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Full Length Research Paper

Suppression of *Fusarium* spp. in a maize and beans intercrop by soil fertility management

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Fusarium **root rot of maize and beans is a common problem in Taita District, Kenya causing reduction in yields to the small scale farmers. The pathogen attacks maize and beans at all growth stages and 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 acceptable in this region due to the small size of farms and prices of fungicides which are out of reach to the small scale farmer. This study is aimed at assessing alternatives to the use of fungicides in controlling root infection by *Fusarium* spp in maize and beans. Field trials were done in Taita District where agriculture contributes to 95% of household income with very little or no fertility inputs in farms. The following were tested in the trial: three kinds of fertilizers, cow manure and *Trichoderma* seed coating. 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 *Fusarium oxysporum* (Schlecht) Snyd. et Hans. and *Fusarium sporotrichoides* Sherb. Addition of soil amendments had a positive effect of reducing root infection and in some cases lowering inoculum density in the soil. Of the four fertilizers tested, Mavuno had the highest yield and was the most effective in suppressing root colonisation by *Fusarium* spp.**

Key words: *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 leads to increased household income from sales. Production of the crops is generally constraint by pests and diseases. Most farmers are small scale and cannot afford expensive inputs for crop protection. Moreover, these pesticides are environmentally not appropriate. Fungal infection of maize and beans not only causes reduced yields through rotting, but can 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 mainly *Fusarium* spp, *Stenocarpell*a spp, *Penicillium* spp and *Aspergillus* spp (Kedera et al., 1992, 1998; MacDonald and Chapman, 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 causes damping-off at the seedling stage, yellowing of the leaves, stunted growth and death if severe. *Fusarium* species are ubiquitous in soils. They are commonly considered as field fungi invading more than 50% of maize grains before harvest (Robledo-Robledo, 1991). Several phytopathogenic species of *Fusarium* are found to be associated with maize including *Fusarium verticillioides* (Sacc.) Nirenberg, *Fusarium proliferatum* (Matsushina) Nirenberg, *Fusarium graminearum* Schwabe and *Fusarium anthophilum* (A. Braun) Wollenweber (Scott, 1993; Munkvold and Desjardins, 1997). Potentially effective crop rotations to maintain the pathogens of the two crops at low levels are not currently

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

Treatment (fertilizer)	Rates of application of fertilizer (Kg/ha)
TSP + CAN	TSP - 900 CAN- 780
Mavuno	1000
Mijingu + CAN	Mijingu - 800 CAN - 80
Cow Manure	10000
Trichoderma harziunum	Seed coat
Control	Nil

Triple Superphosphate (TSP) fertilizer ((17 - 23% P; 44 - 52% P_2O_5). Calcium ammonium nitrate (CAN) contains 27% N and 20% ground limestone. Mavuno is a blend of fertilizers containing 11 nutrients: Nitrogen (N½) 10%, Phosphorous (P_2O_5) 26%, Potassium (K_2O) 10%, Sulphur (SO_4) 4%, Calcium (CaO) 10%, Magnesium (MgO) 4%, and appropriate additions of other Trace Elements like: Zinc, Copper, Molybdenum, Boron and Manganese Mijingu is a rock phosphate (4.8×10^6 Mg with 10.6% P).

acceptable due to the small size of farms and cultural values (Hall and Phillips, 1992).

The purpose of this study was therefore, to evaluate the efficacy of soil amendments in management of root infection by *Fusarium* spp. in maize and beans.

MATERIALS AND METHODS

Study site

The study was carried out in Taita Taveta District as part of the larger 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 2000 m with mean annual rainfall of 800 - 2000 mm. The district covers an area of 16,965 Km^2 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.

