



EVALUATION OF SELECTED SOIL FERTILITY MANAGEMENT INTERVENTIONS FOR SUPPRESSION OF *Fusarium* spp. IN A MAIZE AND BEANS INTERCROP

[EVALUACIÓN DE ALGUNAS ACCIONES SELECTAS DE MANEJO DE LA FERTILIDAD DEL SUELO PARA LA SUPRESIÓN DE *Fusarium* spp. EN UN CULTIVO INTERCALADO DE MAÍZ Y FRIJOL]

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SUMMARY

Fusarium root rot of maize and beans is a common problem in Taita District, Kenya causing economic losses to the small scale farmers. The pathogen attacks maize and beans at all growth stages causing rot at the seedling stage, yellowing of the leaves, stunted growth, and death if severe. Potentially effective crop rotations to maintain the pathogen at low levels are not currently practical due to the small size of farms while fungicides are out of reach to the small scale farmer due to high prices. This study aimed at assessing alternatives to fungicides in controlling root infection by *Fusarium* sp. in maize and beans cropping systems.

Field trials were done in Taita District where agriculture contributes to 95% of household income with limited use of any soil fertility amelioration by farmers. The following were tested in the trials; three types of inorganic fertilizers, cow manure, and *Trichoderma* inoculant. Planting was done during the long and short rains. Soil and roots were collected from the rhizosphere during harvesting and assessed for inoculum density while the roots were evaluated for incidence of infection by *Fusarium* spp. The most common species in both soil and roots were *F. oxysporum* (Schlecht) Snyder et Hans. and *F. sporotrichoides* Sherb. Addition of soil amendments had a positive effect of reduced root infection and in some cases lowering inoculum density in the soil. Of the four integrated soil fertility interventions, Mavuno fertilizer had the highest yield and was the most effective in suppressing root colonisation by *Fusarium* spp.

Keywords: *Fusarium* spp.; root infection; fertilizers; *Trichoderma*; soil amendments.

INTRODUCTION

Maize (*Zea mays* L.) is a staple food of the majority of inhabitants of sub Saharan Africa. In Kenya, maize is grown as an intercrop with common bean (*Phaseolus*

vulgaris L.), an important source of protein. Apart from providing families with cheapest source of starch and protein, maize and bean harvest was source to generate incomes. Production of these crops is constraint by pests and diseases. Most farmers are small scale and cannot afford expensive inputs for crop protection. Moreover these pesticides are not environmentally friendly. Fungal infection of maize and beans not only results in reduced yields through rotting, but may also lead to mycotoxin production. 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 which include *Fusarium* spp, *Stenocarpella* spp, *Penicillium* spp and *Aspergillus* spp (Kedera, et al., 1992, 1998; MacDonald and Chapman, 1997). Several phytopathogenic species of *Fusarium* are found to be associated with maize including *F. verticillioides* (Sacc.) Nirenberg, *F. proliferatum* (Matsushina) Nirenberg, *F. graminearum* Schwabe and *F. anthophilum* (A. Braun) Wollenweber (Scott, 1993; Munkvold and Desjardins, 1997). Root rot severely constrains bean production in Kenya especially where soil fertility is low and bean production is intensive (Otsyula et al., 1998; CIAT, 1992). Root rot is primarily caused by *Fusarium solani* fsp. *phaseoli*, *Rhizoctonia solani*, and *Pythium* species (Nderitu et al., 1997). Root rot pathogens attack beans at all growth stages and cause damping-off at the seedling stage, yellowing of the leaves, stunted growth, and death if severe. *Fusarium* species are ubiquitous in soils and are considered as field fungi invading more than 50% of maize grains before harvest (Robledo-Robledo, 1991). Crop rotations which contribute to minimization of *Fusarium* inoculums in soils is not feasible due to scarcity of land and cultural values (Hall and Phillips, 1992).

The purpose of this study was therefore to evaluate the efficacy of soil amendments in management of *Fusarium* sp. in maize and beans cropping systems.

MATERIALS AND METHODS

Description of the study site

The study was carried out in Taita Taveta District within the global UNEP-GEF funded project: CSM-BGBD (Conservation and Sustainable Management of belowground biodiversity) project. The District is located within the Taita Hills (lat 3°25'; long 38°20') situated in South-Eastern Kenya, Coast Province. The altitude ranges between 1200 to 2000m with mean annual rainfall of 800 - 2000mm. The district covers an area of 16,965 Km² and is divided into five divisions, Wundanyi, Tausa, Voi, Taveta and Mwatate. The study site was in Werugha and Wumingu locations of Wundanyi Division where majority of the farmers are small-scale.

