce, very long, slender, more than three septate and thin-walled microconidia. On PDA, it had a dense white-tan aerial mycelium with dark brown colour. *Fusarium semitectum* had a rapid growing aerial mycelium that was tan in colour with very few microconidia. The macro conidia were borne in aerial mycelium and were spindled shaped, straight to slightly curved. Poliphialides were borne on the aerial mycelium. *Fusarium graminearum* in mature cultures had a dense aerial mycelium which turned yellow and sporodochia were present in thick walled and mature culture with carmine red undersurface. Its macroconidia were distinctively septate, with distinctly foot shaped basal cell.

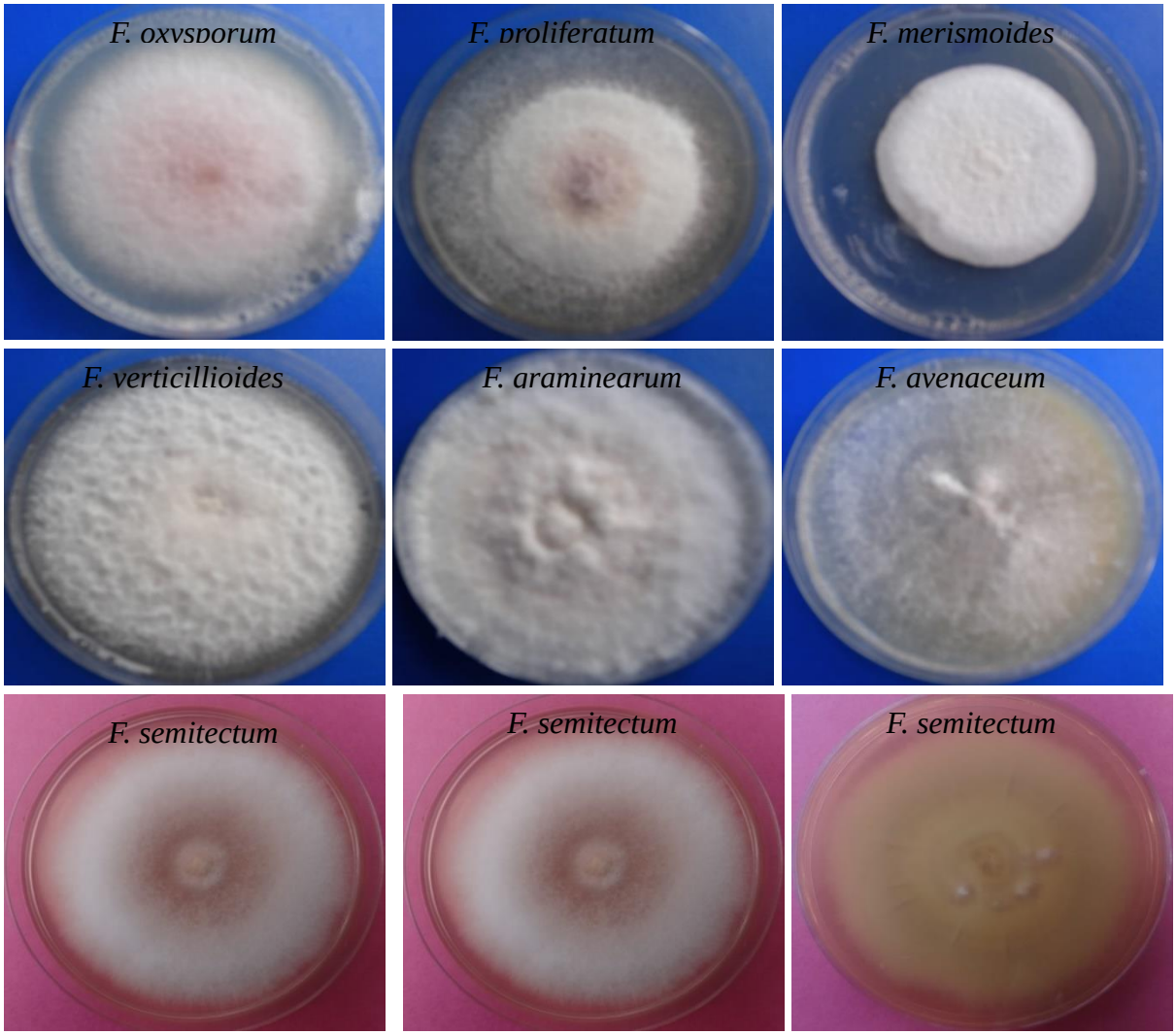


Figure 1. Growth of *Fusarium* species isolated from soil collected from maize fields on PDA

In all the sub counties, *Fusarium verticillioides* was the most predominant fungal pathogen isolated from maize samples. It had abundant microconidia which were single celled, oval shaped, non-septate microconidia. *Fusarium proliferatum* had abundant micro-conidia that were club shaped and with a flattened base which occurred in long chains and its basal cell was foot shaped with branched polyphialids and monophialids. The rare species was *Fusarium chlamydosporum* which had spindle shaped microconidia which was either septate or non-septate with macroconidia that were sickle shaped with a basal cell that was foot shaped and had abundant rough wall chlamydospores (Figure 2).

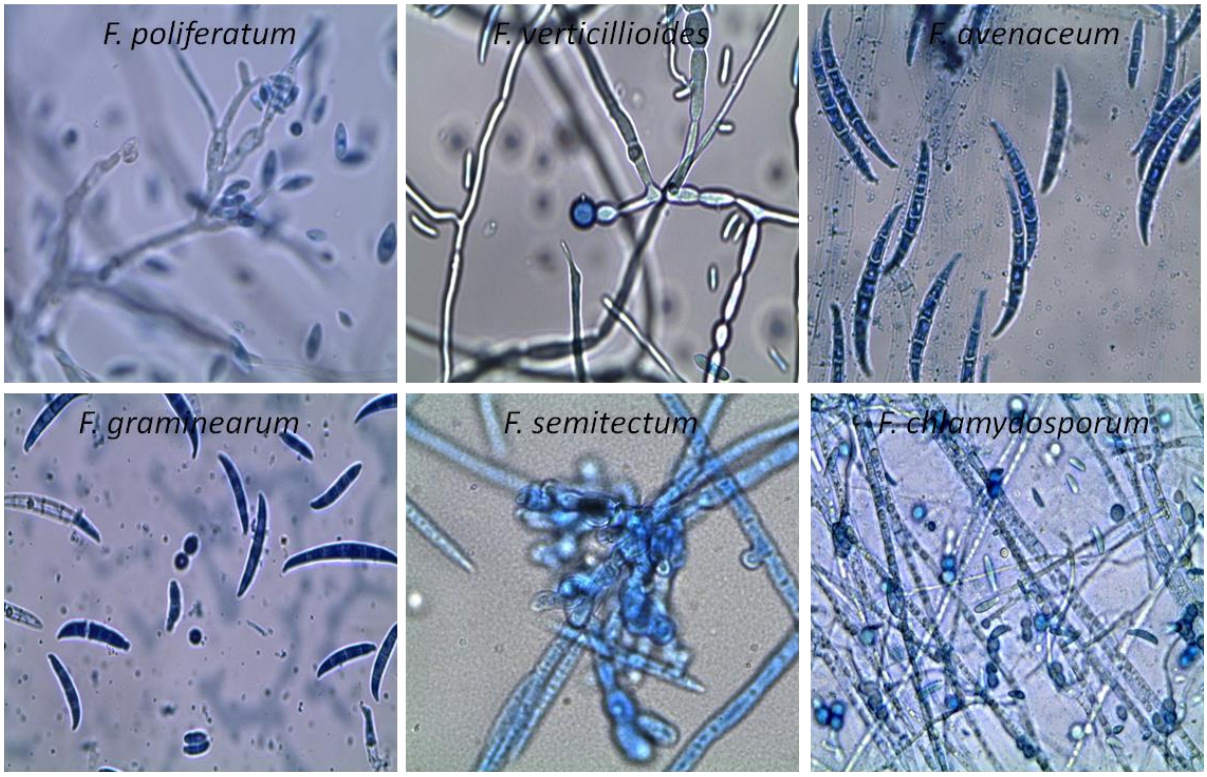


Figure 1. Various conidial types of different *Fusarium* species isolated from maize grain and soil samples from fields in lower Eastern Kenya. (x100 magnification).

Frequency of *Fusarium* species in maize sampled from Lower Eastern Kenya from atoxigenic *Aspergillus flavus* (Aflasafe KE01) treated and untreated maize fields

Incidence of the fungal pathogens varied significantly ($p \leq 0.05$) in maize fields treated with Aflasafe KE01 and untreated maize fields. Maize samples from Aflasafe KE01 treated (5 kg/ha) maize fields recorded lower incidence of the *Fusarium* species compared to untreated maize fields (Figure 3a). A significant difference ($p \leq 0.05$) was observed in the incidence of fungal pathogens between maize grain samples from maize field treated with Aflasafe KE01 (10 kg/ha) and untreated maize field especially in prevalence levels of *F. verticillioides, F. proliferatum and F. oxysporus.* Aflasafe KE01-treated maize fields had significantly lower incidence of *F. verticillioides, F. proliferatum and F. oxysporus* compared to untreated maize fields (Figure 3b).

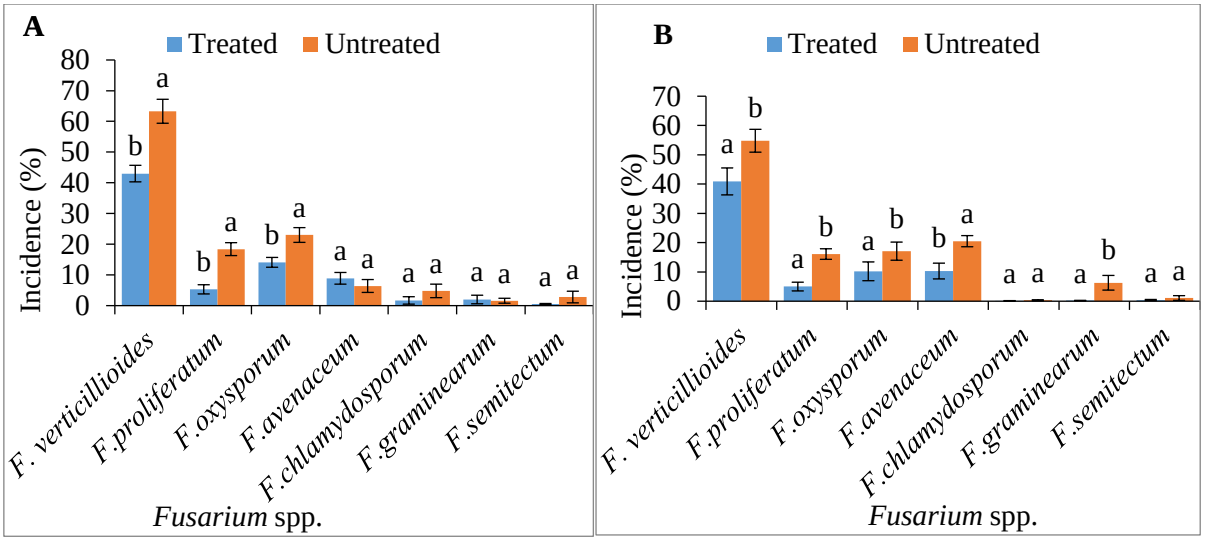


Figure 3. Frequency (%) of *Fusarium* spp. in maize grains sampled at harvest from fields treated with 5kg/ha (A) and 10kg/ha (B) and untreated maize fields.
Error bars represent the standard error of the means.
Means with different letters for each species are significantly different from each other at α≤0.05.

The 5kg/ha rate application of Aflasafe KE01 significantly reduced incidences of *F. verticillioides* and *F. proliferatum* in Kaiti sub-county while in Kathiani sub-county there was a significant difference in the incidence of *F. proliferatum* and *F. oxysporum* between maize samples from Aflsasfe KE01 treated and untreated fields (Table 1). The incidence of *Fusarium verticillioides* varied significantly at (p≤0.05) in treated and untreated maize fields in Kaiti and Kathiani sub-counties but there was no significant difference at (p≤0.05) fields in Nzambani and Wote. *Fusarium proliferatum* and *F. avenaceum* varied significantly in maize fields from Kaiti sub-county only. There were insignificant differences in the population of *F. oxysporum, F. avenaceum, F. chlamydospora, F. graminearum and F. semitectum* between treated and untreated maize fields in all the four sub counties. There was a general decrease in the level of *Fusarium* species in treated maize fields compared to untreated fields (Table 1).

Table 1. Frequency (%) of *Fusarium* spp. in maize grains sampled at harvest from fields treated with 5kg/ha and 10 kg/ha Aflasafe KE 01 in Lower Eastern Kenya.

Fusarium spp.	Treatment	5kg Aflasafe KE 01					10kg Aflasafe KE 01				
		Kaiti	Kathiani	Nzambani	Wote	Mean	Kaiti	Kathiani	Nzambani	Wote	Mean
F. verticillioides	Treated	23.7b	63.2a	74.2a	57.0a	63.3	43.5a	45.2a	73.6a	54.7a	54.3
	Untreated	58.8a	44.9a	62.7a	40.9a	43.1	41.7a	23.4b	75.4a	43a	45.9
F. proliferatum	Treated	1.8b	0.6b	4.9a	13.9a	5.3	4.1b	6b	4.8a	5.2a	5
	Untreated	31.0a	16.7a	0.0a	24.8a	18.1	34a	18.2a	2.8a	9.2a	16
F. oxysporum	Treated	36.0a	4.6b	1.4a	14.5a	14.1	27.8a	20.4a	4.6a	12.1a	16.2
	Untreated	39.7a	20.2a	3.4a	28.5a	23	15.9a	4.5a	18.4a	31.4a	17.6
F. avenaceum	Treated	2.5a	22.8a	8.3a	2.2a	9	23.3a	23a	11.4a	24.3a	20.5
	Untreated	5.4a	12.8a	3.7a	3.7a	6.4	8.3b	23.2a	0a	9.5a	10.3
F. chlamydosporum	Treated	0.0a	0.0a	0.0b	6.9a	1.7	0.5a	0a	0a	0a	0.1
	Untreated	0.0a	0.0a	19.1a	0.0a	4.8	0.1a	0a	0a	1.3a	0.4
F. graminearum	Treated	0.0a	7.9a	0.0a	0.0a	2	0.8a	0b	0a	0a	0.2
	Untreated	0.0a	4.3a	0.0a	2.2a	1.6	0a	20.7a	3.5a	1.2a	6.4
F. semitectum	Treated	0.9a	0.9a	0.0b	0.0a	0.5	0a	0a	0a	1.7a	0.4
	Untreated	0.2a	0.0a	11.1a	0.0a	2.8	0a	0a	0a	4.4a	1.1

Means followed by the same letter(s) within columns for each species in each sub-county are not significantly different (Fisher's protected LSD at $p \leq 0.05$).