Field trials

Field trials were both on station at the Agricultural Training Centre (ATC) and on farm. The former were researcher managed while the latter were farmer managed. This was to ensure that the experiments were run in a practical manner that would allow eventual take up of the technology by farmers. The experiment at the ATC was laid out in a Randomized Complete Block Design (RCD) with treatments replicated 5 times. These treatments were further replicated on farm on 12 slit plots, 6 in each location, to nullify the effect of heterogeneity of farms. The farms were 500 m apart to avoid auto-correlation (Groupe and Theriault Consultants, 1984). Each treatment was a stretch of 5 x 10 m. The test crops were maize intercropped with beans. The maize type was hybrid (H516) with spacing of 90 x 30 cm and planting done with two seeds per hole. Bean type was Mwezi moja with spacing of 75 x 25 cm and planted two seeds per hole. The treatments were Triple superphosphate combined with Calcium ammonium nitrate (TSP + CAN), Mavuno (blend of fertilizer containing 11 nutrients) and Mijingu rock phosphate fertilizers, Cow manure, and *Trichoderma* seed coating (Table 1). The fertilizers were added by broadcasting during planting and top dressing of CAN, TSP 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 pooled from five points in maize root and bean root rhizosphere respectively. The soils were transported in a cool box to the laboratory.

Fusarium density assessment in soil

One gram of soil was added to 9.0 ml of 0.05% water agar (10^{-1}) and shaken. One milliliter of the first dilution was pippeted and added to 9.0 ml of 0.05% water agar (10^{-2}) and shaken. From this last soil dilution, 1.0 ml was taken and pippeted 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 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).

Fusarium infection incidence assessment in plant roots

From each soil sample, 20 small pieces of the thinnest roots were cut approximately 1.0 cm long, washed in 1% sodium hypochlorite for 30 s and twice in 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 was 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 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

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

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

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* spp. were recovered. Out of this, 164 were from the roots and represented 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, had 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

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	*F. oxysporum* *F. crookwellense* *F. sporotrichioides* *F. nygamai* *F. solani*	*F. polyphialidicum* *F. verticillioides F. graminearum* *F. pseudocircinatum*	*F. dlamini*
TSP + CAN	14	24	*F. oxysporum* *F. crookwellense* *F. sporotrichioides* *F. nygamai* *F. dlamini*	*F. polyphialidicum* *F. anthophilum*	*F. chlamydosporum* *F. acuminatum* *F. solani*
Manure	21	24	*F. oxysporum* *F. sporotrichioides* *F. crookwellense* *F. dlamini* *F. chlamydosporum* *F. sambucinum*	*F. polyphialidicum* *F. verticillioides F. avenaceum* *F. arthrosporioides* *F. redolens*	*F. solani* *F. graminearum* *F. subglutinans*
Mavuno	13	31	*F. oxysporum* *F. sporotrichioides* *F. nygamai*	*F. polyphialidicum*	*F. crookwellense* *F. dlamini* *F. chlamydosporum* *F. subglutinans*
Mijingu + CAN	1	3	*F. oxysporum*		*F. compactum* *F. nygamai F. acuminatum F. oxysporum F. nygamai F. solani*
Manure + *Trichoderma* seed coat	8	17	*F. sporotrichioides*	*F. polyphialidicum* *F. dlamini* *F. sterilihyphosum*	*F. crookwellense* *F. torulosum*
Mavuno + *Trichoderma* seed coat	1529		*F. oxysporum* *F. sporotrichioides* *F. dlamini*	*F. crookwellense* *F. polyphialidicum*	*F. solani F. nygamai* *F. chlamydosporum* *F. torulosum*
Trichoderma	21	36	*F. oxysporum* *F. nygamai* *F. solani* *F. crookwellense* *F. torulosum* *F. poea* *F. sambicunatum* *F. verticilloides*	*F. sporotrichioides* *F. semitectum* *F. heterosporum*	*F. dlamini* *F. chlamydosporum* *F. acuminatum* *F. proliferatum* *F. graminearum* *F. thapsinem F. tricinctum*

TSP = Triple Superphosphater. CAN = Calcium ammonium nitrate. Mijingu = rock phosphateMavuno = fertilizers containing 11 nutrients in balanced proportions.

cant 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 (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*

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

Treatment	*Fusarium* inoculum density (CFU) per 10 g of soil	Fisher (LSD)	*Fusarium* 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 + *Trichoderma*	4918 ± 1008	B	78.24 ± 3.44	A
Mavuno + *Trichoderma*	3788 ± 671	B	76.59 ± 2.85	A
Trichoderma 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 (Mean CFU/10 g)	
	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 + *Trichoderma*	71.7	81.8	5317	4700
Mavuno + *Trichoderma*	76.7	76.5	3450	3973
Trichoderma	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 t.

