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Management of *Fusarium verticillioides* Root Infection Court in Maize Using Organic Soil Amendments

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Abstract: In this study the efficacy of various soil organic amendments were evaluated for their potential to manage *Fusarium verticillioides* root infections in maize. The soil organic amendments used were neem cake, sunflower cake, cotton cake, goat manure and farmyard manure. In a field experiment *F. verticillioides* was inoculated to seed holes with different soil organic amendments at planting. Soil and root samples were collected for mycological analysis at 10, 30 and 60 days after silking. Upon maturity maize was harvested at 4 and 8 weeks after physiological maturity where assorted data was collected. Rotten and symptomless maize samples were collected and subjected to mycological and mycotoxin analysis. Significantly high recovery rates of *F. verticillioides* from control soil and roots than in amended soil was evident. At 60 days after silking the percent recovery of *F. verticillioides* was reduced to even zero in some treatments indicating that organic soil amendments have a mechanism of suppressing the survival of *F. verticilliodes* in the soil and hence limit its root infection ability. Mycological analysis on symptomless kernels revealed high recovery of *F. verticilliodes* from in control plots than amended treatments indicating the ability of the amendment to manage root infections of *F. verticilliodes.* Mycotoxin analysis revealed widespread FB_1 contamination across treatments and in both asymptomatic and rotten maize. Average FB_1 in symptomless maize was 333.98 μg kg^{-1} and 357.4 μg kg^{-1} at 4th and 8th weeks after physiological maturity respectively. All rotten maize samples had over 5000 μg kg^{-1} of FB_1. Aflatoxins were only present in three samples at 4th week after physiological maturity. The results show that soil organic amendments could limit root infection by *F. verticillioides* however, it cannot if singly used as a management strategy against the pathogen guarantee 100% eradication of the pathogen and associated mycotoixins. This therefore calls for an integrated approach that could involve use of resistant hybrids, soil solarization, early land preparation, insect control, fungicide treated seed and good post harvest handling practices. Mycotoxin ignorant maize consumers in Africa especially need to be educated on the risks they face if they consume rotten maize given the very high levels of FB_1 revealed in this study and elsewhere.

Key words: Infection court · Fumonisins · Aflatoxins · Organic soil amendments · Ear rot fungi · ELISA · Zea mays · Kenya

INTRODUCTION

The majority of the inhabitants of sub Saharan Africa depend on maize (*Zea mays L.)* as their staple food [1]. Currently maize ear rot ranks highly as a maize production constraint in Kenya and is caused by a variety of fungi that belong to several genera mainly *Fusarium* spp*, Stenocarpella* spp, *Penicillium* spp and *Aspergillus* spp [2-6]. *Fusarium verticillioides* (Sacc) is the most dominant ear rot fungi in maize worldwide [6]. Other than causing reduced maize yields through rotting, *F. verticillioides* is reputed for producing copious amounts of a mycotoxin group called fumonisins. Fumonisins have been shown to posses the ability to cause equine leukoencephalomalacia (ELEM), pulmonary edema, human esophageal cancer and rat liver cancer [7-14]. Of even more concern with *F. verticillioides* is the fact that it can remain symptomless on infected and fumonisin contaminated kernels [15].

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The infection courts of *F. verticillioides* are roots, seeds, silk and wounds caused mostly by insect and bird damage [16-20]. Infection through seed can be managed by seed dressing while silk infection court can be controlled by selecting cultivars with a thick husk and closed ear tips where as predisposition caused my insects can be managed by use of insecticides or repellents. The management of the root infection court is of concern because it occurs early at a very vulnerable stage of plant development and hence allowing the pathogen to spread systemically throughout the plant. Since maize is grown annually or biannually in sub Saharan Africa it is important to therefore develop a mechanism that can efficiently exclude the pathogen from the rhizosphere of the maize plant in order to prevent transmission of innoculum from preceding seasons. The purpose of this study was therefore to evaluate the efficacy of organic soil amendments in management of root infection by *F. verticillioides* in maize. The soil organic amendments used were neem cake, sunflower cake, cotton cake, goat manure and farmyard manure (Fym).