Population of fungal pathogens in maize sampled from atoxigenic *Aspergillus flavus* (Aflasafe KE01) treated farms in lower Eastern Kenya

The population of fungal pathogens varied significantly ($p \leq 0.05$) in maize fields treated with Aflasafe KE01 and untreated maize fields especially in *F. verticillioides, F. proliferatum and F. oxysporum.* Maize samples from atoxigenic *A. flavus* treated (5 kg/ha) maize fields recorded lower populations of *Fusarium* species compared to untreated maize fields (Figure 4a). Application of Aflasafe KE01 at the rate of 5kg/ha significantly reduced incidences of *F. verticillioides* and *F. proliferatum* in Kaiti sub-county while in Kathiani, significant differences were observed in the incidence of *F. proliferatum* and *F. oxysporum* between maize grains from treated and untreated fields (Table 2).

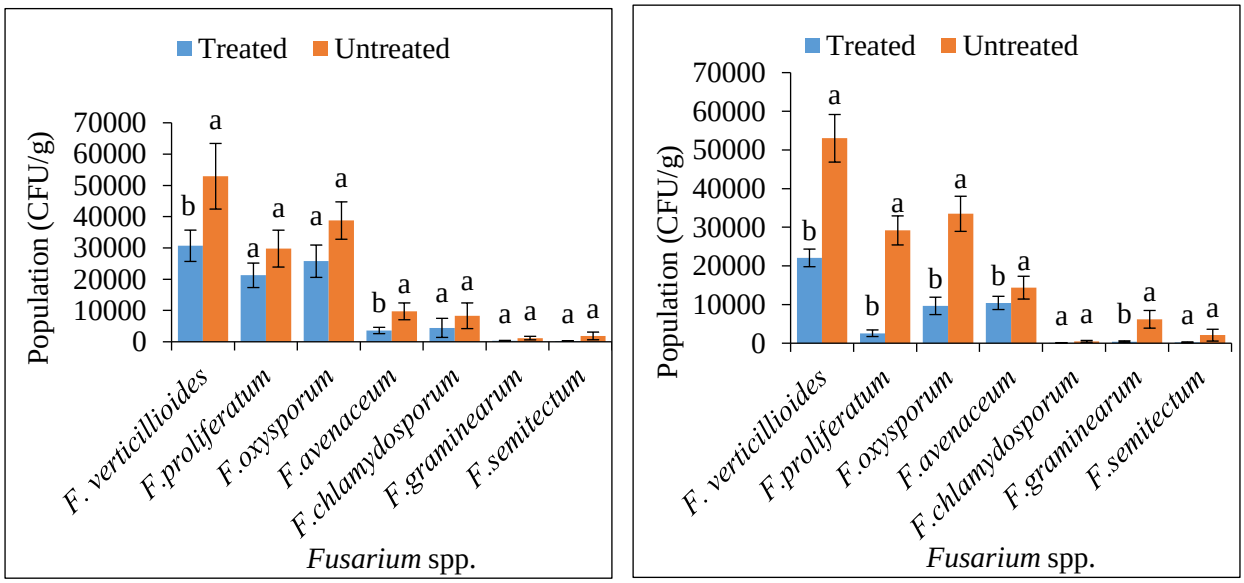
The population of *F. verticillioides* varied significantly at ($p \leq 0.05$) in treated and untreated maize fields in Kaiti and Kathiani sub-counties. However, in Nzambani and Wote, there wERE insignificant differences ($p \leq 0.05$) (Table 2). *Fusarium proliferatum* and *F. avenaceum* varied significantly in maize fields from Kaiti sub-county only. There were insignificant differences in the population of *F. oxysporum, F. avenaceum, F. chlamydospora, F. graminearum and F. semitectum* between treated and untreated maize fields in all the four sub-counties. There was a general decrease in the level of *Fusarium* species in treated maize fields as compared to untreated fields (Table 2).

Table 2. Population (CFU/g) of fungal pathogens in maize kernels sampled from farms treated with 5kg/ ha in four sub-counties in lower Eastern Kenya.

		5 kg/ha				10 kg/ha			
Fusarium spp.	Treatment	Kaiti	Kathiani	Nzambani	Wote	Kaiti	Kathiani	Nzambani	Wote
F. verticillioides	Treated	43333a	23611b	14667a	27500a	21660a	19444b	21889a	25278b
	Untreated	57222a	66667a	59333a	37500a	73889b	44722a	33722a	59722a
F. avenaceum	Treated	556a	11389b	1389a	1111a	14167a	8889b	7500a	11111a
	Untreated	7222a	28056a	556a	3056a	10833a	31389a	0a	15278a
F. graminearum	Treated	0a	1111a	0a	0a	556a	0b	0a	1111a
	Untreated	0a	2778a	0a	1944a	0a	21111a	1389a	2222a
F. semitectum	Treated	278a	556a	0a	0a	0a	0a	0a	833a
	Untreated	278a	0a	0a	0a	0a	0a	0a	8333a
F. chlamydospora	Treated	0a	0a	0b	17778b	278a	0a	0a	0a
	Untreated	0a	0a	33229a	0a	278a	0a	0a	1667a
F. proliferatum	Treated	1944b	33056a	0b	50000a	1944b	3056a	3056a	2222b
	Untreated	99167a	278b	2222a	17500b	55833a	43333b	556a	16944a
F. oxysporum	Treated	43056a	3056b	278a	19444a	20000a	9722b	3889a	5000b
	Untreated	80278a	50556a	4722a	56667a	30278a	24444a	26667a	52500a

Means followed by the same letter(s) within columns in each sub county are not significantly different (Fisher's protected LSD at $p \leq 0.05$).

There was a significant difference ($p \leq 0.05$) in the population of fungal pathogens between the maize grain sampled from fields treated with Aflasafe KE01 (10 kg/ha) and untreated maize fields especially in the population of *F. verticillioides, F. proliferatum and F. oxysporus.* Aflasafe KE01-treated maize fields had significantly lower population of *F. verticillioides, F. proliferatum and F. oxysporum* compared to the untreated maize fields (Figure 4).



Error bars represent the standard error of the means.
Means with different letters for each species are significantly different from each other at $\alpha \leq 0.05$.

Figure 4. The population (CFU/g) of fungal pathogens in maize sampled from farms treated with 5 kg/ha (A) and 10kg/ ha (B) of Aflasafe KE01 in lower Eastern Kenya.

Discussion

From this study, there was high contamination of maize samples with *Fusarium* species. This may be attributed to the conducive weather conditions of negligible rainfall and high temperatures experienced by pre-harvest stages of maize. According to Goertz *et al.* (2010), small amounts of rainfall accompanied by high temperature in the early stages of maize development results in increased infection. Other authors have also reported that high levels of *F. verticillioides* infection are associated with drier, warmer climates (Milani, 2013; Magan and Medina, 2016). According to Doohan *et al.,* (2003) and

Munkvold, (2003), climatic conditions have a direct effect on growth, production and dispersal of inoculum, and also an indirect effect on soil and vegetation type, which may influence saprophytic survival. From this study, it is evident that a range of *Fusarium* species infects maize kernels in lower Eastern Kenya. The observed widespread prevalence of different *Fusarium* species may indicate the possibility of contamination of maize kernels by several mycotoxins other than fumonisin (Logrieco *et al.*, 2021).

Results of this study also indicated that application of atoxigenic *A. flavus* led to a significant decrease in the population and incidence of *F. verticillioides*, *F. graminearum* and *F. proliferatum.* This implies that the atoxigenic *A. flavus* strains present in Aflasafe KE01 reduced the population and incidence of these *Fusarium* species. Similar work done in Brazil showed that the use of an atoxigenic *A. flavus* led to a reduction in the frequency of *F. verticillioides* (Reis *et al.*, 2020). These results suggested a competition for substrate or space between fungi reducing the frequency of *Fusarium* species. Effective establishment of plant with atoxigenic strains could competitively exclude fumonisin-producing strains or prevent them from producing fumonisins (Pereira *et al.*, 2011). The action of competitive exclusion occurs when the non-aflatoxigenic strains effectively compete for space and nutrients, thus excluding their aflatoxigenic counterparts (Kagot *et al.*, 2019). The atoxigenic *A. flavus* strains found in Aflasafe KE01 excluded the toxigenic strain from the niche and competed for nutrients destined for fumonisin biosynthesis.

Other fungal species have been reported to have antagonistic effects that inhibit the growth and proliferation of *Fusarium* species (Wachowska *et al.*, 2013; Samsudin and Magan, 2016; Abdallah *et al.*, 2018). Luongo *et al.* (2005) also reported suppression of saprophytic colonization and sporulation of toxigenic *F. verticillioides* and *F. proliferatum* in maize residues by non-pathogenic *Fusarium* species. Wagacha & Muthomi (2008) suggested that atoxigenic strains of *F. verticillioides* and *F. proliferatum* would be superior bio-control agents for toxigenic strains since they occupy the same ecological niche as the toxigenic

strains in the host plant and share similar growth conditions. This study showed that the concurrent presence of *A. flavus* played a significant role in inhibiting *Fusarium* development on maize kernels across the four sub-counties.

Conclusions

Diversity of the fungal population in lower Eastern Kenya varied among different *Fusarium* species with *F. verticillioides* being the most predominant species isolated across the four sub-counties. *Fusarium grameniearum* was the least isolated *Fusarium* species across all the four sub-counties. Findings from this study showed that application of atoxigenic *A. flavus* (Aflasafe KE01) effectively displaced toxigenic *Fusarium* species in maize in lower Eastern Kenya and thus is a good candidate for a biopesticide.

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Evaluation of pests affecting maize imported across Malawi borders

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Abstract

Cross-border trade is one of the major factors that puts maize at risk as it creates pathways for spread of different maize pests in Malawi. This study was carried out to identify pests associated with imported maize in order to improve phytosanitary measures and reduce the introduction of pests. Random sampling technique was used to collect imported maize at selected borders and commercial cities with the aim of identifying pests present during importation. There were no significant differences (p>0.05) in the population of insect pests before and after incubation in all the surveyed districts. Significant differences were observed after incubation for larger grain borer (LGB) and common maize weevil. The highest percentage infestation (32%) of living LGB was recorded in Karonga and the least in Mzuzu (p<0.05). On the other hand, for maize weevil, the highest percentage infestation (20%) was recorded in Nkhatabay while the least was observed in Lilongwe district (p<0.05). Common fungal pathogen species isolated included *Fusarium* (70%), *Aspergillus* (29%) and *Penicillium* (1%). The highest percentage of kernel infection with *Fusarium* sp. was recorded in Dedza and Lilongwe while for *Aspergillus* sp. were in Mzuzu. The *Aspergillus* sp. isolated were *A. niger* (29%), *A. flavus* (22%) and *A. parasiticus* (5%) while *Fusarium* sp. isolated was *Fusarium verticilloides.* The study provided the status and causes of storage losses by various pests on maize. It is recommended that the phytosanitary system present at various Malawi borders where maize is imported should be strengthened. This can be achieved through human and infrastructure capacity building, strict compliance with importation regulations and improved funding of organizations mandated to ensure phytosanitary compliance.

Key words: *Aspergillus sp.,* consignment, food security, grain storage, random sampling, sanitary and phytosanitary measures.

Introduction

Pests cause significant crop losses worldwide, especially in maize and are barriers to the achievement of global food security and poverty reduction (Reynolds *et al.,* 2015). According to Jimma *et al*., (2016), more than sixty diseases and a number species of insects affect maize worldwide. Global trade and exchange have been identified as major contributors to the dispersal of many pests into different regions of the world where they previously did not exist (Sundstrom *et al*., 2014). Larger grain borer is a known destructive pest that is causing major post-harvest losses in maize in many African countries (Tefera *et al*., 2011). According to Murayama *et al*., (2017), Malawi loses about 40% due to LGB. Other common storage insect pests that affect maize grain and are regulated pests include common maize weevil (*Sitophilus zeamays*) grain moth (*Sitotroga cerealella*) and flour beetle (*Tribolium confusum*) (Murayama *et al*., 2017).

Fungal pathogens are among the causal factors of sixty diseases that affect maize. Ranked second after insects, fungi are among the principal factors that lead to deterioration, poor quality and subsequently, yield loss on farmer's maize across the maize value chain (Suleiman *et al.,* 2015). Common storage fungal pathogens include, *Aspergillus* sp.*, Fusarium* sp. and *Penicillium* sp. which are the most predominant species that attack maize (Odhiambo *et al*., 2013). *Aspergillus, Fusarium, Penicillium* species are known to produce mycotoxins and toxic metabolites. According to Tsedaley and Adugna (2016), these metabolites reduce the quality and viability of maize seed. These fungal pathogens also cause significant plant diseases such as seed rot, seedling blight, Bipolaris leaf spot and Cuvularia leaf spot (Tsedaley and Adugna, 2016).

According to Odhiambo *et al.* (2013), fungal pathogens in maize do not only reduce maize yield but also affect germination, storage and quality, with a potential of affecting trade, human and animal life. Health problems may arise through consuming mycotoxin-contaminated produce (Odhiambo *et al*., 2013). Fungi associated with grain contamination at storage may cause seed deterioration and affect the germination of seed when sown

(Lizárraga-Paulín *et al*., 2013). Development of fungi can be affected by moisture content of the maize, temperature, storage time and degree of fungal contamination prior to storage. Some insects and mites whenever present in the maize grain, facilitate fungal dissemination (Odhiambo *et al.,* 2015). To build a strong phytosanitary system, it is important to know and have a list of pests that can be a useful tool for decision-making and planning of management programs to protect the integrity of a country's resources. The main objective of this study was thus to identify pests that affect maize imported across the borders of Malawi.

Materials and Methods

Sample collection and experimental design and layout

The maize grain samples were collected from the Northern, Central and Southern regions of Malawi, bordering entry points (Dedza, Mchinji, Nkhatabay, Songwe, Muloza and Chiponde as well as the major cities in the regions (Mzuzu, Lilongwe and Blantyre). A total of forty-five maize samples were collected five times from each site. Sampling was done randomly and replicated four times. Complete Randomized Design (CRD) was used in the laboratory to identify live pests and fungal pathogens.