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 + *Trichoderma*	0.471	A	0.941	C
Mavuno + *Trichoderma*	0.882	A	1.706	AB
Trichoderma	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

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 (Figure 1a). However, the fungus was more diverse (Table 7) and rich (Figure 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 (Figures 2a and b).

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 inmany parts of Kenya because of root rot by *F. solani* spp. *phaseoli, R. 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 acreages of smallholder farms, leaving the use of farm amendments as the only affordable option (Hall and Phillips, 1992; Mutitu et al.,1985, 1989; Otsyula and Ajanga,1994)

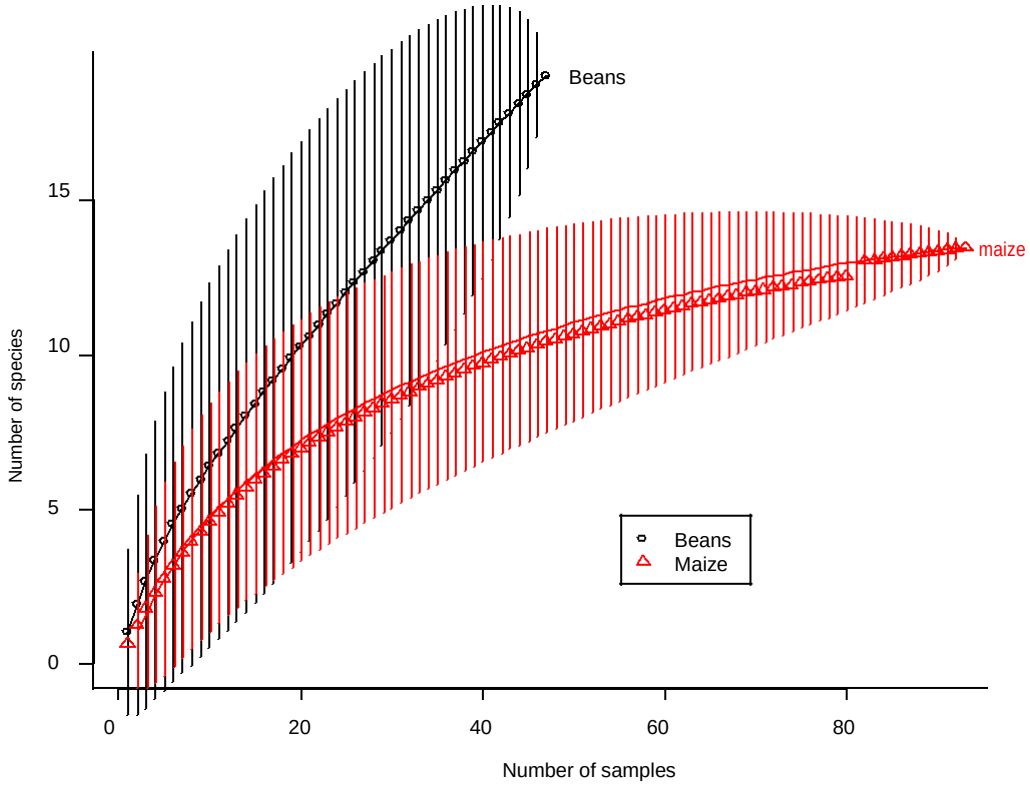
Addition of fertilizer or organic manures may also affect the pathogens themselves. The population of soil *Fusarium* was markedly controlled in plots treated with Mijingu + Can fertilizer followed by manure and *Trichoderma* However, these treatments did not reduce root infection. TSP + CAN and manure promoted occurrence and diversity of *Fusarium* spp. in soil while controlling root infection. The effect of the fertilizer on the fungus could be that of 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 phosphorus (P), particularly in soils with a high 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 Mijingo + CAN in controlling the density of soil *Fusarium*. However, roots from these treatments recorded high infection incidence. 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.