MATERIALS AND METHODS

Isolation and Identification of *F. verticilliodes* from Maize Kernels: A culture of *F. verticillioides* was isolated from maize kernels collected from Tongaren division in Rift Valley province a major maize producing region in Kenya. Isolation of *F. verticilliodes* was by the method described by King, [21]. Summarily, *F. verticilliodes* isolation was on PCNB agar medium that is a selective medium for isolation of *Fusarium* species. It involves dipping seeds in 70 % ethanol then surface sterilizing in 1 % sodium hypochloride for two minutes before rinsing twice in sterile distilled water and drying between sterile filter papers. Five seeds were then plated on PCNB media in triplicates and incubated at 25°C for five days. The colonies of observed fungal growth were sub-cultured on agar media till pure cultures of suspected *F. verticillioides* isolates were obtained. It is important to carry out sub culturing on agar media than other rich media to avoid loss of FB_1 producing abilities of the isolates [18,22].

The *F. verticillioides* suspected colonies were then sub-cultured on Sucrose Nutrient agar (NSA) and placed under Non Ultra Violet light of alternating 12 hours of light and darkness for seven days [23]. Pink coloured cultures were then selected. The cultures were viewed by cutting 1 cm^2 of SNA with the fungal colony and mounting directly on the slide with a drop of water and cover slip (SNA is transparent) then confirmed according to Booth, [24] and Nelson *et al.* [25]. After confirmation a single conidia was isolated onto an agar slant and incubated at 25°C for 3 days before storage at -20°C until needed.

Processing of *F. verticillioides* for Field Infestation: A sterile needle stub on the *F. verticillioides* culture slant at -20°C was inoculated in the middle of a PDA plate and incubated at 25°C for 5 days. A seven millimeter plug was plated on many PDA plates and grown for 10 days. Ten agar plugs of ten days old *F. verticillioides* culture were picked from petri dishes using 7 mm cork borer macerated and then added to each hole containing the respective organic amendments. Each plot comprised of 10 rows with 20 plants in each row. The first row on each side of the plot served as a border row. The control means no organic soil amendment was used whereas treatments comprised of different organic soil amendments used at the rate of one tonne/hectare. Each treatment and the control were replicated four times in a randomized complete block design.

Maize seeds of variety H627 were disinfected by a method by Daniels [26], summarily maize seeds were soaked in sterile distilled water at room temperature for four hours then rinsed in hot water at 50°C for a minute before dipping in hot water at 55°C for six minutes and finally rinsed twice in sterile distilled water. Two seeds were planted in each hole and thinning done to one seed at 4 weeks after planting. The soil at the site (200 g) was analyzed for mineral content at National Agricultural Research Laboratories, Kenya. Weeding was done twice. Maize stalk borer management was done using Bulldock 0.5 GR (Beta- cyfuthrin 0.5 g/Kg) a synthetic pyrethroid insecticide produced by Bayer AG Germany according to manufacturers recommendations.

Soil and Root Sample Collection: Soil and root sample collection was done at 10 days, 30 days and 60 days after silking. At every stage a randomly selected plant from each of the treatments replicates was uprooted and adventitious roots randomly selected from every plant uprooted per plot. In addition, approximately 20 g of rhizosphere soil of uprooted plant was also taken. Each of these samples were put separately in labeled plastic zip sample collection bags then taken to the laboratory where they were stored at 4°C as they awaited mycological analysis. The analysis proceeded within a week following sample collection.

Recovery of *F. verticillioides* from Soil and Roots: Soil samples from the four replicates of each treatment were each mixed thoroughly and 1g of soil weighed for analysis. The number of colonies per gram of soil was determined by dissolving 1g of soil in 9 ml of sterile distilled water in a vial. The vial was then vortexed before pippeting 1ml into another vial containing 9ml of distilled water. This was repeated serially five times followed by aseptically pippeting 1ml from the three last dilutions and dispensing into a separate petri dish with pentachloronitrobenzene agar medium. A petri dish with countable colonies provided the colonies for sub-culturing. The colonies that developed after 5 days were sub-cultured on SNA for confirmation as earlier described. For the roots, they were washed with water dried in sterile filter paper then cut into 2 mm pieces asceptically. *Fusarium verticillioides* was then isolated and identified previously described in *F. verticillioides* isolation protocol from maize Kernels except that twelve root pieces of 2 mm length per treatment/replicate were cultured at 10, 30 and 60 days after silking. The percent recovery of *F. verticillioides* was then calculated.