Identification of insect pests in maize samples and other grain contaminants

Four replications of one hundred kernels from each sample was used in the identification of insect pests and other grain contaminants following the method described by Sserumaga *et al.,* 2015. The samples were incubated in glass jars which had previously been cleaned and disinfected with 3% sodium hypochlorite. Using a magnifying glass, data on damaged kernels at incubation, number of kernels with holes, number of kernels with moulds, number of rotten kernels and total number of grain contaminants was collected from each batch of 100 kernels. Data on living pests such as LGB, common maize weevil, grain moth and flour beetle were also taken. This included the total number of living pests, total number of dead pests and total number of all available pests. After incubating the samples for 30 days at room temperature, data was collected with the same set of parameters afore-mentioned being taken. Percentage infection of damaged

kernels was calculated as follows (Sserumaga *et al.,* 2015):

$$\text{Damaged Kernel \%} = \frac{\text{No. of damaged kernel}}{\text{Total number of kernel}} \text{x}100$$

Determination of *Aspergillus* species and other fungal pathogens in the maize kernels

This procedure involved the use of light plastic sandwich boxes (1,850 ml) which were initially sterilized in 99.9% ethanol and allowed to dry. One hundred kernels collected from each district was replicated thrice. The kernels were surface sterilized in sodium hypochlorite at a concentration of 2.5% for three minutes and rinsed in sterile distilled water in three consecutive petri dishes. The kernels were transferred into sandwich boxes lined with three moistened absorbent paper towel sheets and sterilized in UV light for 15 minutes (Marcos, 2015). The boxes were covered with plastic lids and incubated for 24 hours. To suppress kernel germination, the boxes were kept in a deep freezer (-20^{0}C) for 6 hours. Later the sandwich boxes were incubated at room temperature (25±1^{0}C) for a period of 7 days as described by Tripathi, 2018. Percentage infection of the kernels was assessed as described by Sserumaga *et al*. (2015). The incidence of each fungus species was calculated as follows:

$$\text{Infected Kernels (\%)} = \frac{\text{No. of kernels infected by particular spp.}}{\text{Total number of infected kernels}} \text{X}100$$

Fungal identification was based on macro morphological characteristics such as surface of the colonies, texture and micro morphological characteristics like conidia head, shape and vesicle as used by Adithiya *et al.,* 2017. Fungal growth colonies on the kernels were visualized using stereo-binocular microscope and identified to genus level.

Determination of Colony Forming Units (CFU) by *Aspergillus* and other fungal species in the maize kernels

The serial dilution technique was used to determine the colony forming units (CFUs) of fungal species in the maize kernels (Marcos, 2015). A total of 250 maize kernels were surface sterilized with sodium hypochlorite and rinsed using sterile distilled water thrice. The 250 maize kernels were blended into

fine powder. Ten grams of the milled powder was suspended into 90ml distilled water and shaken for 30 minutes using a mechanical shaker. Suspension of 1ml was transferred into 9ml of distilled water, vortexed and diluted into subsequent 9ml up to 10^{-4} dilution. Dilutions of 10^{-2}, 10^{-3} and 10^{-4} were plated in selective molten potato dextrose agar (PDA) media, gently swirled, mixed and incubated at 37^0 C for 5-7 days as described by Jarvis *et al.*, 1986. Each sample was replicated three times and growth was observed using Jenko dissecting microscope at x2-x10 magnification (Sibakwe *et al.*, 2017).

Colony Forming Units (CFU) was calculated using the formula (Metzger *et al.*, 2003):

CFU/g=A*10^n **/**V)

Where A = Number of colonies

10^n = Level of dilution at which counting was carried out

V = Volume of inoculation

Data analysis

The data collected from assessed maize samples was subjected to Analysis of Variance (ANOVA) using GenStat software package (version 18.2) with locations, treatments and samples as factors and measurements as variables. Means were separated using Tukey's Protected Least Significant Difference (LSD) test at 5% level of significance.

Results

There were no significant differences (p<0.05) in kernels infested by LGB in all the sampled districts before incubation. The highest percentage infestation (32%) of living LGB was observed in samples from Karonga followed by Nkhatabay and Blantyre districts. The least percentage infestation was recorded in samples from Mzuzu (5%), Dedza (7%) and Mangochi (3%). On the other hand, there were significant differences (p<0.05) for the total available living LGB in the maize samples after incubation across sampled districts. The highest percentage infestation (25%) was observed in Karonga district while the least was observed in Mulanje and Dedza districts (Table 1).

The population of common maize weevils did not differ significantly (p>0.05) among the districts before incubation but significantly differed after incubation. The highest

percentage infestation (20%) was observed in Nkhatabay followed by Karonga (19%) whilst the least was recorded from Lilongwe and Mzuzu districts (Table 2).

There was no significant variation in the population of grain moth and flour beetle before and after incubation in the different districts (Table 3 and Table 4). There were no significant differences (p>0.05) in the number of living, dead and total available grain moth in the imported maize kernels before and after incubation across all the districts. Similarly, there were no significant differences (p>0.05) in the number of living, dead and total available flour beetle in the imported maize kernels before and after incubation.

Table 1. Population of LGB infesting imported maize before and after incubation (Sample size: 100 grains)

	Before			After		
District	Live	Dead	Total	Live	Dead	Total
Blantyre	0.4	0.4	0.8	0.8ab	2.3	3.1ab
Dedza	0.0	0.0	0.0	0.1a	0.1	0.2a
Karonga	0.2	0.2	0.4	1.5b	3.1	4.6b
Lilongwe	0.0	0.2	0.2	0.5ab	1.9	2.4ab
Mangochi	0.3	0.1	0.4	0.1a	1.1	1.2ab
Mchinji	0.1	0.3	0.4	0.4ab	1.5	1.9ab
Mulanje	0.2	0.1	0.3	0.3ab	0.7	1.0a
Mzuzu	0.3	0.2	0.5	0.0a	2.1	2.1ab
Nkhatabay	0.5	0.3	0.8	1.00ab	0.9	2.0ab
Mean	0.22	0.2	0.42	0.52	1.52	2.06
CV%	310.9	399.6	331.1	202.8	161.2	138.2
LSD	0.5	0.5	0.7	0.8	1.9	2.1
F pr.	0.571	0.852	0.562	0.002	0.177	0.023

Means were separated by Tukey's Protected Least Significance Difference (LSD) at p=≤0.05, Means followed by same letter(s) within columns are not significantly different.

Table 2. Population of common maize weevil infesting imported maize before and after incubation (Sample size: 100 grains)

District	Before			After		
	Live	Dead	Total	Live	Dead	Total
Blantyre	0.0	0.0	0.0	2.4ab	0.3	2.7a
Dedza	0.5	0.3	0.8	1.7ab	0.5	2.2a
Karonga	1.5	0.4	1.9	3.6ab	0.7	4.3a
Lilongwe	0.5	0.1	0.6	0.7a	0.1	0.8a
Mangochi	0.7	0.1	0.8	1.9ab	0.3	2.2a
Mchinji	0.3	0.0	0.3	1.7ab	0.1	1.8a
Mulanje	0.6	0.1	0.7	1.7ab	0.1	1.8a
Mzuzu	0.6	0.2	0.8	1.1ab	0.1	1.2a
Nkhatabay	0.7	0.2	1.0	3.7b	0.3	4.1a
Mean	0.6	0.16	0.77	2.06	0.27	2.34
CV%	253.0	342.3	239.7	124.4	233.2	122.9
LSD	1.1	0.3	1.4	1.9	0.4	2.1
F pr.	0.375	0.534	0.294	0.020	0.277	0.025

Means were separated by Tukey's Protected Least Significance Difference (LSD) at $p=\leq 0.05$. Means followed by same letter(s) within columns are not significantly different.

Table 3. Population of grain moth (*Sitotroga cerealella*) infesting imported maize kernels (Sample size:100 grains)

	Before			After		
District	Live	Dead	Total	Live	Dead	Total
Blantyre	0.3	0.1	0.4	0.7	0.3	1.0
Dedza	0.2	0.1	0.3	0.4	0.5	0.9
Karonga	0.1	0.0	0.1	0.5	0.1	0.6
Lilongwe	0.4	0.1	0.5	0.4	0.3	0.7
Mangochi	0.3	0.1	0.4	0.5	0.1	0.6
Mchinji	0.1	0.1	0.2	0.4	0.2	0.6
Mulanje	0.8	0.3	1.1	0.9	0.1	1.1
Mzuzu	0.1	0.1	0.2	0.1	0.1	0.2
Nkhatabay	0.5	0.1	0.6	0.5	0.1	0.6
Mean	0.3	0.5	0.4	0.5	0.2	0.7
CV%	264.4	378.3	313.3	268	289.6	252.4
LSD	0.6	0.3	0.8	0.9	0.4	1.3
F.pr	0.425	0.643	0.365	0.906	0.483	0.95

Means were separated by Tukey's Protected Least Significance Difference (LSD) at (p=≤0.05).

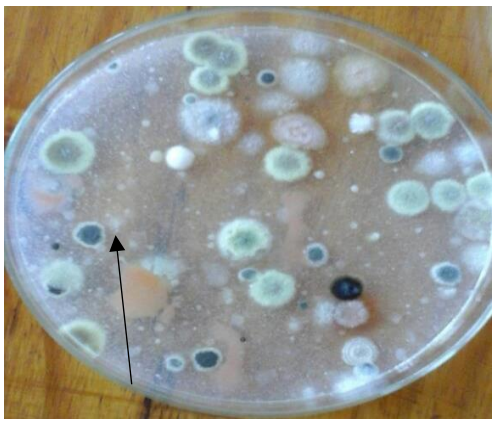
Table 4. Population of flour beetle (*Tribolium castaneum)* infesting imported maize kernels (Sample size: 100 grains)

District	Before			After		
	Live	Dead	Total	Live	Dead	Total
Blantyre	0.1	0.0	0.1	0.2	0.0	0.2
Dedza	0.6	0.2	0.5	0.9	0.0	0.9
Karonga	0.1	0.1	0.1	0.1	0.1	0.2
Lilongwe	0.2	0.0	0.2	0.5	0.0	0.5
Mangochi	0.1	0.0	0.1	0.0	0.0	0.0
Mchinji	0.2	0.0	0.2	0.1	0.0	0.1
Mulanje	0.1	0.0	0.1	0.0	0.0	0.0
Mzuzu	0.3	0.1	0.3	0.3	0.0	0.3
Nkhatabay	0.1	0.0	0.1	0.1	0.0	0.1
Mean	0.2	0.1	0.1	0.2	0.01	0.3
CV%	308.9	602.1	337.2	337.2	1161.9	336.1
LSD	0.4	0.2	0.4	0.6	0.1	0.6
F.pr	0.147	0.212	0.43	0.101	0.439	0.131

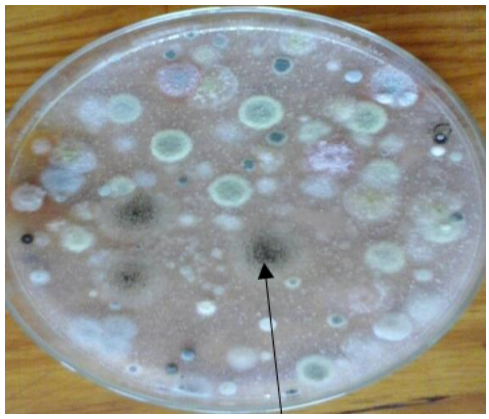
Means were separated by Tukey's Protected Least Significance Difference (LSD) at $p=\leq 0.05$.

Identified fungal pathogens

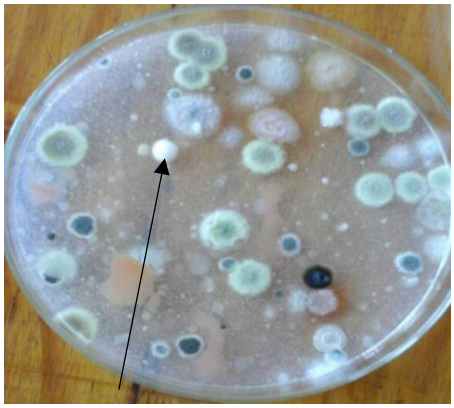
Mycological analysis of the maize samples showed a wide range of fungal pathogens that affected kernels from each district. Common fungal pathogens identified during direct plating and serial dilution included *Aspergillus* species (*Aspergillus flavus, Aspergillus niger, Aspergillus parasiticus*), *Fusarium* species and *Penicillium* species (Figure 1).



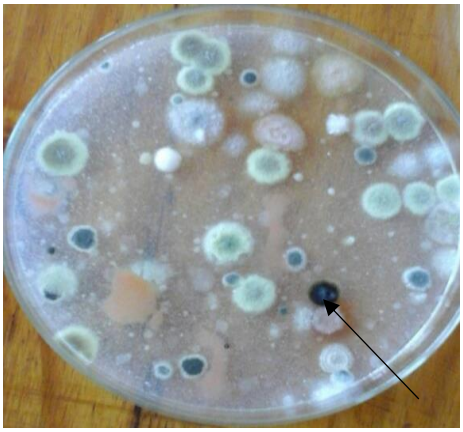
Aspergillus parasiticus



Aspergillus niger



Penicillium



Fusarium verticilloides

Figure 1. Different *Aspergillus* colonies and other fungal species isolated from maize observed at Biotechnology laboratory at Chitedze agricultural research station

The total kernel infection by different fungal pathogens varied significantly (p<0.05) across the districts (Table 5). Kernels with highest fungal contamination were from Mzuzu (15%) followed by Dedza (13%), while the least were from Nkhatabay (10%). Significant differences (p<0.05) of kernels infected with *A. flavus, A. niger* and *Fusarium* were observed across different districts. The highest percentage of kernels contaminated with *A. flavus* were from Mzuzu (26%) followed by Nkhatabay (22%) and Mchinji (18 %). The highest percentage of kernels contaminated by *Aspergillus niger* (22%) were from Mzuzu, followed by Mangochi (16%) and the least from Lilongwe (15%). The highest percentage of kernels contaminated with *Fusarium* were from Dedza and Lilongwe while Mchinji had the least (8%). However, no significant differences were noted in kernels contaminated with *A. parasiticus* and *Penicillium* species (Table 5).

Table 5. Percentage of kernels affected with different fungal species.

District	*A.flavus*	*A.niger*	*A.parasiticus*	*Fusarium*	*Penicillium*	Ttl of infect. Kernels
Blantyre	0.6a	20.2cd	0.2	45.3ab	0.2	66.56ab
Dedza	0.9a	16.9bcd	1.1	62.5b	0.1	81.57bc
Karonga	2.9abc	14.8bc	0.2	53.8ab	0.2	71.88b
Lilongwe	1.7ab	6.3ab	0. 9	61.5b	0.3	70.71ab
Mangochi	0.7a	25.8de	1.1	45.3ab	0.1	73.02b
Mchinji	0.4a	21.7cd	0.8	40.7a	0.1	63.69ab
Mulanje	1.3ab	23.5cd	0.9	47.9ab	0.2	73.78b
Mzuzu	4.2c	36.7e	0.3	54.7ab	0.0	95.89c
Nkhatabay	3.5bc	0.00a	0.4	46.1ab	0.0	49.96a
Mean	1.8	18.4	0.63	50.9	0.13	71.9
CV	121.9	51.5	142.0	34.1	323.9	121.9
LSD	1.580	6.861	0.687	12.55	0.331	1.580
F pr.	<.001	<.001	0.014	0.005	0.722	<.001

Treatments with different letters are significantly different @ p<0.05. Means were separated by Tukey's protected LSD -Least significance different @ 5% confidence.