Establishing of the field trials

Field trials were established at the Agricultural Training Centre (ATC) and on selected farms. The experiment at the ATC was laid out in a Randomized Complete Block Design (RCBD) with treatments replicated five times. These treatments were established in selected twelve farms that were 500m apart and considered as replicates. One block constituted by the treatments described earlier were installed in each farm and measured 5 x 10m. Maize variety (H516) was sown at a spacing of 90 x 30cm with two seeds per hole. The bean variety was Mwezi moja planted at a spacing of 75 x 25 cm and two seeds per hole. The treatments were Triple superphosphate combined with Calcium ammonium nitrate (TSP + CAN), Mavuno fertilizer (a blend of fertilizer containing 11 nutrients) and Mijingu Rock Phosphate (MRP) fertilizers, Cow manure, and *Trichoderma* seed coating (Table 1). The fertilizers were added by broadcasting during planting and top dressing of CAN and Mavuno done after first round of weeding. Planting was done during the long rains which occur between March and May and short rains between October and December. Soil and root samples were collected during harvesting from each treatment. Samples were bulked from five points in maize root and bean root rhizosphere respectively. The soils were transported in a cool box to the laboratory.

Assessment of *Fusarium* Density in Soil

One gram of the sampled soil was added to nine ml of 0.05% water agar (10^{-1}) and shaken. One milliliter of the first dilution was pipetted and added to nine ml of 0.05% water agar (10^{-2}) and shaken. From this last soil dilution 1.0 ml was taken and pipetted to each of two Malachite Green Agar (MGA) plates and incubated at room temperature for six days. The colonies formed were counted (Leslie and Summerell, 2006).

A small piece of growth at the edge of the colonies identified as *Fusarium* was transferred to Potato Dextrose Agar (PDA) plates incubated at room temperature for five days and then transferred to Spezieller Nährstoffarmer Agar (SNA) and Carnation Leaf Agar (CLA) for identification. Isolates of *Fusarium spp* obtained from SNA and CLA media were identified using the text references and taxonomic keys of Burgess *et al.*, (1994) and Booth (1971).

Table 1. Rates of application of soil amendments in plots.

Treatment (fertilizer)	Rates of Application of fertilizer
TSP + CAN	50 Kg P/ha 100 Kg N/ha
Mavuno	50 Kg P/ha 100 Kg N/ha
Cow Manure	10 Tones/ha
Mijingu	800kg/ha
<i>T. harziunum</i>	Seed coat
Control	Nil

Triple Superphosphate (TSP) fertilizer (44-52% P₂O₅). Calcium ammonium nitrate (CAN) contains 27% N. Mavuno is a blend of fertilizers containing 11 nutrients: Nitrogen (N $\frac{1}{2}$) 10%, Phosphorous (P₂O₅) 26%, Potassium (K₂O) 10%, Sulphur (SO₄) 4%, Calcium (CaO) 10%, Magnesium (MgO) 4%, and appropriate additions of other Trace Elements like: Zinc, Copper, Molybdenum, Boron and Manganese

Assessment of *Fusarium* Infection Incidence in Plant Roots

From each soil sample, 20 small pieces of the thinnest roots were cut approximately one centimeter long, washed in 1% sodium hypochlorite for 30 seconds and sterilized distilled water before drying in sterilized paper towels. Five root pieces were transferred to two MGA plates each and incubated at room temperature for five days. Total infection incidence were calculated by considering the total number of root pieces as the 100% and the number of roots infected as the percentage of incidence (Singleton *et al.*, 1992). A small piece of agar at the edge of the colonies identified as *Fusarium* was transferred to a PDA plate incubated at room temperature for five days and transferred to SNA and CLA for identification.

Statistical Analysis

Analysis of variance tests were done to establish the effect of soil amendments on the occurrence of the fungus, soil fungal density and root infection. Fisher's Least Significance Difference (LSD) was used to

compare treatment group means. Shannon's diversity indices were applied to compare fungal species diversity. Species accumulation curves were generated by plotting the total number of species recorded per sample from bean and maize rhizosphere soils and roots from all the treatments.