Field Maize Harvesting and Data Collection: Three rows per plot were harvested at 4-weeks and 8 weeks harvest time points after physiological maturity. Information collected in the field included plant diameter, plant height, percentage lodging, ear rot severity and incidence, borer incidence. Ear rot severity of ear rot was evaluated using a seven-class rating scale by Harris, [27], where 1=0%, 2= 1-3%, 3=4 –10%, 4= 11-25 %, 5=26-50, 6=51-75%, 7= 76-100% of kernels exhibiting visible symptoms of infection such as rot or mycelial growth. Symptomless and rotten maize kernels were taken from each of the four treatments and used for reisolation of *F. verticillioides* and mycotoxin analysis. Transportation and drying of maize samples was done in cotton bags and laboratory benches respectively until moisture content fell between 11-13 %. Moisture content below 13 % prevents growth of saprophytic fungi and hence maintains the integrity of the sample. The symptomless and rotten maize samples were divided into a representative sample using a Pascal's Cascade Rotary Divider (Model 1) with a medium cone cap. Isolation and identification of *F. verticillioides* was performed on each treatment replicate separately as earlier described, whereas for mycotoxin analysis equal amounts of each treatment at every harvest time interval were mixed to make a sample.

Mycotoxin Analysis: A total of 6 symptomless maize samples and 6 rotten maize samples was analysed for both Fumonisin B_1 and aflatoxin B_1. A representative kernels from each treatment were obtained using a Pascal's Cascade Rotary Divider (Model 1) after combining treatment subsamples. From the final treatment sample, 200 g of dry maize kernels was ground fine using a coffee blender with ethanol cleaning between samples and stored at 0°C until analysis. A representative sample of 2 g of maize flour was weighed after mixing the whole sample in a blender at high speed for 3 min and put into a 20 ml vial containing 10.5 ml of 50:50 methanol to water. The vial was then vortexed vigorously for 5 min followed by centrifugation for 3 min at 3000 rpm. All the supernatant was pippeted into a fresh tube and centrifuged for 10 min at 20000 rpm.

Carbon$_{18}$ (C_{18}) from Isolute® –International Sorbent Technology Ltd. UK, mounted on a solid-phase extraction manifold (Vacmaster®) were equilibrated by passing 10 ml of methanol: water (10:90) solvent. Ten milliliters of supernatant was then carefully pippeted into C_{18} extraction columns for solid phase extraction. The samples were passed through the column and the column washed by passing through 10 ml of distilled water, followed by drying with air. The mycotoxins in the column were eluted with 2 ml of methanol, which was diluted 1:10 with phosphate buffered saline (PBS) solution, before analysis by direct competitive microplate enzyme-linked immunosorbent assay (ELISA) procedure.

The ELISA assays were performed as described for FB_1 by Usleber et al. [28] and AFB_1 by Gathumbi et al. [29]. The microtitre plate wells (Maxisorp® Nunc, Denmark), were coated with anti-mycotoxin antibody solution in 0.1M sodium bicarbonate buffer and incubated overnight at room temperature. Free protein binding sites were blocked with 3% fetal calf serum in phosphate buffer solution (200 µL/well) for 30 min. The wells were then washed three times with NaCl-Tween (8.5 g NaCl and 250 µL of Tween-20 in 1 liter of water) solution. Aliquots (50 µL) of diluted maize extracts or respective mycotoxin standards were added into the well, followed by addition of aliquots (50 µL) of the respective mycotoxin horse radish peroxidase conjugate. The plates were incubated for two hours at room temperature, washed and an enzyme substrate solution (100 µL) added. The enzyme reaction was stopped by addition of 1M sulfuric acid (100 µL) and absorbance read at 450 nm using an ELISA reader (Uniskanii® Labsystems, Finland), absorbance values were analyzed with a competitive ELISA software 6.

Data Analysis: Data in percentage was arcsine transformed whereas for the severity rating the indexing value was used before being subjected to analysis of variance of Variance using the statistical package Genstat. For presentation purposes in the results the percentages are used for easy reading and understanding of the manuscript.

RESULTS

The purpose of the soil analysis was to ensure that the macro and micro elements do not occur at levels toxic to maize. The levels were all adequate for maize growth and shown in Table 1.