The number of CFU that were observed during mycological analysis differed across the districts. Significant differences (p<0.05) were observed in the number of CFU of *A. niger*, *A. parasiticus* and *Fusarium* (Table 6). However, no significant differences were noted in the number of CFU of *A. flavus* and *Penicillium* species. The largest CFU of *A. flavus* were from Nkhatabay while the least were observed in Mchinji (24). Dedza had the largest number of CFU of *A. niger* and *A. parasiticus* (32%) while Nkhatabay (25%) and Mulanje (18%) had the least, respectively. The number of CFU of *Fusarium* species was higher in Karonga (24%) followed by Mchinji (15%) while the least were from Blantyre (10%) district. There were no significant differences in total CFU among the districts.

Table 6. Number of CFU by different *Aspergillus* species and other fungal pathogens affecting the maize

District	*A.flavus*	*A.niger*	*A.parasiticus*	*Penicillium*	*Fusarium*	Total CFU
Blantyre	847bc	246ab	436ab	18.3	244.5a	1790
Dedza	104a	768c	1297b	23.3	313.5a	2506
Karonga	418ab	213ab	249ab	34.9	1454.7b	2370
Lilongwe	442ab	475bc	132ab	2.3	617.5ab	1670
Mangochi	692abc	321abc	175ab	15.7	501.6a	1705
Mchinji	101a	364abc	998ab	80.9	917.4ab	2461
Mulanje	415ab	464bc	61a	34.5	604ab	1579
Mzuzu	222a	367abc	151ab	84.9	551.0a	1377
Nkhatabay	1022c	0.0a	529ab	10.7	782.5ab	2344
Mean	473.7	357.6	447.6	33.9	665.2	1978
CV%	192.4	111.3	230.4	284.3	115.2	85.0
LSD	658.7	287.7	745.1	69.69	553.6	1214.6
F pr.	0.068	<.001	0.011	0.195	0.002	0.431

Treatments with different letters are significantly different @ $p<0.05$, Means followed by same letter(s) within columns are not significantly different. Means were separated by Tukey's protected LSD, CV = Coefficient variation, CFU Total: Total fungal colonies available in maize samples.

Discussion

Cross-border agriculture has been identified as one of the pathways that transmit pests across regions. The results of this study have provided key evidence that pests are transmitted into Malawi as maize is traded with other countries. The implication of these results suggests that imported maize grain is an important pathway in transmission of both regulated and non-regulated maize pests which affect quality and quantity of maize value chain.

During the study, A*spergillus* was found to be the most predominant fungal pathogen, corroborating findings by Tsedaley and Adugua (2016) who showed that the most predominant

fungal species isolated from maize kernels belonged to the *Aspergillus* spp. Ozay *et al*. (2008) and Nyasetia (2015) also noted that *Aspergillus* spp and other fungal pathogens had contaminated maize and hazel nuts kernels at storage. The presence of fungal pathogens such as *Aspergillus* is influenced by the percentage of the available factors. These include; damaged kernels, presence of foreign matter and impurities, presence of microorganisms, insects and mites, period of storage moisture content, the relative humidity, storage atmosphere and length of storage (Nyasetia, 2015; Ozay *et al*., 2008). Because of these conditions, the corn kernels lose mass, volume and strength and experience nutritional degradation, discoloration, development of unpleasant odors, heat and chemical changes. High *Aspergillus* population levels may also be attributed to late harvesting (Wanjiku, 2016).

During the study, it was observed that storage methods can increase the population of living pests. These storage facilities especially when unsealed, create a favorable environment for breeding and multiplication of pests. These results concur with Tefera *et al*., (2011a) who found that there was an increase of population of living pests due to storage environment. In addition, the study by Tefera *et al*., (2011) also suggested that temperatures between 27°C-32°C and 38°C increased the population of maize pests at storage. The current study however, contradicts with the findings of Bell (2014) who observed that population of insect pests decreased between 27°C-32°C and 38°C. According to Suleiman *et al*., (2015) storage of maize while relatively moist and warm may lead to rapid deterioration of the grains and promote the growth of microorganisms. In tropical and subtropical countries, a large portion of grain is harvested and stored under hot and humid conditions and most farmers lack proper knowledge, equipment and methods of drying grains (Matumba *et al*., 2015). Subsequently, maize being hygroscopic in nature, tends to absorb or release moisture (Matumba *et al*., 2015, Wanjiku, 2016) leading to increased maize moisture contents and increased deterioration. Given that the districts in which this study was carried out experience hot and humid conditions, this may have created conducive

environment for the development of fungal pathogens.

Conclusion and recommendations

This study showed that cross-border trade is responsible for the spread of fungal pathogens and living pests associated with maize imported into Malawi. The detection of various living pests and pathogens before and after maize kernel incubation is a cause for concern because the pests affect maize across the value chain hence reducing the quantity and marketable quality of the produce. The results from this research should be considered in order to improve the effectiveness of Malawi's phytosanitary system. Imported maize should be sampled and analyzed for pests at borders before being released for consumption in various districts. Other imported materials posing the same threat should also be closely monitored.

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Impacts of selected Climate Smart Agricultural Practices on African Indigenous Vegetables in Kenyan drylands

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Abstract

Climate change has had significant impacts on the cultivation of African Indigenous Vegetables (AIVs) resulting in insufficient yields and food insecurity. African indigenous vegetables are important food crops used in combating malnutrition and food insecurity. The AIVs have the potential to provide **nutrition and sustain smallholder farmers' livelihoods**. However, little is known about the impacts of Climate-Smart Agriculture Practices (CSAPs) on the yield levels in AIVs. This study was therefore conducted to evaluate the impacts of selected CSAPs on the yield levels in cowpeas (*Vigna unguiculata.* L) and black nightshade (*Solanum nigrum*. L) in Kenyan drylands. Six treatments consisting of organic manure, commercial organic fertilizers, irrigation, mulching, inorganic fertilizers and controls were used. Research plots measuring 3m by 3m were set out in a randomized complete block design and sowed with certified cowpeas seeds and well-established black nightshade seedlings. Treatments were applied at land preparation and at 7 days after crop germination. Data was collected on plant height at vegetative stage and the overall primary yield at crop maturity. Analysis of variance (ANOVA) was conducted on the quantitative data collected and analyzed using Genstat software. Post hoc analysis was carried out for significant means using Tukey's Honest Significant Difference (HSD) test at $p \leq 0.05$. The results revealed significant differences in both the plant height and primary yield across all treatments ($p \leq 0.05$). The AIV yield levels were significantly influenced by the CSAPs products used. Plots treated with organic manure, mulching and commercial organic fertilizers had significantly higher yields. Control plots had the least amount of yields.

Key words: ANOVA, Climate change, food security, malnutrition and soil degradation.

Introduction

Generally, there is limited capacity in controlling the rate of climate change within the 2°C threshold necessitating a need to cope with its effects (Rogelj *et al.* 2011) Sub-Saharan Africa (SSA) is the most affected by climate change (Cline, 2007). Climate change has had a significant impact on the cultivation of AIV resulting in insufficient yields and food insecurity (Fox *et al.,* 2004). Soil degradation has also been identified as a leading cause of reduced agricultural production among smallholder farmers (Kimaru, 2003). Among the soil nutrients, nitrogen is the most important and the most deficient mineral element. The use of inorganic fertilizers has been reported to cause an increase in soil pH causing more acidity in the soil. Most crops perform well in pH levels of 6.0-7.0. Low pH levels lead to aluminium toxicity, which interferes with the uptake of other elements such as phosphorus, molybdenum and reduction in soil microbial activities (Kimaru, 2004). It is therefore important to perform soil tests before applying inorganic fertilizers. Good soil fertility improves vigorous vegetative growth and increases leaf production. Climate-smart agricultural practices play a crucial role in enhancing resilience, reducing greenhouse gas emissions, increasing productivity per unit area and mitigating environmental degradation (FAO. 2017). Despite the crucial role played by CSAPs, their adoption by small-scale farmers has been poor globally (Lipper *et al.,* 2014).

Out of the 45, 000 plant species available in SSA, 1000 of them can be eaten as leafy vegetables (Maundu *et al*., 1999). According to Muhanji *et al* (2011), there has been active cultivation of AIVs in SSA for many generations as part of the food systems. African indigenous vegetables form part of Kenyan culture and cuisine. Common indigenous vegetables include; cowpeas, leaf amaranth, black nightshade, jute mallow, *Crotalaria* species, and Cleome species (Abukutsa-Onyango, 2007). These vegetables have the potential to provide nutrition and sustain smallholder farmers' livelihoods**.** According to Ekesi (1999), Kenya has more than 200 species of indigenous vegetables. Indigenous vegetables are naturally rich in nutrients such as

vitamins, minerals and micronutrients (Afari-sefa *et al*., 2012). African indigenous vegetables have several medicinal values and health benefits such as managing stomach problems, constipation, respiratory diseases and skin ailments (Kokwaro, 2009). The black nightshade (*Solanum nigrum* L) and cowpeas *(Vigna unguiculata* L*)* vegetables are rich in vitamins, minerals and proteins. These leafy vegetables also contain essential phenols and alkaloids which include; nicotine, quinine, cocaine and morphine, known for their medicinal properties. The vegetables are able to provide a wide range of food and main dish accompaniments (Maundu *et al*., 1999). Despite their growing popularity and diverse health benefits, research on the production aspects of AIVs has not been done (Yang *et al*., 2009).

African indigenous vegetables are mainly produced on subsistence basis and are mostly planted around the house, together with bananas, maize, cassava and sorghum (Kimiywe e*t al*., 2007). Most vegetable production is rain-fed (Banwat, 2012). Cowpea is the most important legume owing to its main economics (Langyintuo *et al.,* 2003). It is an economically and nutritionally important vegetable, which can be harvested for tender and less fibrous leaves (Odhiambo *et al*., 2021) Black nightshade (*Solanum nigrum.* L) is the second most important AIV vegetable in Kenya after cowpeas.

Agriculture is particularly vulnerable to natural and environmental disasters (FAO. (2018). Crop cultivation in ASALs is heavily dependent on local weather dynamics, climate, land and water to thrive. The ever-alarming levels of weather extremes such as droughts and floods for the last 10 years have negatively affected agricultural production in Kenya, mostly in the ASALs. Murang'a South sub-county is centrally located in the ASAL region. The region has low soil fertility levels, water scarcity and very fragile soils making it a suitable site for this study. African Indigenous Vegetables are highly dependent on good farming practices for good yields (Womdim *et al.,*2012). Therefore, the purpose of this study was to determine the effects of selected CSAPs on the yield of two African indigenous vegetables; cowpeas and black nightshade in Kenyan drylands.

Materials and methods

Study site description

The research work was carried out in Ithanga location, Murang'a south sub-county in Murang'a County. Murang'a County lies between latitudes 0° 34′ South and 1 ° 07′ South and longitudes 36° East and 37° 27′ East. Ithanga location is found in the eastern part where semi-arid conditions prevail. The area has two rainfall seasons per year; March to May (long rains) and October to December (short rains). Ithanga has average temperatures ranging from 21-35˚C (Figure 1). The study area is characterized by humic nitisols and a dense population of averagely 404.5 people per square km (Kiboi *et al.*, 2018). On-farm experiments were conducted during the short rainy season of October to December 2021 and long rainy season of March to May 2022.

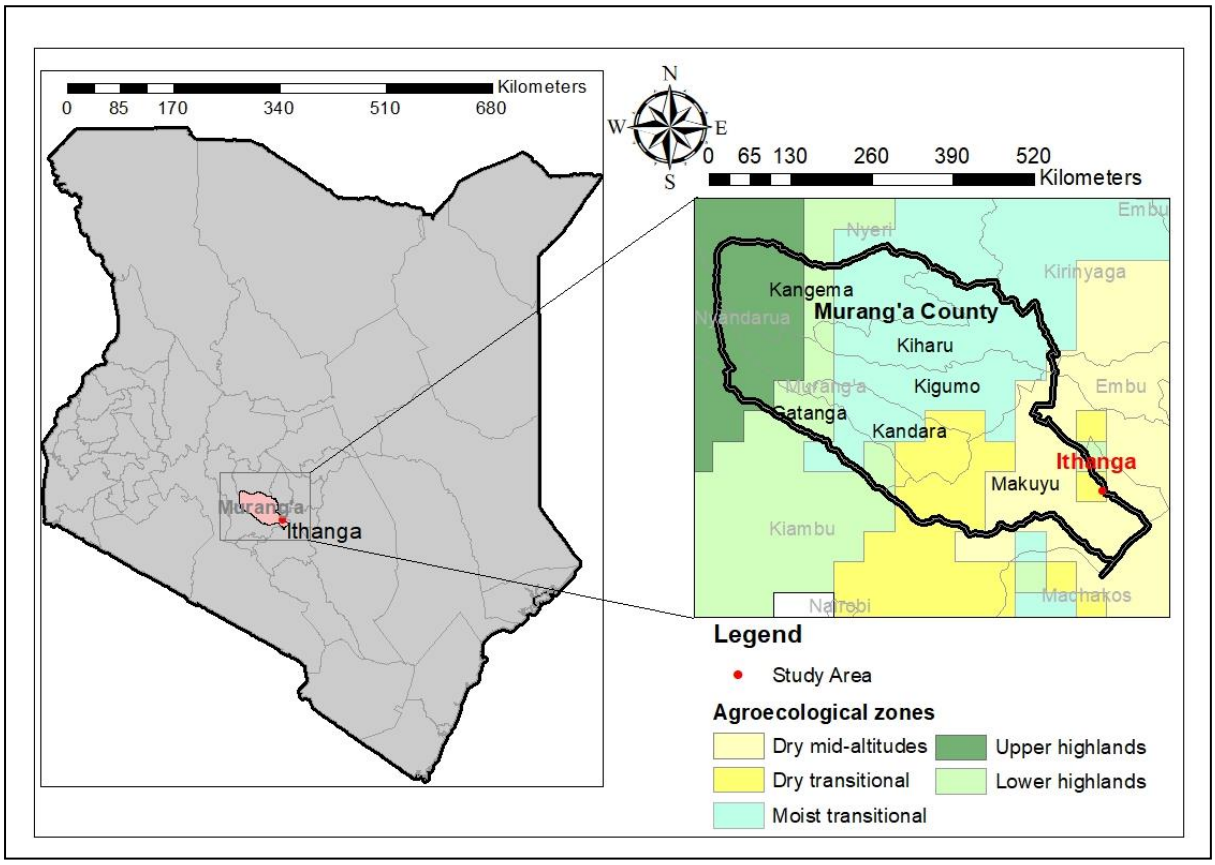

Figure 1. Map of Murang'a County showing Ithanga location, the study site.