RESULTS

A total of 303 isolates of *Fusarium* sp. were recovered, of which 164 were from the roots representing 18 species while 110 were from the soil and represented 22 species (Table 2). The most frequently isolated species were *F. oxysporum* and *F. sporotrichioides* in both soil and roots. The frequency of isolation and diversity of *Fusarium* varied with treatment (Table 3). The fungus was more abundant and diverse in plots treated with *Trichoderma* and Manure. Plots treated with Mijingu + CAN recorded the least frequency of isolation and diversity. Plots treated with *Trichoderma* had the highest frequency of isolation and diversity of the fungus in the roots too. Mavuno and Mavuno + *Trichoderma* also recorded values higher than the control. *Fusarium* was most rare and least diverse from roots in Mijingu + CAN treated plots.

Fusarium inoculum density in soil varied significantly across treatments ($p < 0.001$, Table 4). Plots treated with Mijingu + CAN fertilizer had the least amount of inoculum followed by those treated with *Trichoderma* seed coat and Manure. The highest inoculum density was recorded in plots treated with TSP + CAN. Mavuno, and Mavuno + *Trichoderma* recorded the lowest inoculum levels. Root infection varied significantly with treatment ($p = 0.052$). Mijingu + CAN had the highest infection incidence while TSP + CAN the least. The mean values (Table 5) showed variation with *Trichoderma* treatment recording the highest root infection for beans while Mijingu + CAN treatment recorded the highest root infection for maize. There was a significant interaction between crop type and treatment on *Fusarium* root infection. Soil inoculum density was highest from the TSP + CAN for both bean and maize rhizosphere and least in Mijingu + CAN. This difference was significant at $p < 0.001$. Crop type alone did not significantly affect the soil *Fusarium* abundance.

Soil amendments significantly influenced *Fusarium* richness in the roots, but not in the soil rhizosphere (Table 6). *Trichoderma* treated plots had the highest number of species in the roots followed by Mavuno + *Trichoderma*. Plots treated with TSP + CAN and Manure recorded levels lower than Control while Manure + *Trichoderma* treatment presented the lowest root infection. Mavuno and Mijingu + CAN plots had levels of root infection similar to Control plots according to Fisher's LSD grouping. Species diversity

was influenced by crop type with bean rhizosphere being more diverse with *Fusarium* compared to maize rhizosphere as shown by Shannon indices (Table 7) and species accumulation curve (Fig 1a). However, the fungus was more diverse (Table 7) and rich (Fig 1b) in maize than bean roots. Fertilizer treatment influenced species accumulation in soils and roots with *Trichoderma* and manure treated plots having the highest number of species both in the soil and roots (Fig 2a, b).

Table 2. Frequency of isolation of *Fusarium* spp. from soil and roots of maize and beans.

Fungal Species	Frequency of isolation	
	Soil	Roots
<i>F. oxysporum</i>	31	66
<i>F. sporotrichioides</i>	24	46
<i>F. crookwellense</i>	12	11
<i>F. polyphialidicum</i>	9	0
<i>F. nygamai</i>	6	17
<i>F. dlamini</i>	4	11
<i>F. verticillioides</i>	3	1
<i>F. solani</i>	3	12
<i>F. sambucinum</i>	3	2
<i>F. torulosum</i>	2	3
<i>F. avenaceum</i>	2	0
<i>F. anthropilum</i>	1	0
<i>F. sterilihyphosum</i>	1	0
<i>F. semitectum</i>	1	0
<i>F. arthrosporioides</i>	1	0
<i>F. latevitium</i>	1	0
<i>F. redolens</i>	1	0
<i>F. chlamydosporum</i>	1	10
<i>F. pseudocircinatum</i>	1	0
<i>F. poae</i>	1	2
<i>F. heterosporum</i>	1	0
<i>F. graminearum</i>	1	2
<i>F. acuminatum</i>	0	3
<i>F. proliferatum</i>	0	2
<i>F. subglutinans</i>	0	2
<i>F. compactum</i>	0	1
<i>F. tricinctum</i>	0	1
<i>F. thapsinem</i>	0	1
TOTAL	110	193

Table 3. Effect of soil amendments on diversity of *Fusarium* species in soil and roots of maize and beans.