The effect of soil amendments on *F. verticillioides* recovery from the roots and soil during the field experiment revealed significant (P=0.05) differences between treatments at 10 days, 30 days and 60 days after silking. The recovery of *F. verticillioides* from the roots at 10 days after silking was 2.5, 22.0, 22.5, 25.0, 27.5 and 35% in goat, cotton, Fym, sunflower, neem and control respectively. The number of colonies recovered from soil 10 days after silking ranged between 45000 in control to 0 colonies in cotton. In Fym and goat 800 colonies were recovered whereas 300 and 16000 were recovered in neem and sunflower respectively. Percentage recovery from roots at 30 days after silking was significantly (P=0.05) different between treatments and ranged between 40% in control to 6.7% in Fym. Cotton, goat, neem and sunflower revealed 16.7, 23.3, 23.3 and 33.3% respectively. The number of *F. verticillioides* colonies recovered from soil at 30 days after silking were significantly different and were 700 in cotton, 3300 in Fym, neem and sunflower. For goat and control 6700 and 33300 colonies were recovered respectively. The percentage recovery of *F. verticillioides* from roots 60 days after silking revealed no recovery (0.0%) in cotton and Fym, 6.7% in sunflower and neem, 13.3% in goat and 23% in control. The number of *F. verticillioides* colonies recovered from the soil ranged between 0 to 10330. Whereas number of colonies recovered for sunflower cake, goat manure, neem, Fym, cotton and control were 3670, 67, 330 and 10330 colonies. Both root and soil *F. verticillioides* recovery showed significant differences. Generally there was reduction in recovery of *F. verticillioides* in soil and the roots of treatments with time. The highest percentage recoveries of *F. verticillioides* were in the control (Table 2).

At 4 weeks harvest time point, there was no significant (P=0.05) difference between treatments in stem diameter, plant height, total rot incidence, Fusarium ear rot incidence and severity, Sternocarpella ear rot and severity and percent lodging. Despite this, higher values were recovered in amended soil with regard to stem diameter and plant height. The control for instance had 2.86 cm for stem diameter whereas cotton and goat manure revealed 3.078 cm and 3.12 cm respectively. The least plant height was from the control and was 99.9 inches whereas the highest was 120.7 inches and was reported in neem (Table 3). Total rot incidence was not significantly different at 4 weeks harvest time point and ranged from

Table 1: Soil analysis of the experimental site plot

Nutrient	Soil PH	Total nitrogen %	Organic C %	P. ppm	K. Me %	Ca. Me %	Mg. Me %	Mn. Me %	Cu. ppm	Fe. ppm	Zn. ppm	Na Me %
Value	5.51	0.4	2.14	14	1.02	6.50	2.40	1.59	1.62	91.58	5.37	0.4

Table 2: Effect of soil amendments on *F. verticillioides* recovery from soil and roots at 10, 30 and 60 days after silking

	[1]*F. verticillioides* recovery from soil and roots					
	10 days[2]		30 days[2]		60 days[2]	
Treatments	% rec roots	No. of colonies/gm of soil	% rec roots	No. of colonies/gm of soil	% rec roots	No. of colonies/gm of soil
Control	35.0b	45000d	40.0d	33300c	23.0c	10330d
Cotton	22.0b	0.0a	16.7b	700a	0.0a	330b
FYM	22.5b	800b	6.7a	3300ab	0.0a	670b
Goat	2.5a	800b	23.3c	6700b	13.3b	0.00a
Neem	27.5b	300a	23.3c	3300ab	6.7a	3670c
Sunflower	25.9b	16000c	33.3c	3300ab	6.7a	0.00a

[1]Numbers are means of four replicates

[2]Numbers are significantly different (P=0.05) if followed by different letter (s) and in the same column.

Table 3: Effect of soil organic amendments on maize ear rot at four weeks after physiological maturity.

Treatments	Stem Diameter (cms)	Plant Height (inches)	Total rot incidence %	*Fusarium* rot % incidence	*Stenocarpella* rot incidence %	Mean ear rot Severity%	*Stenocarpella* rot severity %	*Fusarium* rot severity %	% Lodging	% *F. verticilliodes kernel r*ec
Control	2.860a	99.9a	42.8a	21.0a	21.8a	50.75e	50.3a	51.2a	13.9a	78.8c
Cotton	3.078a	108.0a	58.0a	35.5a	22.5a	42.75ab	43.5a	42.0a	6.9a	52.5b
Fym	3.115a	106.6a	47.0a	31.2a	15.7a	44.00bc	43.3a	44.2a	11.8a	40.0a
Goat	3.120a	113.4a	38.8a	21.0a	18.2a	40.25a	41.2a	39.2a	12.6a	42.5a
Neem	2.857a	120.7a	45.8a	32.8a	13.0a	45.75cd	55.8a	45.0a	8.0a	55.0b
Sunflower	3.110a	107.6a	51.5a	36.2a	12.8a	48.00de	48.8a	46.2a	6.0a	37.5a

[1]Numbers are means of four replicates.