Source of vegetable seeds

The certified cowpea variety M-66 and giant leaf variety of black nightshade used in this study are known to be drought-resistant, high yielding and have robust vegetative growth. Ten grams of the hybrid black nightshade seeds were sourced from the local agrovets and propagated on-site. Two kilograms of cowpeas seeds were sourced from KALRO-Katumani.

Experimental layout

The experiments were set out in a randomized complete block design (RCBD) consisting of six treatments replicated three times. The six treatments were; well decomposed organic manure, organic fertilizer (Lisha organic), surface irrigation, mulching (dry grass), farmer practices (CAN and NPK 17.17.17 inorganic fertilizers) and control (no treatments). For each of the focal crops, 18 experimental plots were set up. Square plots measuring 3m by 3m were sowed with 243 seeds for cowpeas and 81 seedlings for black nightshade. They were planted with a spacing of 30cm between plants and 30cm between rows for uniform crop density across all plots. 30g of organic manure was applied per hole at land preparation. Two weeks after crop germination, 5g of inorganic fertilizer (a mixture of NPK 17:17:17 and CAN 26% in 50% ratio) procured from the local agrovets was applied. Mulching using dry grass material was done 2 weeks after planting. Surface irrigation was done once every week using plastic watering cans.

Planting and crop management

Three cowpeas seeds were sowed per hole to increase the chances of germination. After thinning and gapping crops, the population was maintained at 162 plants per plot. Yellow and blue stick cards were used for monitoring insect pests. Black nightshade seedlings were first introduced in the nurseries for effective management during early stages of growth and to ensure seedling quality. Weeds were removed physically by handpicking. Two weeding regimes were carried out. Thinning of excess plants was done one week after seed germination. Weak and deformed plants were removed and discarded. Gapping was done to maintain the seed population at 162 plants per plot.

Fertilizer/manure application

Soil tests were conducted prior to planting and the fertilizers (organic/inorganic fertilizers) applied in accordance with the soil test results. The soil tests were conducted at the Kenya soil survey laboratories located at NARL–KALRO in Kabete.

Pest and disease control

Pest control was done through application of broad-spectrum biopesticides (pyrethrin with garlic extracts) supplied by Juanco SPS Limited based in Ngong, Kajiado County. Preventive disease control was done by fungicides application. Two regimes of fungicide application using Ridomil gold MZ 68 WG were done at 2 weeks and 5 weeks of growth.

Data collection and analysis

Efficiency of the selected CSAPs was determined by comparing yields across treatments. Data was collected on plant heights at vegetative stage and the overall primary yield at crop maturity. Weight of harvested leaves, plant height and primary yield were recorded and compared across all treatments. Data was collected over 3 months' duration for two consecutive seasons. Recorded data was entered in Microsoft excel spreadsheet, analyzed and compared across the six treatments. Analysis of variance (ANOVA) was conducted on the quantitative data collected using Genstat software (Genstat-Edition 22). Post hoc analysis was carried out for significant means using Tukey's Honest significant difference (HSD) test at $p \leq 0.05$.

Results and discussion

Effects of CSAPs on plant height in cowpeas and black nightshade

There were significant differences in heights of cowpeas plants across the treatments ($p \leq 0.05$). Five treatments were tested in the experiments and results were compared with control plots that had no treatment products applied to them. The plant heights ranged from 24.73cm to 34.20cm. Plants in plots treated with commercial organic fertilizers and organic manure had significantly taller plants (34.2±1.58cm and 30.17±2.55cm respectively) compared to plants that had been mulched with dry grass materials (24.73±1.99cm). However, plants in plots treated with organic fertilizer and manure did not differ significantly with plants exposed to

farmer practices, irrigation and controls. (table 1). The experiments used commercial organic fertilizer rich in N, P and K. Studies done by Shah *et al.* (2001) showed a clear correlation between nitrogen application and growth parameters such as height, leaf size and crop biomass which was correlated with the plant's photosynthetic activities. Plots treated with dry grass mulching had the shortest plants followed by farmer's practices (26.00±0.68cm) and controls (28.33±1.80cm). There was no significant difference in height of plants that were mulched, under farmer practices, irrigated and controls. This study contradicts the work done by Kuru *et al*. (2020) who revealed that mulching using dry grass materials significantly increased the plant height in maize and beans in experiments carried out in Wairaka, Jinja, Uganda.

At vegetative stage, there were significant differences in black nightshade plant height across all the treatments ($p \leq 0.05$). Irrigated plots had the highest plant height at 26.33±1.41cm followed by organic fertilizers (20.00±0.73cm) and organic manure (19.33±2.12cm) respectively. Farmers' practices plots had the least plant height followed by mulching and controls respectively (table 1). The three treatments did not have significant effect on black nightshade height. The plant heights ranged from a high of 26.33±1.41cm in irrigated plots to 12.35±2.05cm in farmer's practices plot. There was no significant difference in plant heights among organic manure, organic fertilizer, mulching, farmer practices and control plots. In addition, there was no significant difference in plant height among organic manure, organic fertilizer, irrigated and control plots. Plants in plots under irrigation however showed significantly higher heights compared to plants under mulching and farmer practices. The results agree with Saleh *et al*. (2018) who found out that irrigation water increased the vegetative growth such as plant height and the number of leaves in two bean cultivars.

Differences in plant heights in the two crops can be attributed to effects of treatments applied. Farmers' practices plots were applied with CAN and NPK inorganic fertilizers. The use of inorganic fertilizers has been reported to cause a decrease in the soil pH

causing more acidity in soil and this may have negatively affected the plant height. Most crops perform well in pH levels of 6.0-7.0. Low pH levels lead to Al toxicity, which interferes with the uptake of other elements such as P, Mo, and reduction in the soil microbial activities (FAO, 2017). Black nightshade does well in the pH range of 6.0-6.5. Good soil fertility improves vigorous vegetative growth and increases leaf production (Zhao *et al.,* 2009).

Table 1. Mean plant height in cowpeas and black nightshade at vegetative stage.

Treatment	**Cowpeas Mean height (cm)±SE**	**Black nightshade Mean height (cm)±SE**
Control	28.33±1.80ab	18.58±2.35ab
Farmer practices	26.00±0.68ab	12.35±2.05a
Irrigation	29.00±1.10ab	26.33±1.41b
Mulching	24.73±1.99a	15.17±2.88a
Organic Fertilizer	34.20±1.58b	20.00±0.73ab
Organic Manure	30.17±2.55b	19.83±2.12ab
***p* Value**	**0.00608 ****	**4.875e-05 *****

Means followed by the same letters within a column are not significantly different according to the LSD test at p=0.05

Effects of CSAPs on primary yield in cowpeas over two growing seasons

During the short rains (October to December 2021), there were significant differences in the amount of primary yield recorded across the various treatments ($p \leq 0.05$). Mulching (3341.67±0.29g) recorded significantly higher yields followed by irrigated plots (3048.33±0.28g), organic manure (2756.67±0.73g), organic fertilizers (2441.67±0.75g) and farmer's practices (1600±0.75g). Control plots (988.33±0.57g) had the least amount of primary yields compared with the other treatments (table 2). Mulching with dry grass material provided a prolonged moist environment, smothered all weeds and ensured continued supply of nutrients through the decaying biomass. This explains the relatively higher yields recorded in the mulching plots. Gradual organic matter mineralization releases essential

nutrients into the soil, which are made available for plant absorption and growth. The findings from this research corroborate results by Niang *et al*. (1996) who noted that adding fresh *Tithornia* biomass increased maize yield. Similar results were also obtained by Buyushan *et al*. (2002) who used polythene mulch and dried sorghum straws which considerably improved the crop height, dry matter weight and the quantity of leaves in maize.

Farmer's practices plots were applied with CAN and NPK inorganic fertilizers in a ratio of 1:1. Use of fertilizers increases the soil's natural fertility (El-Aziz, 2007). Fertilizers are designed to directly meet plant needs by altering aspects of the soil's structure and pH. The quantity and quality of plant growth are greatly improved when the right fertilizers are applied to soils (Liu, 2010). Low yields recorded in control plots was due to lack of soil fertility improvement products that were used in the other treated plots. According to Cockroft *et al*. (2000) crop yields can be tripled by the use of irrigation water. Well-irrigated crops have increased photosynthetic rates and are able to also withstand attack by some insect pests leading to higher yields as recorded in this study. These results agree with those obtained by Fageria (2006). Dry soils caused by reduced rainfall or lack of soil cover such as mulching leads to low productivity. During dry weather, lack of sufficient moisture makes it difficult for the plants to take up essential nutrients from the soil for better crop performance. Irrigated plants tend to be less stressed and have better chances of reaching physiological maturity (Stewart, 1990). Optimal nitrogen presence in the soil due to application of inorganic fertilizers and organic manure led to increased photosynthetic activity and thus yields (Nduwimana *et al*., 2020).

During the long rains (March to May 2022), there were significant differences in primary yield recorded across all treatments ($p \leq 0.05$). Plots treated with organic manure produced significantly higher yields (2825± 0.39g) followed by organic fertilizer (2263.33± 0.77g) and mulching (2195±1.89g). Controls had the least amount of yields at 471.67± 0.57g followed by farmer's practice (1080±0.78g) and irrigated plots (1911.67±0.75g) (table 2). Low yields

recorded in control plots was due to lack of soil fertility improvement products and moisture that were increased in the other treatment plots. The differences in primary yield in cowpeas under different treatments can be attributed to effects of the products applied. Climate-smart agricultural practices/products reduce pest populations, improve plant health and thus yield levels. Further, the study findings indicate that there was significant difference in the yields recorded in control and farmer's practices plots over the two seasons. An inadequate supply of primary (N, P, and K) nutrients leads to poor yields (Burney *et al.,* 2012). This means that despite the differences in rain and weather patterns over the two seasons, failure by farmers to implement CSAPs will continuously result in poor yields. Mulching, irrigation, organic manure and organic fertilizer recorded significantly different yields over the two seasons with season one having comparatively higher yields. This could be explained by the heavier and extended rains, which were witnessed in the October to December 2021 short rainy season. The rains diluted the nutrients supplied by mulching materials, organic manure and organic fertilizer and made them readily available for the plant's uptake. The weight of harvested cowpea leaves was positively impacted by grass mulch (table 2). These findings agree with Jodaugienė *et al.*(2010) and Lorenzo *et al.* (2011) who recorded increased crop yields attributable to mulching.

Table 2. Mean primary weight in cowpeas under different CSAPs over two growing seasons

Cowpeas	Short rainy seasons (Oct-Dec 2021)	Long rainy season (March-May 2022)
Treatment	**Mean weight (g) ±SE**	**Mean weight(g) ±SE**
Control	988.33±0.57a	471.67±0.57a
Farmer Practices	1600±0.75b	1080±0.78b
Irrigation	3048.33±0.28e	1911.67±0.75c
Mulching	3341.67±0.29f	2195±1.89cd
Organic Fertilizer	2441.67±0.75c	2263.33±0.77d
Organic Manure	2756.67±0.73d	2825±0.39e
***p*-value**	**1.609e-05 *** **	**1.609e-05 *** **

Means followed by the same letters within a column are not significantly different according to the LSD test at $p \leq 0.05$.

Effects of CSAPs on primary yield in black nightshade over two growing seasons

During the short rains (October to December 2021), there were significant differences in the primary yield recorded among all the treatments ($p \leq 0.05$) (table 3). There was a significant difference between the yield levels across the two seasons and across treatments. During the long rainy season, significantly higher primary yields were recorded ($p \leq 0.05$) compared to short rainy season ($p \leq 0.05$). During short rainy season, irrigated plots recorded the highest yields (1825±1.79g) followed by organic fertilizer (1683.33±4.34g) and mulching (1528.33±1.71g) respectively. However, there was no significant difference between yields from plots where mulching was done and where organic manure was applied. Controls (791.67±1.22g) had the least amounts of yields followed by farmer's practices (1026.6±2.02g) and organic manure (1453.33±1.34g) (table 3).

According to Burney *et al*. (2012), irrigation makes it easier to use other productivity-boosting inputs and intensifies smallholder-farming methods. Irrigation water dilutes available nutrients and makes them available for plant uptake thus irrigated fields produced the highest yields. However, the heavy and extended rains witnessed in the short rainy season from October to December 2021 negatively affected the yields by causing nutrient leaching and erosion of nutrients through surface runoff. Surface runoff leads to soil erosion of highly nutritious top soil and plant nutrient depletion. Leaching removes nutrients from surface soils (top soil) and moves them to deeper layers making them inaccessible to the plants. The downward movement of the nutrients further denies plants essential minerals meant for their proper growth. This leads to stunted plants and thus poor yields.

During the long rainy season (March–May 2022), plots treated with organic manure had significantly higher yields (3226.67±3.54g) followed by plots treated with commercial organic fertilizer (2758.33±3.19g), mulching (2690±2.1g), irrigation (2306.67±2.12g), farmers' practices (1875±1.11g) and controls (1373.33±0.77g) respectively (table 3). The moderate and evenly distributed rainfall experienced during this season effectively diluted all the applied products and made them available for the plant's uptake. Control plots recorded the least amount of yields followed by farmer's practices plots that had been treated with inorganic fertilizers (table 3), though the difference was significant. Plots treated with organic manure had significantly higher yields compared to all the other treatments. Soil organic carbon found in organic manure is the main component of soil organic matter (Yemefack *et al*.,2006). Soil organic matter affects plant growth since it is a source of energy and triggers nutrient availability through mineralization process (Yemefack *et al*., 2006). Soils applied with organic manure have robust microbial activity (Jabeen *et al*., 2018) which further improved the crop yield. Further, Ahmad *et al*. (2022) discovered that soils' water holding capacity is highly improved by the soil organic matter and this probably explains why plots applied with organic

manure recorded higher yields in the second season.

Significantly, higher yields were recorded during the long rainy season compared to short rainy season across all treatments. There were heavier rains witnessed during short rainy season in the study sites. This created damp soils and probably caused nutrient leaching. Black nightshade requires well-drained soils for proper nutrient uptake and growth. The damp soils lowered the soil temperature and reduced the plant's metabolic rates leading to poor growth and low yield. The long rainy season had modest rainfall, which provided well-drained soils which resulted in better crop performance. The highest yield in crops relies on the plant's maximum photosynthetic productivity (Singh and Singh, 2007).

Table 3. Mean primary weight in black nightshade under different CSAPs over two growing seasons.