Treatment	Total frequency of isolation		Species isolated		
	Soil	Roots	Soil and Roots	Soil	Roots
Control	17	28	<i>F. oxysporum</i> <i>F. crookwellense</i> <i>F. sporotrichioides</i> <i>F. nygamai</i> <i>F. solani</i>	<i>F. polyphialidicum</i> <i>F. verticillioides</i> <i>F. graminearum</i> <i>F. pseudocircinatum</i>	<i>F. dlamini</i>
TSP + CAN	14	24	<i>F. oxysporum</i> <i>F. crookwellense</i> <i>F. sporotrichioides</i> <i>F. nygamai</i> <i>F. dlamini</i>	<i>F. polyphialidicum</i> <i>F. anthophilum</i>	<i>F. chlamydosporum</i> <i>F. acuminatum</i> <i>F. solani</i>
Manure	21	24	<i>F. oxysporum</i> <i>F. sporotrichioides</i> <i>F. crookwellense</i> <i>F. dlamini</i> <i>F. chlamydosporum</i> <i>F. sambucinum</i>	<i>F. polyphialidicum</i> <i>F. verticillioides</i> <i>F. avenaceum</i> <i>F. arthrosporioides</i> <i>F. redolens</i>	<i>F. solani</i> <i>F. graminearum</i> <i>F. subglutinans</i>
Mavuno	13	31	<i>F. oxysporum</i> <i>F. sporotrichioides</i> <i>F. nygamai</i>	<i>F. polyphialidicum</i>	<i>F. crookwellense</i> <i>F. dlamini</i> <i>F. chlamydosporum</i> <i>F. subglutinans</i> <i>F. compactum</i> <i>F. nygamai</i> <i>F. acuminatum</i>
Mijingu + CAN	1	3	<i>F. oxysporum</i>		<i>F. oxysporum</i> <i>F. nygamai</i> <i>F. solani</i> <i>F. crookwellense</i> <i>F. torulosum</i>
Manure + <i>Trichoderma</i> seed coat	8	17	<i>F. sporotrichioides</i>	<i>F. polyphialidicum</i> <i>F. dlamini</i> <i>F. sterilihyphosum</i>	<i>F. oxysporum</i> <i>F. nygamai</i> <i>F. solani</i> <i>F. crookwellense</i> <i>F. torulosum</i>
Mavuno + <i>Trichoderma</i> seed coat	15	29	<i>F. oxysporum</i> <i>F. sporotrichioides</i> <i>F. dlamini</i>	<i>F. crookwellense</i> <i>F. polyphialidicum</i>	<i>F. solani</i> <i>F. nygamai</i> <i>F. chlamydosporum</i> <i>F. torulosum</i>
<i>Trichoderma</i>	21	36	<i>F. oxysporum</i> <i>F. nygamai</i> <i>F. solani</i> <i>F. crookwellense</i> <i>F. torulosum</i> <i>F. poea</i> <i>F. sambucinum</i> <i>F. verticillioides</i>	<i>F. sporotrichioides</i> <i>F. semitectum</i> <i>F. heterosporum</i>	<i>F. dlamini</i> <i>F. chlamydosporum</i> <i>F. acuminatum</i> <i>F. proliferatum</i> <i>F. graminearum</i> <i>F. thapsinem</i> <i>F. tricinctum</i>

TSP = Triple Superphosphate r

CAN = Calcium ammonium nitrate

Mijingu = rock phosphate

Mavuno = fertilizers containing 11 nutrients in balanced proportions

Table 4. Effect of soil amendments on *Fusarium* density and root colonisation.

Treatment	<i>Fusarium</i> inoculum density (CFU) per 10g of soil	Fisher (LSD)	<i>Fusarium</i> incidence in roots (Mean of frequency)	Fisher (LSD)
Control	4895 ± 781	B	78.33 ± 3.10	A
TSP+CAN	7662 ± 972	A	77.05 ± 3.08	A
Manure	1195.2 ± 401.1	C	82.05 ± 2.99	A
Mavuno	3500 ± 481	B	76.33 ± 3.64	A
Mijingu + CAN	675 ± 92.1	C	90.00 ± 6.55	A
Manure + <i>Trichoderma</i>	4918 ± 1008	B	78.24 ± 3.44	A
Mavuno + <i>Trichoderma</i>	3788 ± 671	B	76.59 ± 2.85	A
<i>Trichoderma</i> seed coat	1012.9 ± 318.8	C	89.18 ± 2.42	A
P-value	<0.001**		0.052	

**Significant at all probability level 0.01

Table 5. Influence of crop type and soil management on *Fusarium* density.