[2]Numbers are significantly different (P=0.05) if followed by different letter (s) and in the same column.

Table 4: Effect of soil organic amendments on maize ear rot at eight weeks after physiological maturity.

Treatments	Stem Diameter (cms)	Plant Height (inches)	Total rot incidence %	*Fusarium* rot incidence %	*Stenocarpella* rot incidence %	Mean ear rot Severity %	*Stenocarpella* rot severity %	*Fusarium* rot severity %	% Lodging	% *F.verticilliodes r*ecovery from *kernels*
Control	2.78	102.1a	57.5a	39.2c	18.2a	51.25d	49.5a	53.0d	22.5a	65.0b
Cotton	2.95bc	120.4c	43.2a	15.2a	28.0a	46.65bc	46.4a	48.4c	24.0a	45.0a
Fym	2.88b	111.9b	43.8a	18.2ab	25.0a	44.57b	43.2a	45.9bc	23.9a	45.0a
Goat	2.91b	119.3c	51.2a	20.8b	30.5a	40.97a	44.2a	42.7ab	17.8a	42.5a
Neem	3.06c	122.3c	42.8a	14.5a	28.2a	39.65a	44.6a	39.6a	22.2a	62.5a
Sunflower	3.52d	119.7c	48.5a	15.5a	39.5a	48.42c	55.0a	41.8a	17.20a	42.5a

[1]Numbers are means of four replicates.

[2]Numbers are significantly different (P=0.05) if followed by different letter (s) and in the same column.

58.5 to 38% in goat manure. Fusarium rot incidence was also not significantly different. Control and goat manure revealed 21%, Fym 31.2%, neem 32.8%, cotton 35.5% and sunflower 36.2%. For Sternocarpella rot incidence, control and cotton had the highest incidence of 21.8 and 22.5 respectively. Sunflower, neem, goat and Fym recorded 12.8, 13, 18.2 and 15.7% respectively. Even though the control recorded higher *Sternocapella* rot severity, *Fusarium* rot severity and lodging than the amended soils there was no significance difference between treatments (Table 3). Mean ear rot severity was significantly different between treatments; the control had the highest percentage of 50.75%. The other treatments sunflower, neem, goat, Fym and cotton revealed 48, 45.75, 40.25 and 42.75% respectively. The percentage recovery of *F. verticillioides* from kernels was significantly different at 4 weeks harvest time point (Table 3).

The control revealed 78.8% whereas cotton, Fym, goat, neem and sunflower revealed 52.5, 40, 42.5, 55 and 37.5%, respectively (Table 3).At the 8th week harvest interval there was significant difference in stem diameter between the control and all other treatments. The lowest to highest stem diameter measurements were control (2.78 cm) Fym (2.88 cm), goat (2.91c m), cotton (2.95 cm) neem (3.06 cm) and sunflower (3.52 cm). Plant heights were also significantly different between treatments and the control. The control average height was 102.1 inches and was the lowest and significantly different from all other treatments. The highest value was 122.3cm in neem. Total ear rot incidence was not significantly different between treatments. The lowest total rot incidence was 42.8% in neem and highest was 57.5% in control. *Fusarium* ear rot incidence revealed significant difference between treatments. The lowest and highest Fusarium rot incidences were 14.5% and 39.2% in neem and control respectively. *Sternocarpella* rot incidence was highest in sunflower 39.5% and lowest in control 18.2%. However there was no significant difference between treatments.

Mean ear rot severity was significantly different. It ranged between 51.25% in control to 39.65% in neem. Although no significant difference was revealed between treatments with regard to *Sternocarpella* rot severity, the range was between 55.0% in sunflower to 43.2% in Fym. *Fusarium* rot severity was significantly different between treatments and ranged from 53.0% in control and 39.6% in neem (Table 4). No significant differences were revealed in percentage lodging between treatments. Percent lodging ranged from 7.2% in sunflower to 24.0% in cotton. Percentage recovery of *F. verticillioides* from kernels was significantly different between treatments with control and neem revealing the highest recovery of 65% and 62.5% respectively the rest of the treatments revealed recovery rates of between 42% and 45% (Table 4).