Black nightshade	**Short rainy season (Oct-Dec 2021)**	**Long rainy season (March -May 2022)**
Treatment	**Mean weight (g)±SE**	**Mean weight (g) ±SE**
Control	791.67±1.22a	1373.33±0.77a
Farmer Practices	1026.6t±2.02a	1875±1.11b
Irrigation	1825±1.79c	2306.67±2.12c
Mulching	1528.33±1.71b	2690±2.10d
Organic Fertilizer	1683.33±4.34bc	2758.33±3.19d
Organic Manure	1453.33±1.34b	3226.67±3.54de
***p*-value**	**7.014e-05 *** **	**0.0001969*** **

Means followed by the same letters within a column are not significantly different according to the LSD test at $p \leq 0.05$.

Conclusion and recommendations

Yield levels in the two indigenous vegetables were significantly influenced by the CSAPs used. Plots treated with organic manure, mulching and commercial organic fertilizers produced higher yields across all crops and growing seasons. Control plots had the least yields. These findings suggest that farmers can achieve excellent yield results with the adoption of CSAPs. More research is needed on the long-term effects of CSAPs on soil structure and fertility. The study further recommends that farmers should use sustainable farming practices that preserve soil fertility and structure and increase AIVs crop yield.

Acknowledgements

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Farmer perception, knowledge and management of the scale insect pest complex infesting crops and trees in Coastal Kenya

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Abstract

Scale insects and mealybugs (Hemiptera: Sternorrhyncha: Coccomorpha) are serious plant sap-sucking pests affecting a wide range of cultivated crops and tree species. The insects are highly diverse and can have devastating effects on yields due to lack of farmer awareness and inappropriate management practices. Recent alien mealybug invasions in Kenya include among others, papaya mealybug (*Paracoccus marginatus*), a highly invasive pest that is spreading quickly. Farmer experience with diverse crop pests can support development of a successful pest management strategy and contribute to reduced impacts on both crop yields and agro-biodiversity. This socio-economic survey was carried out in three coastal counties of Kenya (Mombasa, Kilifi and Kwale) to establish the impact of scale insect and mealybug pests on farmer livelihoods and to document the perception, knowledge and management practices used by smallholder farmers. Data from oral interviews using ODK were administered to respondents and analysed using Excel and GENSTAT. It emerged that 26% of the respondents were familiar with scale insects and 51% with mealybugs, respectively. Of these, 78.13 % and 94% acknowledged having encountered scale insects and mealybugs, respectively, on their farms. The farmers confirmed that scale insect pests affected a high diversity of crops and trees. About 56% of the respondents used pesticides to control the pests while 25% did not apply any management strategy. The remaining 19% practiced cultural control methods such as field sanitation and intercropping with less susceptible crops. Pesticides were reported to be moderately effective (41-70%) at controlling scale insects and mealybugs. Other management options reported by farmers included farm hygiene, the use of high-pressure water jets, and applications of ash and bio-pesticides such as home-made neem extract. Based on the findings, it is recommended that capacity building for farmers and input providers be undertaken to enhance the knowledge of scale insect and mealybug pest biology, symptoms of crop and tree attack, host ranges and management practices. Additonally, training on the use of low-risk pest control products and innovative control methods should be undertaken to reduce the impact of the pest on crop production and agro-biodiversity.

Keywords: Farmer knowledge, management, mealybugs, scale insects and socio-economic survey.

Introduction

Scale insects (including mealybugs) are pests belonging to Order Hemiptera, superfamily Coccoidea (Gullan and Cook 2007). They are serious pests of several crop and fruit species including cassava (*Manihot esculenta*), papaya/pawpaw (*Carica papaya*), citrus, egg-plant (*Solanum melongena*), soursop (*Annona muricata*) and ornamentals, among others (Franco *et al.* 2009; Mazzeo *et al.* 2014). These pests cause damage by sucking plant fluids from leaves, stems and sometimes roots (e.g. in the case of groundnuts) (Kondo *et al.* 2008). The coffee mealybug (*Planococcus kenyae*) attacks coffee and a large number of wild and cultivated plants including yam (*Dioscorea rotundata*), pigeon pea (*Cajanus cajan*), passion fruit (*Passiflora edulis*), sugarcane (*Saccharum officinarum*) and sweet potato (*Ipomoea batatas*) (Watson and Ouvrard, 2019).

In the last seven years the scale insect population in Kenya has greatly increased, with these pests being reported in most parts of the country, causing serious yield losses in crops and trees including native species. In the coastal region, an invasive mealybug pest, papaya mealybug, *Paracoccus marginatus* Williams & Granara de Willink, was first reported on papaya in 2016 (Macharia *et al*., 2018). However, the problem has since escalated to affect other crops and fruit trees in other areas. Yield losses of affected crops have been estimated to be as high as 91% and it is feared that the percentage may increase after recent identification of 66 more potential scale insect pest species new to Kenya, most of them non-native (Watson *et al*., 2021; Macharia *et al.,* 2021).

In Africa, a number of farmers still depend on indigenous methods to manage pest problems (Abate *et al*., 2000). However, in Coastal Kenya majority of the farmers were reported to use pesticides as their only method to control scale insects and mealybugs since they had inadequate knowledge of any other form of pest control (CABI, 2020). However, there are several impediments to the successful management of these pests. Scale insects have a waterproof layer of wax coating their bodies which repels aqueous contact pesticides and their habit of feeding on leaf undersides and

in other cryptic sites can make them unreachable by regular pesticide application techniques (Ouvrard *et al*., 2013). Unfortunately, most farmers are not aware of these pest characteristics making it difficult for them to apply pesticides effectively. Understanding of these challenges could help farmers in future management of the pests. Therefore, this survey was carried out to understand how farmers perceive and understand the pests especially in management perspective.

A socio-economic survey was carried out in three coastal counties (Mombasa, Kilifi and Kwale) to establish the perception and knowledge of scale insects including mealybugs by smallholder farmers and the impact on their livelihoods. Information on farmers' current perceptions of scale insect control practices and available resources may provide essential data on acquired skills and indigenous knowledge for the successful development of pest management strategies and contribute to rural development at the county level.

Kilifi and Kwale counties are the main farming areas in the coastal region of Kenya due to the availability of sufficient land and a larger workforce (Okutoyi, 2021). Earlier studies in the area by Wekesa *et al*. (2017) observed that farming activities have been declining over time. This is mainly due to unpredictable rainfall and increased problems with weeds, insect pests and diseases, making the people to turn to alternative sources of livelihood. Presence of new pests such as scale insects exacerbates the problem further. In Mombasa, most of the areas is mainly residential with the major economic activity being fishing and ecotourism. Farming though present, occupies a small percentage. There is lack of information on whether the farmers at the coastal region are aware of these insect pests and actions that they undertake to deal with them.

Methodology

Study sites

The study was carried out in three different coastal Counties: Mombasa, Kwale and Kilifi (Figure 1), in mid-2019. In all the three Counties data was collected from 12 sub-counties. Farmers were selected randomly with the guidance of the county agriculture extension officers.

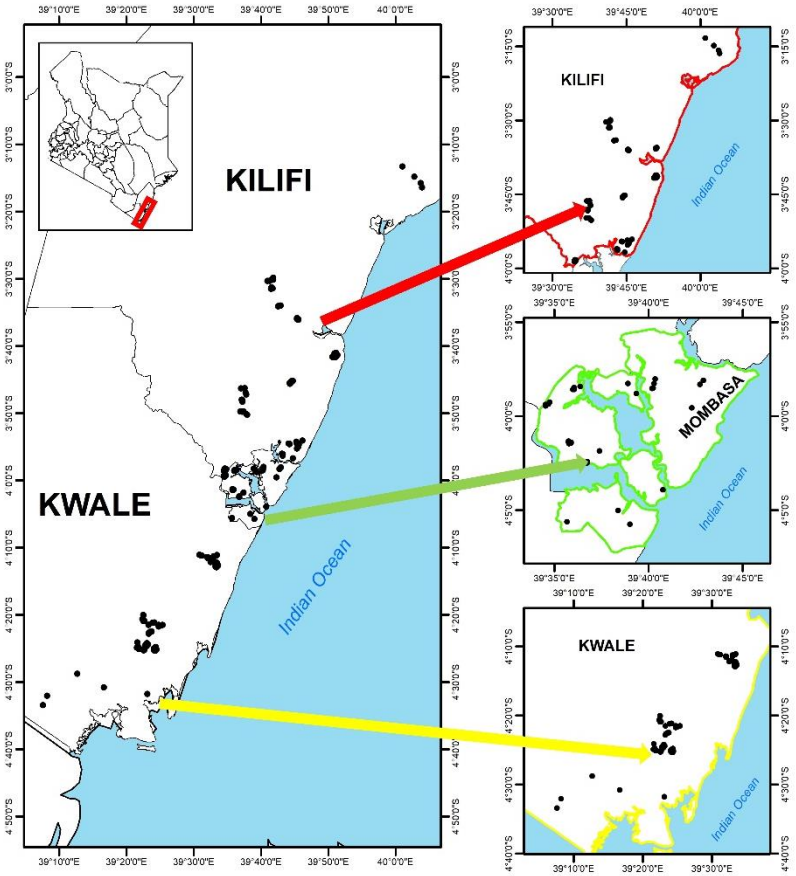

Figure 1. Map of the study sites

Study design and data collection

A formal structured questionnaire was developed on ODK (Open Data Kit) collect v 1.30.1 for recording the data. The questionnaire was jointly developed by all seven partner institutions of the Darwin Initiative project (Kenya Agricultural and Livestock Research Organization (KALRO), Kenya Plant Health Inspectorate Service (KEPHIS), Kenya Forestry Research Institute (KEFRI), Centre for Agriculture and Biosciences International (CABI), National Museums of Kenya (NMK), University of Nairobi (UoN) and Natural History Museum, U.K. (NHM)) and covered aspects of both agriculture and forestry. The study was carried out using face-to-face oral interviews, where enumerators engaged the respondents to capture appropriate responses to target questions. This allowed the use of multiple languages, with English as the basic language used

to design the questionnaire and train enumerators, and a general-purpose language for communication. However, Kiswahili as the native language in the coastal region, was used in case-to-case interviews as warranted.

Enumerators were trained prior to data collection to standardize responses and minimize skewness. During this period, areas of enumeration and team leadership aspects were agreed upon. The questionnaire was translated onto the ODK system so that enumeration was paperless, with immediate submissions after enumeration and discussion with team leaders to confirm that all aspects were captured. A target of 250 respondents was set before the enumeration process across the three Counties. The parameters captured included: age, gender, crop production constraints, pest infestation and management. Data was extracted from the ODK server and converted to MS Excel for curation. The results were shown as Excel spreadsheets and GENSTAT was used for basic analyses.

Results and discussion

Details of the respondents

In all the three Counties, a total of 238 respondents were interviewed: 41% from Kwale County, 39% from Kilifi and 20% from Mombasa (the smallest and most urbanised county). There was cross-gender representation in all the counties, although the majority of respondents were male. The study by Wekesa *et al*. (2017) established that participation of women in decision-making in the coastal regions of Kenya was limited, as they required consent from the men. An additional youth representation in Kilifi (24%), Kwale (20%) and Mombasa (13%) was recorded (Figure 2).

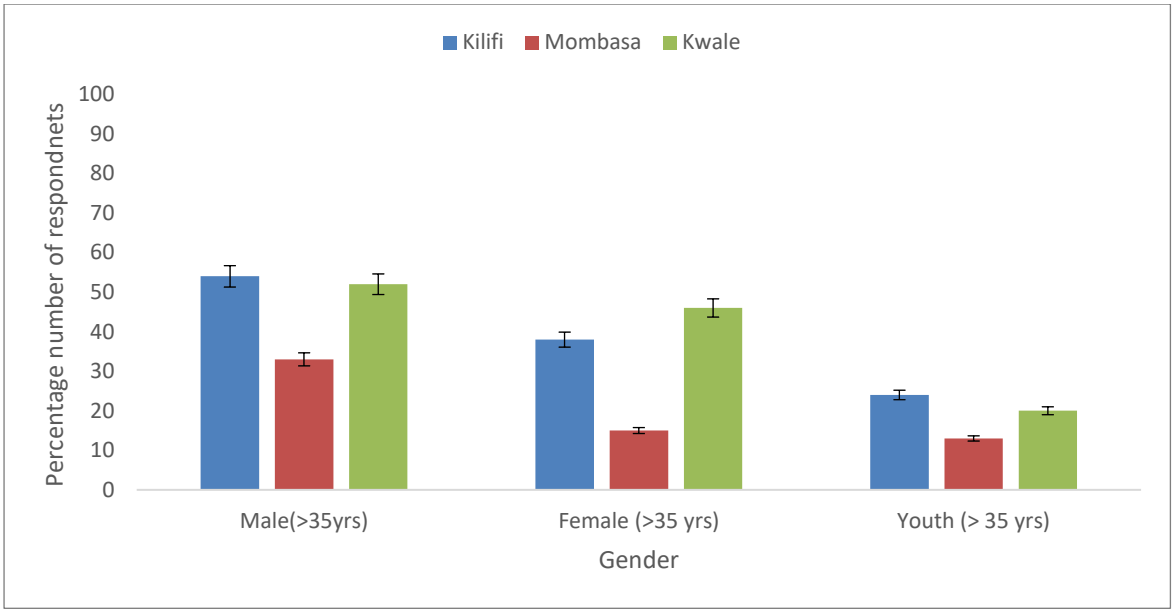

Figure 2. Gender representation from the respondents

The major constraints of crop production were found to be insect pests (45%), diseases (21%), drought (20%) and climate (5%). Other constraints included insuficient farm inputs, lack of markets and low yields (together accounting for 7%) (Figure 3). Scale insects and mealybugs were among the pests reported to have caused challenges to crop production in the area. However, out of the 238 farmers interviewed, only 26% (n=62) were familiar with scale insects and 51% (n=121) were familiar with mealybugs. Of those who were aware of scale insects 78.13% and 94.3% could correctly distinguish scale insects and mealybugs respectively, from a group of other insects. Some soft scale insects (family Coccidae) have a barnacle-like appearance and are covered with a waxy coating that hides and protects the adult and its eggs, making it difficult to recognize it as an insect (*Shorthouse, 2003).* In contrast, mealybugs have no hardened covering but a white cottony/powdery wax coating with wax extensions (filaments) around the margins of the small, soft body (Held, 2019); the colonies look like clusters of cotton wool on the above-ground portion of plants, making them more visible to the naked eye. This probably explains why more respondents indicated that they identified the mealy bugs more than the scale insects.