Treatment	Root infection incidence (Mean)		Soil inoculum density (MeanCFU/10g)	
	Bean	Maize	Bean	Maize
Control	80.0	77.5	4457	5114
TSP + CAN	72.9	79.1	6914	8036
Manure	86.1	80.0	1586	1000
Mavuno	74.3	77.4	2114	4193
Mijingu + CAN	60.0	100.0	400	767
Manure + <i>Trichoderma</i>	71.7	81.8	5317	4700
Mavuno + <i>Trichoderma</i>	76.7	76.5	3450	3973
<i>Trichoderma</i>	97.0	84.9	1203	909
Crop P - value		0.721**		0.4341**
Crop X Treatment P - value		0.016*		<.001*

Table 6. Effect of soil amendment on *Fusarium* species richness.

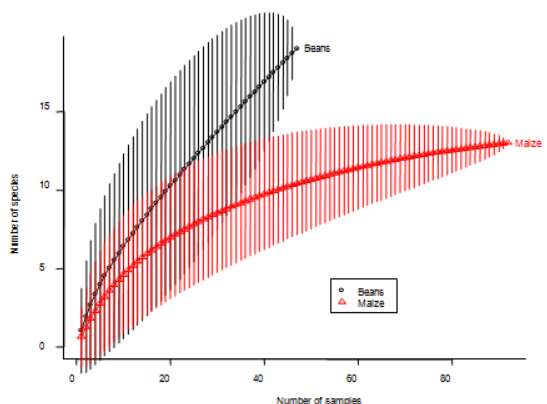
Treatment	Soil		Roots	
	Mean Richness	Fisher's (LSD)	Mean Richness	Fisher's (LSD)
Control	0.81	A	1.333	ABC
TSP + CAN	0.667	A	1.143	BC
Manure	1.000	A	1.143	BC
Mavuno	0.619	A	1.476	ABC
Mijingu + CAN	0.25	A	1.25	ABC
Manure + <i>Trichoderma</i>	0.471	A	0.941	C
Mavuno + <i>Trichoderma</i>	0.882	A	1.706	AB
<i>Trichoderma</i>	1.235	A	2.118	A
P-value	0.468**		0.014*	

*Significant

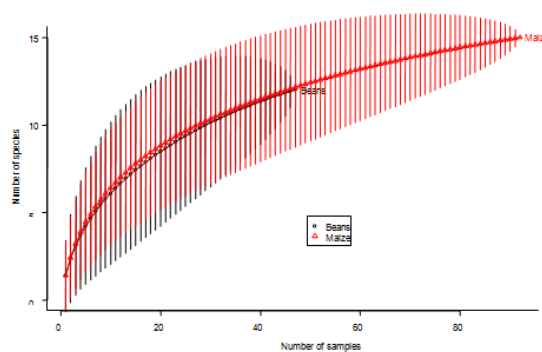
**Not significant at 0.01 level of probability

Table 7. Variation of *Fusarium* species richness with crop type: Shannon diversity indices.

Crop Type	Soil		Roots	
	Bean	Maize	Bean	Maize
Total Shannon Index	2.308	2.150	1.874	2.023
Mean Shannon Index	0.218	0.136	0.283	0.347

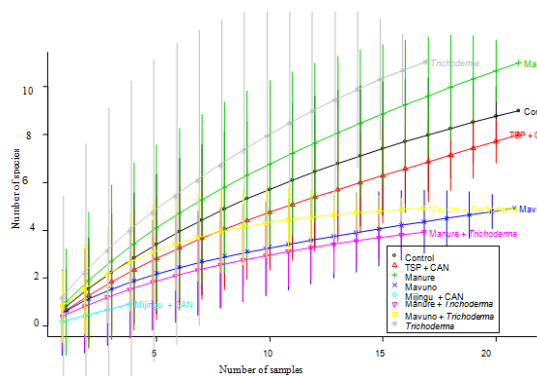


(a) In Soil

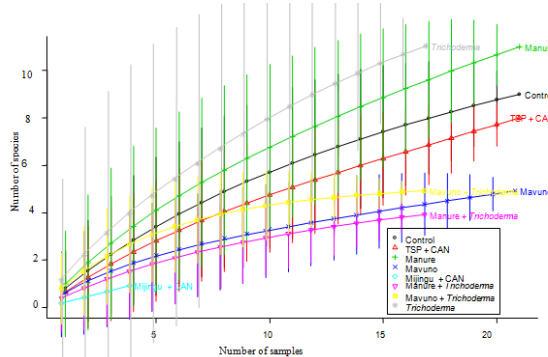


(b) In Roots:

Figure 1. Variation of species accumulation with crop type: Species accumulation curves.