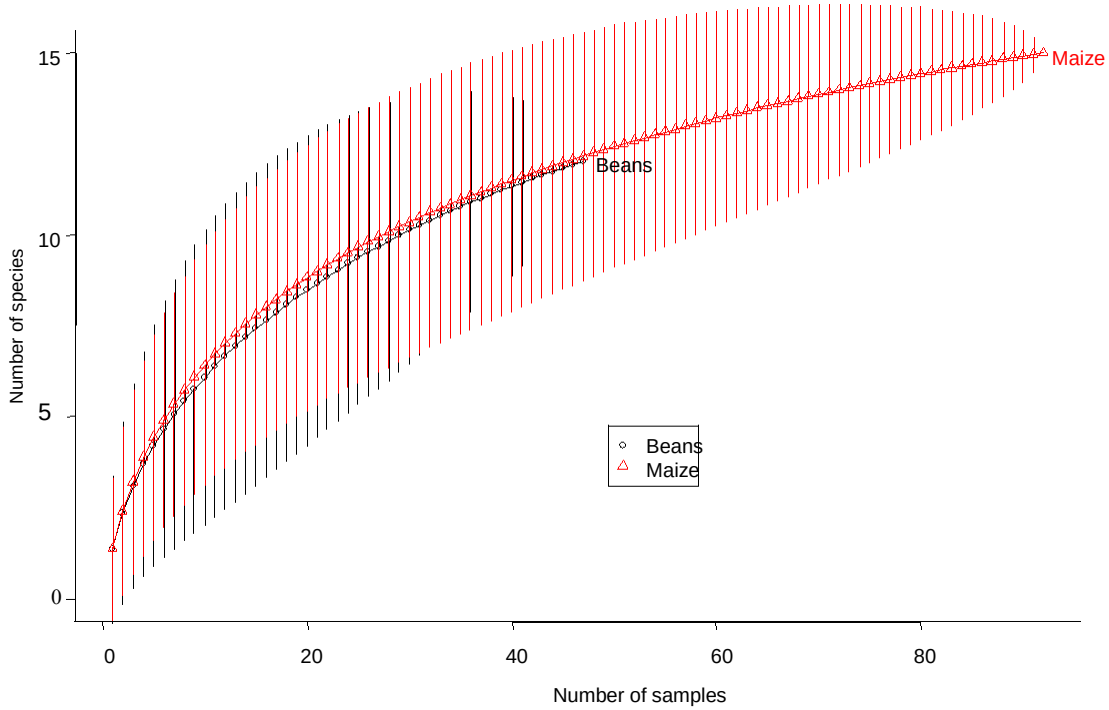
The interaction between crop rhizosphere and soil management influenced the diversity of *Fusarium*. Crop type alone did not have an effect. *Fusarium* species diversity was higher in bean than maize rhizosphere. However maize roots showed higher diversity of *Fusarium* root infestation than beans suggesting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

Conclusion

Soil management system can be used to control *Fusarium* root infections. The type chosen should depend on crop type and the fungus to be controlled. Overall Mavuno rated highest in this case followed by TSP + CAN.