Fumonisin B_1 and aflatoxin B_1 level were determined in 24 samples of both symptomless and rotten maize harvested at 4th and 8th weeks after physiological maturity.

Table 5: Levels of fumonisin B_1 and aflatoxin B_1 at 4th and 8th weeks harvest time points after physiological maturity in maize grown with different organic soil amendments

	4th week harvest time point after physiological maturity				8th week harvest time point after physiological maturity			
	Symptomless maize		Rotten maize		Symptomless maize		Rotten maize	
	FB_1	AFB_1	FB_1	AFB_1	FB_1	AFB_1	FB_1	AFB_1
Control	135.5	4.1	>5000	ND	725.1	ND	>5000	ND
Cotton	200.5	ND	>5000	ND	211.0	ND	>5000	ND
FYM	254.5	2.6	>5000	ND	217.5	ND	>5000	ND
Goat	298.0	ND	>5000	ND	642.5	ND	>5000	ND
Neem	191.5	ND	>5000	ND	120.0	ND	>5000	ND
Sunflower	919.5	3.0	>5000	ND	228.5	ND	>5000	ND
Mean	333.98	3.2	>5000	ND	357.4	ND	>5000	ND

[1]Numbers are in µg kg^{-1};

[2]Numbers are an average of duplicate sample analysis

[3]ND means AFB_1 absent or below the detectable level of 0.085 parts per billion (ppb).

The variety revealed 100% contamination with FB_1 in both symptomless and rotten maize samples. The lowest FB_1 level was 120.0 µg kg^{-1} from symptomless maize grown in neem treated soil whereas the highest FB_1 level in symptomless maize was 919.5 µg kg^{-1}in sunflower treated maize. Mean FB_1 levels at 4th and 8th week harvest time point in symptomless kernels was 333.98 and 357.4 µg kg^{-1}, respectively. All rotten samples revealed FB_1 level greater than 5000 ug kg^{-1}. Aflatoxins only occurred in symptomless maize at 4th week harvest interval in control, Fym and sunflower treated plots and were 4.1 µg kg^{-1}, 2.6 µg kg^{-1} and 3 µg kg^{-1} respectively. The rest of the samples including rotten kernels were negative for AFB_1. Mean AFB_1 level at 4th week harvest interval was 3.2 µg kg^{-1}.

DISSCUSION

The significantly high recovery of *F. verticillioides* from control soil and roots than in amended soil indicates that the organic amendments have some ability to manage the root infections by the pathogen and also limit survival of the pathogen in the soil. The recovery of *F. verticilliodes* in the roots after inoculating the soil confirm earlier reports that roots are one of the pathogens' infections courts.The results further show the importance of roots as an infection court in the maize plants. On the other hand the recovery of *F. verticilliodes* from the control up to 60 days after silking indicates that this pathogen has the ability to survive in the soil for quite some time. The recovery in the amended soils is greatly reduced to even zero indicating that organic soil amendments have a mechanism of suppressing the survival of *F. verticilliodes* in the soil. From our results continuous maize cropping as is the case in Kenya may increase the incidence of *F. verticilliodes* in the field.

Earlier studies have shown a positive correlation between stalk and cob rot severity and conservation tillage [30-31]. Though not investigated in this study it is evident that conservation tillage increases availability of host debris on which ear rot fungi easily survives. Ploughing of host debris into the soil soon after harvesting as revealed by Martin and Johnson [33], Payne, [34] and Flett *et al.,* [30] can reduce ear rot innoculum. The findings of this study indicate that ploughing could only be effective in an integrated approach by either addition of organic soil amendments or maybe if preceded with soil solarisation, a hydrothermal disinfection technique that has been successfully used to suppress soil borne ear rot fungi and *Macrophomina phaseolina* [35].

The lack of significant differences in plant diameter, plant height, indicate that the nutrients in the soil were not limiting prior to organic soil amendment application hence failure of the study to capture this aspect. This aspect is also supported by the soil analysis of the field soil done to ascertain the nutrient content. It would have been important to carry out this study on a farmer's fields but since the soil was to be inoculated with the pathogen use of a research station was ideal given the available capacity to disinfect the field. Fields on research stations are used for a variety of researches some of which involve application of high amounts of fertilizers. The lack of significant difference in total rot, *Fusarium & Sternocarpella* rot may be due to other channels of ear rot infections like the silk and ears and given that

the same variety was used the effect of the artificial *F. verticilliodes* innoculum could have been masked by this multiple infection courts.