(a) In Soil



(b) In Roots:

Figure 2. *Fusarium* spp. accumulation curves across treatments in soil and roots.

DISCUSSION

Fusarium is of widespread distribution in soils and root tissues. Soil fertility management influenced the occurrence, diversity and abundance of this fungus. Plots treated with Mavuno fertilizer recorded the least root infection followed by TSP + CAN, Mavuno + *Trichoderma* and Manure + *Trichoderma*. These fertilizers may have controlled root infection by improving plant growth due to nutrient availability (Kapkiyai *et al.*, 1996). Low soil fertility has been reported to cause poor bean production in many parts

of Kenya because of root rot by *Fusarium solani* sp. *phaseoli*, *Rhizoctonia solani* and *Pythium* species (CIAT, 1992, Gitu,1992). Application of cultural methods such as crop rotations to maintain the pathogen at low levels have not been successful due to the small acerages of smallholder farms leaving the use of farm amendments as the only affordable option (Hall and Phillips, 1992; Mutitu *et al.*,1985 and 1989; Otsyula and Ajanga,1994)

Addition of fertilizer or organic manures may also affect the pathogens themselves. The population of

soil *Fusarium* was markedly controlled in plots treated with Mijingu + Can fertilizer followed by Manure and *Trichoderma*. However these treatments did not reduce root infection. TSP + CAN and manure promoted occurrence and diversity of *Fusarium* sp. in soil while controlling root infection. The effect of the fertilizer on the fungus could be encouraging competitive fungi to grow in the soil thereby reducing population of *Fusarium*. Alternatively the fertilizer may have made the environment of the soil non-conducive for *Fusarium* proliferation. Kimani *et al.*, (2001) reported that manures or other organic inputs applied to the soil control the rate, pattern and extent of growth and activity of soil organisms and provide the source of carbon, energy and nutrients for the synthesis of soil organic matter. Manure can increase the humus content of soils by 15-50%, depending on soil type, in addition to increasing soil aggregate stability and root permeability (Kapkiyai *et al.*, 1996). Kapkiyai *et al.* (1996) reported that manuring restocks the particulate organic matter fraction better than fresh crop residues. Manure also acts as a buffer, thus improving nutrient uptake for crops grown in acid soils. Using manures alleviates aluminium toxicity and improves the availability of nutrients such as P, particularly in soils with a high phosphorus (P) fixation, and sulphur (S). Manure also supplies essential elements such as Mg, and trace elements which may not be available in commonly used inorganic fertilizers. This indicates that manure still remains an important fertilizer and source of nutrients both to the plant and soil organisms.

Trichoderma as a bioherbicide also had promising effects. The bio-inoculant, *Trichoderma*, and the organic amendment, manure were second to Mijingu + CAN in controlling the density of soil *Fusarium*. However, roots from these treatments recorded high infection incidences. Addition of *Trichoderma* with other fertilizers reduced root infection more than the bio-inoculant or the fertilizer applied alone. *Trichoderma* as seed coat alone is not sufficient as a control of *Fusarium*. The fungus should be mixed with other fertilizers as manure and mavuno to promote its growth and effectiveness.

The interaction between crop rhizosphere and soil management influenced the diversity of *Fusarium*. Crop type alone did not have an effect. *Fusarium* species diversity was higher in bean than maize rhizosphere. However maize roots showed higher diversity of *Fusarium* root infestation than beans suggesting that the type of crop species is important when choosing a disease management strategy.

CONCLUSION

Integrated soil fertility management interventions could be used to control *Fusarium* root infections. The type of intervention chosen could be based on crop type and the fungus to be controlled. Overall, Mavuno fertilizer rated highest in this case followed by TSP + CAN which implied that chemical fertilizer use and low fertility could be the major cause for high *Fusarium* incidence in soil and use of the fertilizers would reduce the abundance.

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