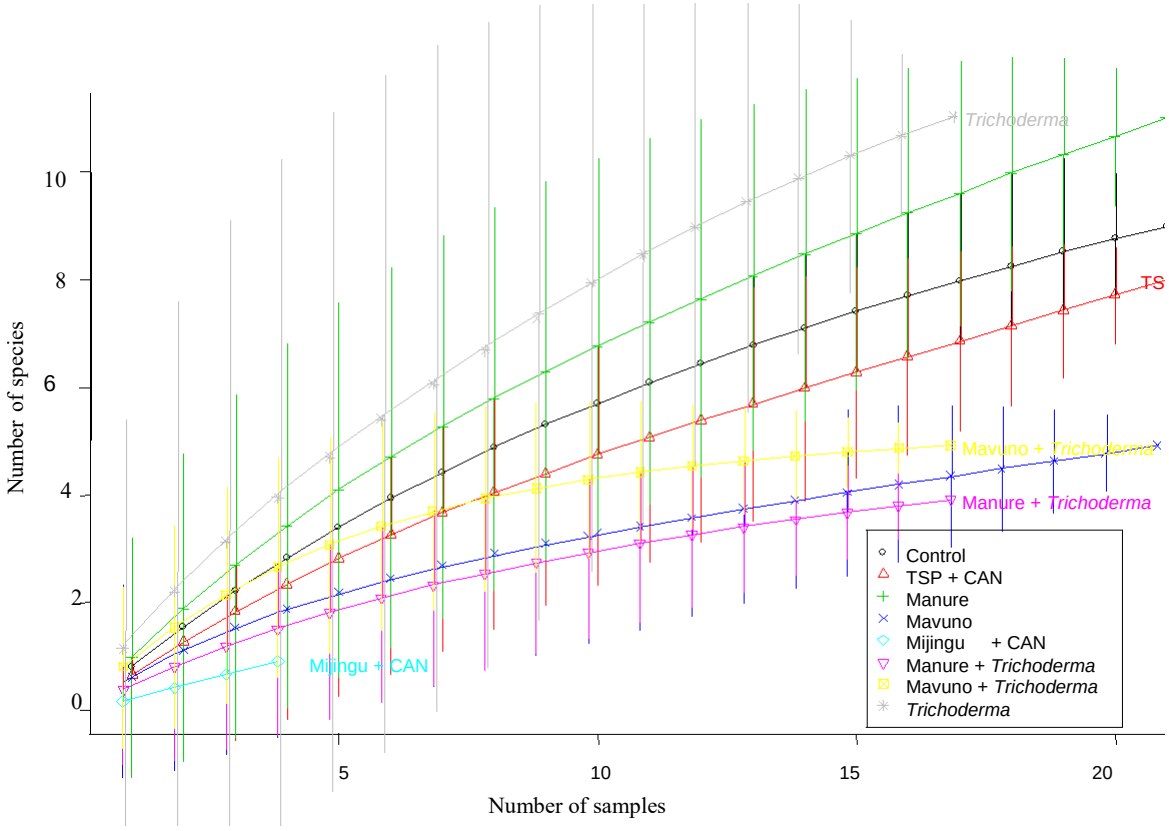


(a) In Soil

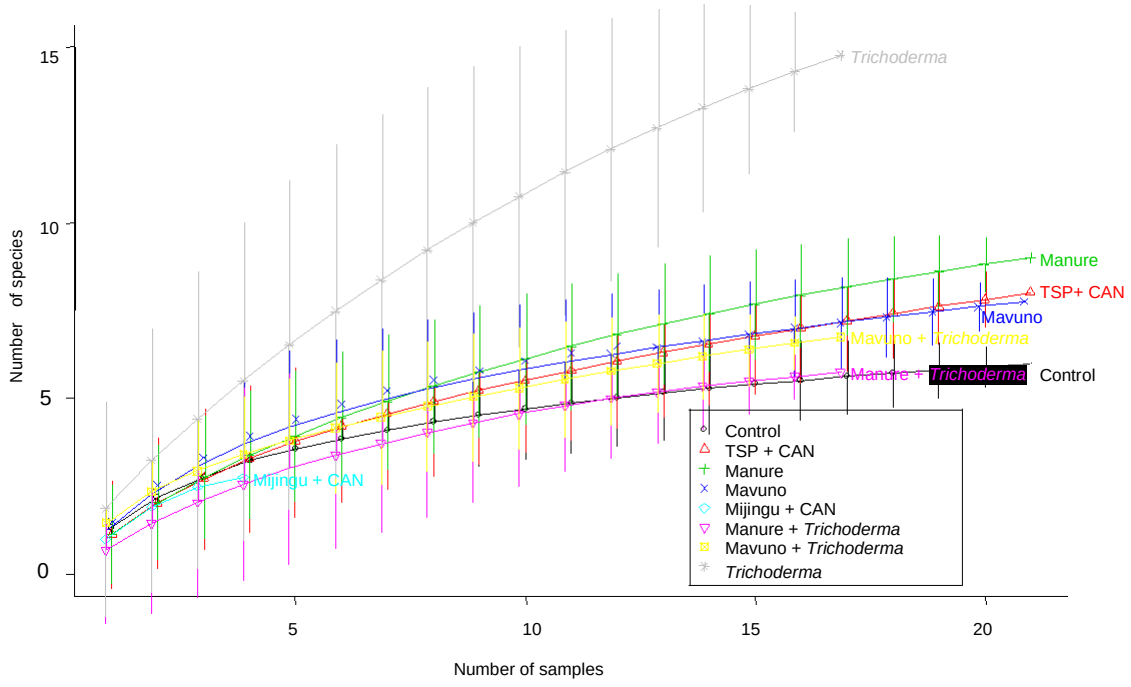


(b) In Roots:

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.

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