Fusarium pathogens may enter the maize ears through the silks [36] or via wound caused by birds and insects [37]. Our analysis of bird damage data and stalk borer incidence (data not shown) revealed no significance difference. Lodging which was not significantly different between treatments could have led to bending of ears to touch the soil and hence could have been infected with the pathogen in the soil. However this needs further investigation. Further more due to the asymptomatic nature of *F. verticilliodes* infections [15] it was not possible to capture the treatment differences in the field. Upon mycological analysis of recovery of *F. verticilliodes* from asymptomatic kernels it was noted that the control revealed very high recoveries compared to amended treatments indicating the ability of the amendment to manage root infections and further limit systemic spread of *F. verticilliodes* from maize roots up the plant.

Though in our work we did not analyse the organic soil amendments for presence of any possible microbial antagonists or competitors of *F. verticilliodes* earlier studies have shown that if fungal antagonists are sprayed on plants at the flowering stage or applied in the soil they eradicate or limit the growth of toxin producers [38-41]. One mechanism by which organic amendments promote biological control is by enhancing the activities of the biocontrol agent. *Bacillus subtilis* is known to inhibit the growth of fungi during their endophytic growth phase [42-43]. Contradictory studies have speculated high concentrations of mycotoxins in organic agriculture as compared to conventional production hence this needs further investigation [41,44-46]. Identifying the exact control echanism could illuminate scientists with new insights in managing the fungi.

The widespread FB_1 detection in the maize could have been because the *F. verticillioides* isolate was prolific in producing FB_1 and also that the conditions at the site and the genetic constitution of the variety used were favourable for accumulation of the toxin. The detection of FB_1 in symptomless maize is not surprising as *F. verticillioides* can infect maize asymptomatically and also it could be because of other *F. verticillioides* isolates *and* Fusarium species that also produce moderate amounts of fumonisins like *Fusarium proliferatum* which could have infected the maize through the same or other infection courts like silk or insect wounds. Fumonisin accumulation usually occurs in the field and is influenced by climate, hybrid characteristics insect damage and cultural practices [20,47-52]. Timing of harvest and doing it earlier could result in reduction of mycotoxin conconcentrations that are known to accumulate in the field [53]. The risk of fumonisin contamination may begin very early during ear development and production increases throughout the development and physiological maturation of the infested maize ear [20]. Fumonisin accumulation continues in maize kernels after harvest prior to drying, especially during the pre-storage period of harvested and moist grains [54,55].

The high levels of FB_1 in rotten maize to the excess of 5000 μg kg^{-1} is even more worrying as farmers in Kenya use rotten maize for various purposes such as livestock feed, brewing and occasionally mix it with syptomless maize and then sale it to unsuspecting/ignorant consumers [3]. It is estimated that the average daily intake per person for people living in sub Saharan Africa is 400 g. These results therefore indicate that the average fumonisin intake per day for individuals consuming symptomless maize planted in organically amended soil would be 135 μg daily while one who consumes rotten maize would be exposed to 2000 μg per day. This value is much higher than the acceptable limit of about 10 μg per kg body weight of 60 kg person [56].

In this study the rotten maize had FB_1 levels beyond 5000 μg kg^{-1} indicating that the organic amendments are not effective in reducing FB_1 levels in rotten maize. The acceptable limit for FB_1 level in Kenya is 1000 μg kg^{-1} in both feed and feedstuff. That means that none of the rotten maize was suitable for use as feed or food in Kenya. It is of concern that despite the fact that Kenyans consume forty times more maize or its products than Europeans, the Kenya Bureau of Standards (KEBS) has set higher acceptable FB_1 levels than Europe [57]. Because of frequent aflatoxicoses in Kenya, we decided to also find out the levels of AFB_1 in the maize. The study established that most of the AFB_1 levels in the samples fell lower than 20 μg kg^{-1} the maximum tolerance levels set by Kenya this could have been due to lack of favorable environmental conditions (hot and humid conditions) for *Aspergillus* species that produce AFB_1 [58]. Most of the Asian countries have their minimum acceptable range for AFB_1 set between 5 to 35 μg kg^{-1}, Latin America ranges between 2 to 35 μg kg^{-1} where as North America ranges between 5 and 20 μg kg^{-1} [57]. The results further indicate that the frequent aflatoxicoses experienced in Kenya [33] could majorly be due to poor post harvest handling of kernels.

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