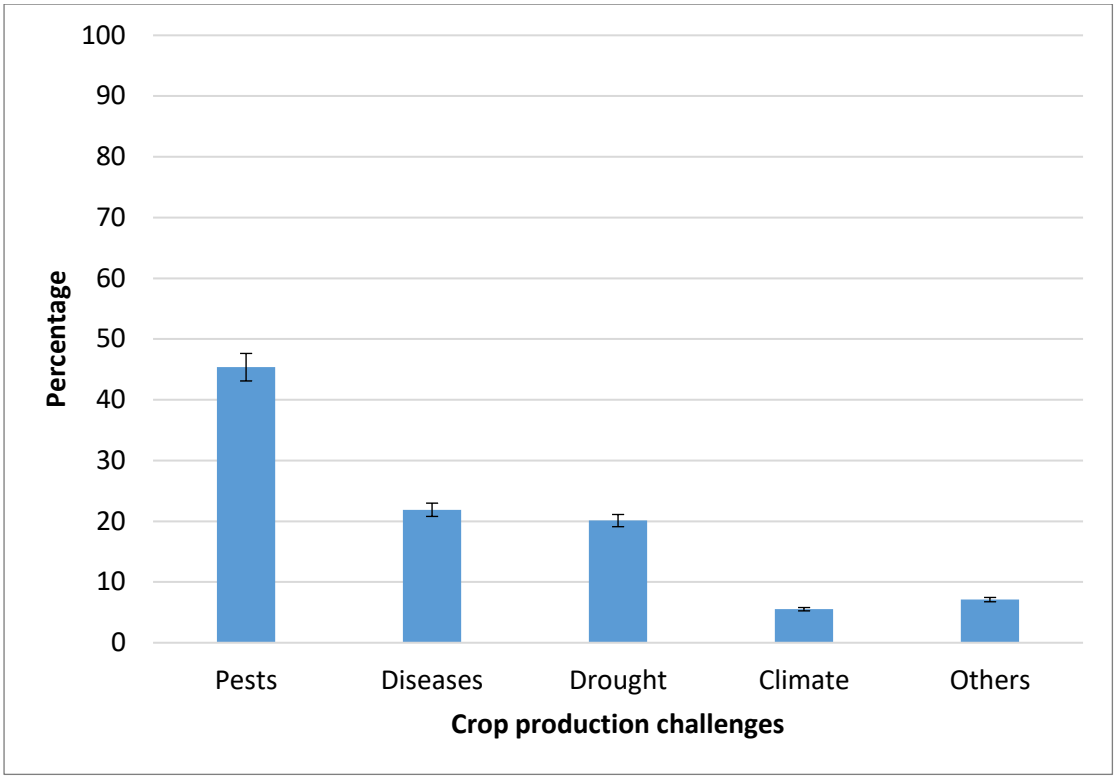

Figure 3. Crop production constraints

Amongst the host plants investigated, papaya was the most infested by both mealybugs and other scale insects, followed by cassava and citrus (Figure 4); both were reported to cause a lot of damage to the host plants recorded. However, it was noted that damage caused by presence of both pests was much more than what was caused by occurrence of each pest individually (Figure 5). The most common symptoms were leaf withering, leaf yellowing, stunted growth, drying of tissues and defoliation. Compared to other types of scale insects, mealybugs caused the most damage (Figure 5). A previous study in coastal Kenya indicated that an alien invasive species, papaya mealybug (*Paracoccus marginatus*) was introduced in 2016 (Macharia *et al.,* 2018). More recent survey conducted in the area identified additional seventeen alien scale insects and mealybug species in Kenya (Macharia *et al*., 2021), feeding on many crop species including cassava, sugarcane and egg-plant, among others thus adding to the burden of crop pests already experienced by farmers in this region.

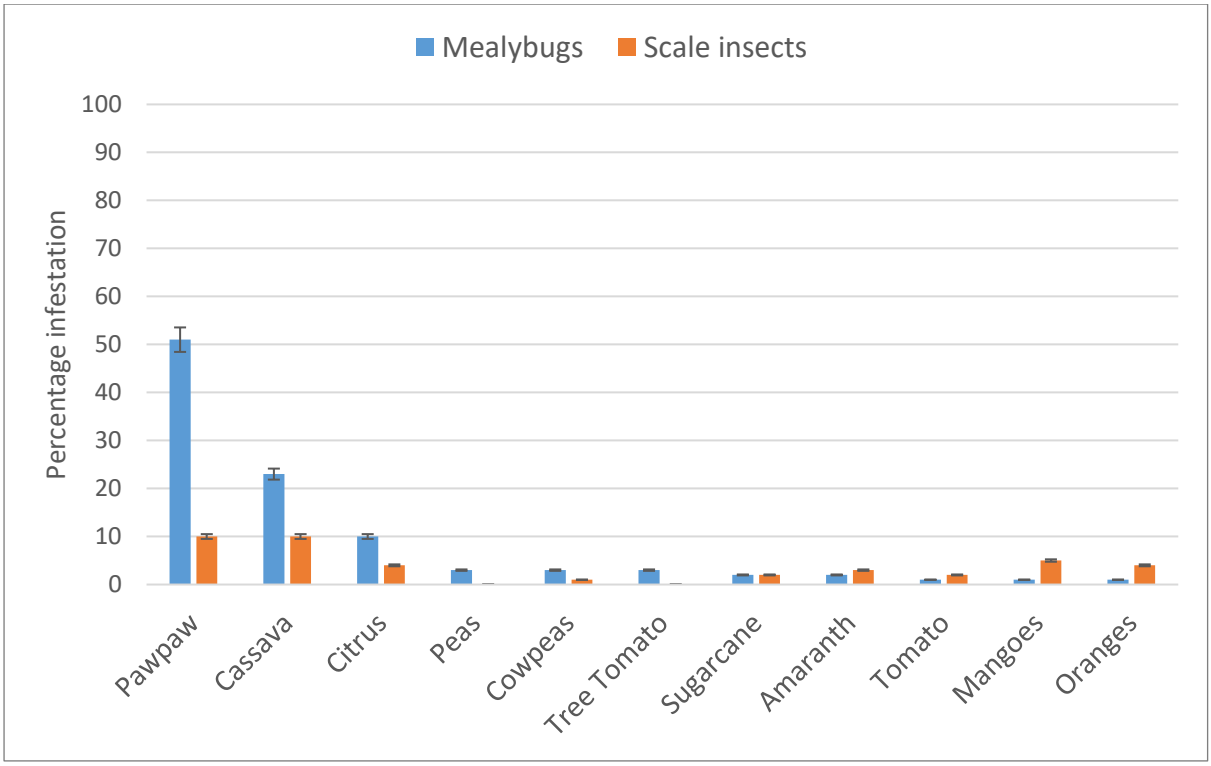

Figure 4. Percentage of plants infested with mealybugs and scale insects

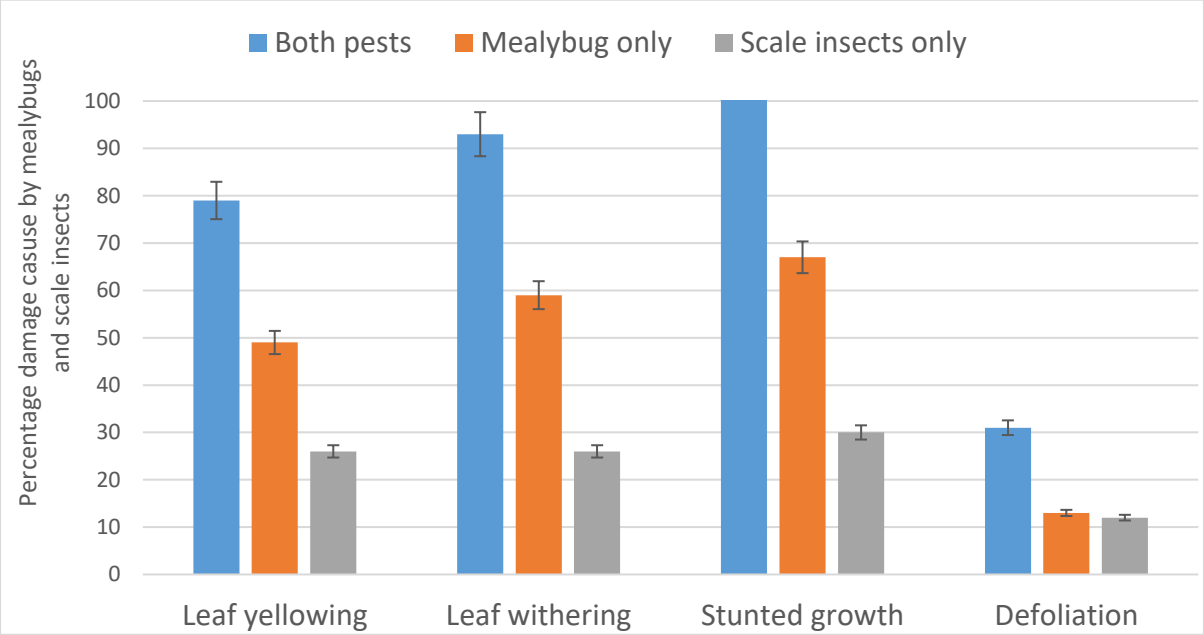

Figure 5. Percentage damage due to mealybug and scale insect infestations

Although over half of respondents (56%) indicated that they mainly used insecticides to target all insect pests on the farm, a considerable number (25%) did not use any control method (Figure 6a). Most of those using insecticides (39%) intended to kill fall armyworm; only 6% targeted other species of caterpillars. Although other insect pests were also targeted, the number of instances was generally very low (Figure 6b).

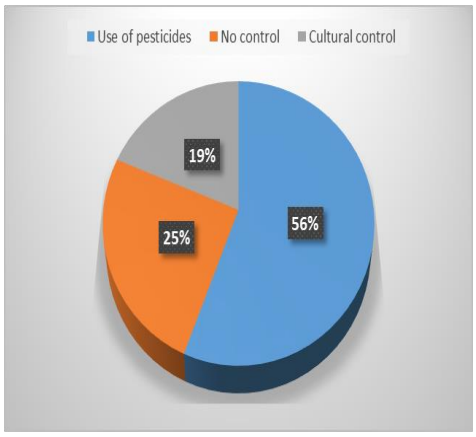

Figure 6a. Pest control methods employed

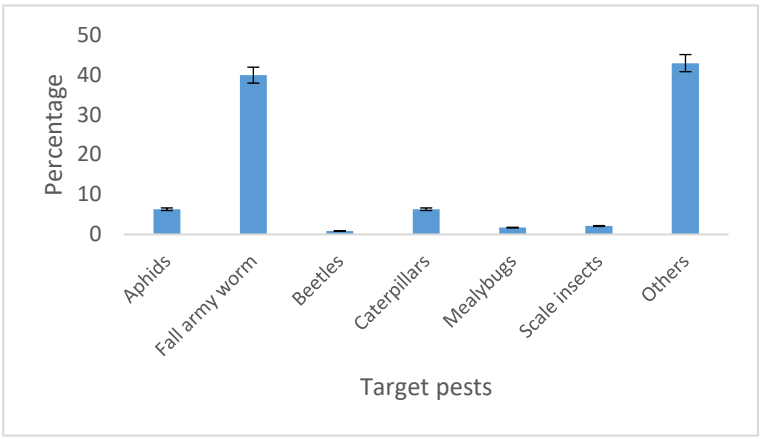

Figure 6b. Target pests for control

Pesticides play a significant role in food production by protecting crops from pest attack, increasing yields and can determine the number of times per year a crop can be grown on the same land particularly for countries that face food shortages. Earlier studies in Kenya (Abong'o *et al.*, 2014) indicated that the rapid expansion of agriculture due to population growth has resulted in increased demand for agro-chemicals which have become an integral part of plant, livestock and public health protection. Unfortunately, majority of farmers use broad-spectrum pesticides. This increases the risk of selection for resistance to pesticides in non-target insect pests, due to sub-optimal dosages. On the other hand, some farmers are known to use non-chemical methods of pest control because of the risk of toxic effects of pesticides and their high cost (Marete *et al.*, 2021).

Majority of the respondents indicated that agrovets (29%) and extension officers (27%) were their main sources of information on pesticide use. Others

depended on their own experience and advice from neighbours. Unfortunately, very few (3.55%) of them read pesticide labels (Figure 7), hence were not able to follow proper guidelines for safe application and effective use with reference to target pests. Previous studies in Kenya reported that farmers relied on several sources of pesticide information, which to some extent resulted in malpractice (Marete *et al.,* 2021). Earlier studies in Africa and other developing countries (Damalas and Eleftherohorinos, 2011; Lalah *et al.*, 2018) indicated that there is a lack of knowledge and qualified agricultural extension workers to help explain to farmers how to safely handle pesticides. Farmers who received proper information were able to apply pesticides in the right way and use host-specific pesticides hence reducing the toxic effects of pesticide exposure (Marete *et al.*, 2021).

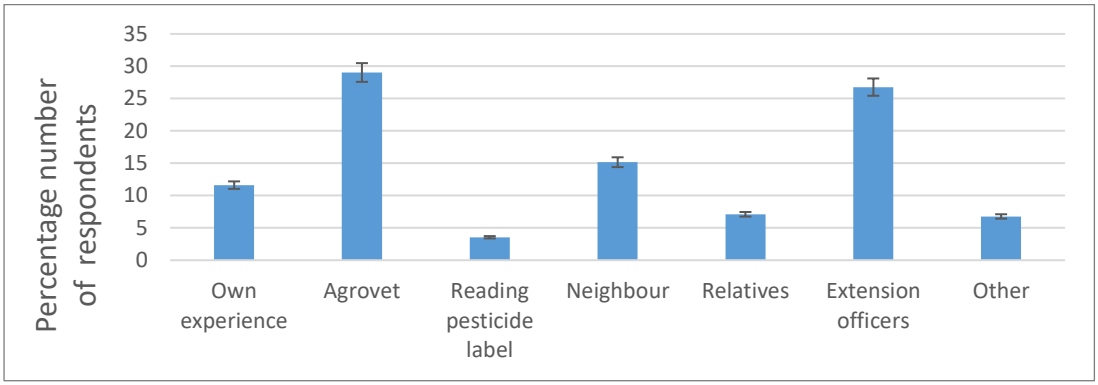

Figure 7. Source of information for pesticide use

Conclusion

A diversity of crops (both cultivated and uncultivated) are affected by scale insects and mealybugs, hence posing serious threat to the livelihood of most households in the coastal counties. Farmers rely on pesticides to manage these pests although in most cases broad spectrum insecticides are used to maximise management of other pests in the farm. Farmers in these counties access information about pests from various sources which is a good sign that they are kept informed especially on pest management issues. However, failure to read the pesticide labels was recorded as a challenge that may lead to unsafe use of pest control products. The survey showed that farmers in

coastal counties of Kenya had a major challenge in identification of scale insects and mealybugs. Some farmers may not be able to manage these pests appropriately because they may not even be aware they exist. This has been documented as a major challenge in early detection and management of scale insects in the counties.

Recommendations

Based on the findings from this study, it is recommended that the capacity of extension officers and other information providers in the coastal counties on the identification and proper management of scale insects and mealybugs be built. Factsheets, manuals and other relevant documents should be developed, published and distributed to various strategic areas for visual recognition of the target pests and how they occur on the farms, to enhance awareness of these pests. National institutions should work with the identified county governments to develop effective management practices for the target pests. Further to this, parasitoids, predators and other natural enemies of scale insects and mealybugs should be identified and practices that enhance the population of the natural enemies should be developed and encourged to enable sustainable control. There is need to train affected farmers on use of specific insecticides for specific target pests, to avoid misuse of chemicals on the farm.

Acknowledgements

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Atoxigenic *Aspergillus flavus* (Aflasafe KE01) application reduces Fumonisin contamination in maize in lower Eastern Kenya

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Abstract

Management of fumonisin contamination in maize has been a challenge largely because there are no effective management measures for *Fusarium* ear rots. Maize varieties that are resistant to fumonisin contamination are also lacking. This study was conducted to determine the efficacy of atoxigenic *Aspergillus flavus* (Aflasafe KE01) on fumonisin contamination of maize. The study was carried out in four sub-counties in lower Eastern Kenya. Twenty-four maize fields were selected in each sub-county; where 12 fields were treated with Aflasafe KE01, while 12 fields comprised the untreated controls. Aflasafe KE01 was applied at a rate of 5 kg/ha and 10kg/ha by hand broadcasting in maize fields two to three weeks before tasselling of maize. Fumonisin level in the maize samples was determined using Accuscan Pro-reader enzyme-linked immunosorbent assay (ELISA). The results showed that application of Aflasafe KE01 reduced fumonisin in the maize from the Aflasafe KE01 treated fields by up to 68% compared to samples from untreated fields. About 62.5% of the maize fields treated with 5kg/ha of Aflasafe KE01 met the European Commission regulatory threshold of ≤2ppm for total fumonisin as compared to about 45% from the control fields. This indicates that Aflasafe KE01 is a potential biopesticide for the management of fumonisin in maize. Therefore, efficacy of Aflasafe KE01 to reduce fumonisin contamination of other key staples in Kenya should also be evaluated.

Key words: Aflasafe KE01, atoxigenic *Aspergillus* sp., fumonisins, maize.

Introduction

Maize is the staple crop in Kenya, contributing up to 21% of the total value of primary agricultural commodities (Kang'ethe *et al*., 2020). According to FAO statistics, maize crop occupies 48.5% of arable land (FAOSTAT, 2019). However, maize is prone to mycotoxins such as aflatoxins and fumonisins (Cinar and Onbasi, 2019). Fumonisins are toxic metabolites produced by *Fusarium verticillioides* and *Fusarium proliferatum* and is common in maize-based food and feed (Fandohan, 2003; Kamle *et al*., 2019). In Kisii county, a study by Alakonya *et al.* (2009) reported fumonisin B1 levels of 3,600–11,600ng/g in maize. Acute aflatoxicosis outbreaks in humans in Kenya have been reported from time to time and in different parts of the county (Korir and Bii, 2012; Muthomi *et al*. 2012; Okoth and Kola, 2012). Fumonisins are carcinogenic and have been linked to oesophageal cancer (Kimanya, 2015). Fumonisin epidemics occur commonly in dry years and are favored by warm, dry weather during grain-filling stage of maize growth. Droughts at the beginning of the growing season and wet weather during pollination and silking stages can favor fumonisin synthesis in harvested maize grain (Ariño *et al*., 2009). To reduce human exposure and deaths caused by fumonisin, there is an urgent need to manage/control its metabolism in maize. Fumonisins are managed by prevention of *F. verticillioides* infection.

To manage mycotoxigenic fungi and mycotoxin production, different approaches have been used including physical, chemical and cultural methods. These methods range from the use of synthetic fungicides (UlHaq *et al*., 2020), breeding for resistant maize cultivars (Lanubile *et al*., 2014) and cultural approach to avoid pre- and post-harvest attack of fungi (Munkvold, 2003; Njeru *et al*., 2019). However, agronomic practices for fumonisin content reduction are ineffective when conditions for fungal growth are optimal (Robertson-Hoyt *et al*., 2007). Biological control methods have become popular because of their ability to reduce the primary fungal soil inoculum and the infection rate in root systems of maize plants (Etcheverry *et al*., 2009).

Non-pathogenic *Fusarium* strains have been moderately applied as biocontrol

agents in suppressing the growth of toxigenic strains of *F. proliferatum* and *F. verticillioides* in maize (Luongo *et al.*, 2005). This method uses competitive exclusion which is made possible by the presence of toxigenic and atoxigenic strains of *A. flavus* populations. Application of non-toxigenic inoculants in the soil around the crops ensures competition with toxigenic strains for infestation sites on the growing plant. This is achieved by identifying and successfully introducing harmless atoxigenic strains that show competitive gain over toxigenic strains. Atoxigenic strains of *A. flavus* virtually eliminate the highly toxigenic strains thereby reducing aflatoxin contamination (Agbetiameh *et al.*, 2019; Senghor *et al.*, 2020). The objective of this study, therefore, was to assess the effectiveness of atoxigenic *Aspergillus flavus* (Aflasafe KE01) on the reduction of fumonisin contamination in maize in Lower Eastern Kenya.

Materials and methods

Description of the study area

The study was conducted in Nzambani sub-county (Kitui County), Kathiani sub-county (Machakos County) and Wote and Kaiti sub-counties (Makueni County) in lower Eastern parts of Kenya (latitude between 4°N to 4°S, longitude 34° to 41°E). These regions receive an average rainfall of between 150 mm to 650 mm p.a. On average, Machakos and Kitui counties receive 500 to 700mm p.a and 500 to 1050 mm p.a. respectively. The soils in these areas are sandy to loamy sand texture with low organic matter contents, low water retaining capacity and low plant nutrients thus making is susceptible to erosion (Gachimbi *et al.*, 2005). Makueni county has several agro-ecological zones (AEZs) with altitudes ranging from 790-1770masl and receives about 600-1050mm of average annual rainfall (Jaetzold *et al.*, 2010).

Experimental design and application of atoxigenic *Aspergillus flavus* (Aflasafe KE01)

The farms were selected randomly within each sub-county. The experiments were conducted using maize planted by the farmers who consented to take part in the study. Each of the four sub-counties had 24 maize fields where 12 fields were treated with Aflasafe KE01 while the other 12 were control fields. Within

each area, control fields were a maximum of 100m from treated fields. Aflasafe KE01 was obtained from the International Institute of Tropical Agriculture (IITA). Six of the individual farmers' farms were treated with Aflasafe KE01 at an application rate of 5 kg/ha while the other six were treated with 10 kg/ha. Aflasafe KE01 was broadcast by hand in selected fields 2-3 weeks prior to tasselling of maize. The experiment was carried out in one maize cropping season across the four sub-counties. Data collected from the experiment included the population of *Fusarium* species in the soil and grain samples and the fumonisin levels in the maize grains.

Collection of maize cob samples

Maize cobs were sampled from the maize fields to quantify the amount of fumonism present in grains. Maize cobs were sampled at harvest ensuring a minimum of eight cobs from each farm. Maize cobs were picked from the farm using a zigzag approach. The maize cobs were sun dried avoiding contact with the ground. Thereafter the dry maize was manually shelled by hand and dried in an oven at 45°C for two days and then crushed to fine powder using a Bunn coffee mill grinder (Bunn omatic Corporation, Spring Field Illnois, USA). The ground maize sample was thoroughly mixed for fumonisin analysis. The samples were stored in the refrigerator at 4 °C.

Analysis of fumonisins in maize kernels

AccuScan Neogen Reveal Q+ was used for fumonisin analysis. A sample of 10g of ground maize was mixed with 50ml of 65% ethanol then shaken vigorously in a centrifuge for three minutes and allowed to settle and then sieved using a Whatman No. 1 filter paper. Red and clear sample cups were placed into a labeled sample rack. Two hundred microlitres of the sample diluents was placed in the red dilution cup and mixed with 100µL sample extract in a sample cup and mixed by a pipette up and down five times. One hundred microlitres of the diluent sample extract was then transferred into a new well labeled clear sample cup. The new reveal Q+ for fumonisin test strip was placed into the sample cup with the test strip coming into contact with the liquid and the timer set for 6 minutes after which it was removed from the sample cup and read within 1 minute by fully

inserting in the AccuScan pro-reader which automatically analyzed the cartridge. The test strips were read in the Reveal AccuScan or Reveal AccuScan III Reader within one minute of completion of the 5-minute incubation. Reaveal Q+ was designed for quantitative analysis of fumonisin with a limit of detection that ranges from 0.3 to 6ppm. Samples with quantities above 6ppm were determined through serial dilution and the result acquired multiplied by the number of dilutions. After each dilution, the above procedure on taking reading from the Reveal AccuScan III Reader was followed.

Data analysis

The data on fumonisin level was subjected to analysis by GenStat 15th edition to determine significant difference in fumonisin levels in Aflasafe KE01 treated and untreated maize fields. Differences between treated and untreated fields were separated using Fishers protected LSD ($p \leq 0.05$). Fumonisin categorization levels provided by Food and Drug Administration (FDA) of ≤ 2 = low, 2-4 = medium and >4 = high fumonisin level (above acceptable level in maize) was used to analyze fumonisins.

Results

Efficacy of field application of atoxigenic *A. flavus* (Aflasafe KE01) in reducing fumonisin contamination of maize grains at harvest

Maize sampled at harvest from Aflasafe KE01 treated fields were contaminated with varying levels of fumonisin. About 63.5% of the maize fields treated with 5kg/ha and 10kg/ha of Aflasafe KE01 met the threshold set by European Commission (≤2ppm) for fumonisin (Table 1). A total of 66.6% of the maize samples met the standard set by the US FDA (≤4ppm) while 33.3% was above the set standard of (≤4ppm) in the maize samples from fields treated with 10kg/ha of Aflasafe KE01 (Table 1). Generally, there was a reduction in fumonisin levels due to the application of Aflasafe KE01. There was significant difference in fumonisin levels in maize sampled from Aflasafe KE01 treated and untreated maize fields from Kaiti and Nzambani using the 10 kg/ha application rate with a 60 % and 65.5% decrease in the fumonisin level respectively (Table 2).

Table 1. Proportions (%) of fumonisin contamination levels falling under different categories for maize sampled at harvest from treated and untreated fields with atoxigenic *A. flavus* (Aflasafe KE 01).

Rate of Aflasafe	Sub-county	Treatment	Fumonisin level (ppb)			
			≤ 2	2-4	>4	Range
5kg/ha	Wote	Treated	83.3	0.0	16.7	0-6
		Untreated	66.7	16.7	16.7	0-11.5
	Kaiti	Treated	50.0	16.7	33.3	02-6.2
		Untreated	16.7	50.0	33.3	0-8.2
	Kathiani	Treated	16.7	50.0	33.3	0-6.5
		Untreated	16.7	33.3	50.0	0-8
	Nzambani	Treated	100.0	0.0	0.0	0-0.6
		Untreated	83.3	16.7	0.0	0-3.3
10kg/ha	Wote	Treated	83.3	0.0	16.7	0-4.1
		Untreated	100.0	0.0	0.0	0-0.6
	Kaiti	Treated	50.0	0.0	50.0	0-5.4
		Untreated	16.7	0.0	83.3	0-9.3
	Kathiani	Treated	50.0	16.7	33.3	0-7.5
		Untreated	83.3	0.0	16.7	0-7.5
	Nzambani	Treated	66.7	0.0	33.3	0-7
		Untreated	83.3	16.7	0.0	0-2.9

The fumonisin level categories are ≤ 2ppb is low, 2-4ppb is medium and >4ppb is high.

Table 2. Fumonisin level (ppb) in maize kernels sampled at harvest from atoxigenic *A. flavus* (Aflasafe KE01) treated and untreated fields in Kaiti, Nzambani Kathiani and Wote sub-counties in alternate seasons

	Treatment	Sub county			
		Kaiti	Kathiani	Nzambani	Wote
5 Kg/ha	Treated	2.7a	3.7a	0.25a	2.5a
	Untreated	3.7a	4.2a	0.8b	3.1a
% Reduction[a]		**27.0**	**11.9**	**68.7**	**19.3**
10 Kg/ha	Treated	2.4a	1.8a	1.0a	0.5a
	Untreated	6.0b	3.5a	2.9b	0.9a
% Reduction[a]		**60**	**48.5**	**65.5**	**44.4**

[a] Indicates reduction in levels of fumonisin in maize grains sampled from aflasafe KE01 treated and untreated fields. Means followed by the same letters within a column are not significantly different ($p \leq 0.05$) for each sub county.

Discussion

The higher fumonisin levels noted in untreated fields is as a result of dominant occurrence of *F. verticillioides* and *F. proliferatum* in maize samples from these fields. Most isolates of *F.verticillioides* and *F. proliferatum* are major fumonisin producers (Chulze *et al.*, 2015; Tsehaye *et al.*, 2017). The infection of maize grains with fumonisin in this study could be attributed to the high incidence of *F. verticillioides* and *F. proliferatum* (Misihairabgwi *et al.*, 2019). Additionally, weather in the lower Eastern parts of Kenya provides optimal conditions for the production of fumonisins in maize.

The results from this study showed that maize samples from treated fields had lower fumonisin levels compared to maize from untreated fields. This implies that the fumonisin level in maize grains was reduced due to the application of Aflasafe KE01. Lauren *et al.* (2004) reported a reduction in the population of *F. verticillioides* and *F. proliferatum*, and significant inhibition of fumonisin Bl (FBI) production by the presence of *F. gramineanum*. In a study by Kaur *et al.* (2010) strains of non-phytopathogenic *Fusarium* were combined with other biocontrol agents to obtain an effective reduction in fumonisin levels in crops. Other studies have reported a decrease in fumonisin levels in maize as a result of the interaction of *A. flavus* and *Fusarium* species (Dwivedi and Enespa, 2013; Camiletti *et al.*, 2018; Giorni *et al.*, 2019; Reis *et al.*, 2020). Single application of Aflasafe has been shown to be effective for up to 3 years and in several crops subsequently planted on the same plot (IITA, 2009). This implies that, Aflasafe KE01 should not necessarily be applied every season for effective control of the *Fusarium* species and fumonisim production in maize.

Conclusion

The level of Fumonisin in from Aflasafe KE01 treated fields were reduced by up to 68% compared to samples from untreated fields implying that Aflasafe KE01 is effective in reducing fumonisin contamination in maize and is therefore a promising biocontrol product in managing fumonisin contamination.

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