INFLUENCE OF SOIL FERTILITY AMENDMENT PRACTICES ON *EX-SITU* UTILISATION OF INDIGENOUS ARBUSCULAR MYCORRHIZAL FUNGI AND PERFORMANCE OF MAIZE AND COMMON BEAN IN KENYAN HIGHLANDS

[INFLUENCIA DE LAS PRÁCTICAS DE MEJORAMIENTO DE LA FERTILIDAD DEL SUELO SOBRE LA UTILIZACIÓN *EX SITU* DE MICORRIZAS ARBUSCULARES NATIVOS Y PRODUCTIVIDAD DEL MAIZ Y FRIJÓL COMÚN]

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SUMMARY

Arbuscular Mycorrhizal Fungi (AMF) are important in agriculture and have received attention as they are considered a part of an active and diverse soil biological community essential for increasing the sustainability of agricultural systems. However, most of agricultural practices have a negative impact on AMF association and agricultural soils are AMF impoverished. Interventions to replenish AMF include re-introduction through inoculation or manipulation of existing AMF to increase density. A major problem with inoculation is that there is possible competition with native (indigenous) AMF species. Indigenous AMF will be more adapted to the soil environment than introduced strains but with conflicting results on the effects of AMF inoculation on crop yield, more field studies for different ecological areas are required. The objective of the study was to compare the effect of inoculating crops with indigenous AMF applied applied singly or combined with other Soil Fertility Amendment Practices (SFAP) on root colonisation and subsequent performance of maize (*Zea mays* L.) and common bean (*Phaseolus vulgaris* L.). Analysis was also done on the best soil amendment practice that encourages crop colonisation by AMF. This was tested under field experiment and compared with control treatment (no soil amendment practice) and three other soil fertility amendment practices used singly or in combination with AMF; (1) MAVUNO (macro- and micronutrients and secondary nutrients) fertilizer, and (2) Calcium Ammonium Nitrate (CAN) and Triple Super Phosphate (TSP) (3) cattle manure. Maize and bean performances were determined and compared between the treatments for a period of two consecutive seasons with the experiment replicated in two benchmark sites of Embu district (highlands of central Kenya) and Taita-Taveta district (coastal highlands). Soils at Embu have high soil pH than at Taita which results in low phosphorus levels and possible micronutrients deficiencies. Even though no significant differences were observed from root colonisation by AMF with application of SFAP, significant differences were observed at the crop yield. Bean crop was more responsive to AMF inoculation than maize in terms of yield. Combination of AMF inoculant with other organic and inorganic fertilizers resulted in higher crop yield compared to AMF applied singly. Thus, utilisation of indigenous AMF species has potential to constitute an environmentally friendly method of soil fertility amendment over time to improve maize and bean production potential of small-scale holders but consideration should be done on the local soil nutrients conditions, other soil fertility amendment practices in use and the targeted crop.

Keywords: Arbuscular Mycorrhizae Fungi; Soil fertility amendment practices; indigenous species; inoculation; crop yield; colonisation intensity.

RESUMEN

Los hongos arbusculares micorrizicos (AMF) son importantes en la agricultura y han recibido atención al considerarse que como parte de una activa y diversa comunidad biológica del suelo que es esencial para lograr la sustentabilidad de los sistemas agrícolas. Sin embargo, la mayoría de las prácticas agrícolas tienen efecto negativo en la asociación de AMF y los suelos resultan empobrecidos de AMF. Prácticas para restablecer AMF incluyen re-introducción a través de la inoculación o manipulación de los AMF existentes para incrementar su densidad. Un problema con la inoculación es la posible competencia con AMF nativos. Los AMF nativos estarían más adaptados a las condiciones del suelo en comparación con las cepas introducidas pero hay resultados contradictorios en los efectos de la inoculación de AMF sobre la producción de los cultivos y más estudios para diferentes áreas ecológicas son requeridos. El objetivos del presente estudios fue comparar los efectos de inocular con AMF nativos, aplicados de manera aislada o en combinación con prácticas de mejoramiento de la fertilidad del suelo, sobre colonización de raíces y la subsecuente producción del maíz (*Zea mays L.*) y fríjol común (*Phaseolus vulgaris L.*). Se evaluó cual fue la mejor práctica de manejo que propició la colonización del cultivo por AMF. Se realizó un estudio de campo donde se comparó un control (sin prácticas de mejoramiento) y tres prácticas de mejora de fertilidad, solas o en combinación con AMF: (1) MAVUNO, macro y micro nutrientes y fertilización secundaria, (2) CAN+TSP, Nitrato cálcico amoniacal + super fosfato triple, (4) excretas de bovino. Se comparó la producción del maíz y fríjol entre tratamientos por dos períodos de cultivo consecutivos en dos sitios; el distrito de Embu (altiplano de Kenya) y el distrito de Taita-Taveta (zona costera del altiplano). Los suelos de Embu tienen un pH más alto que los de Taita lo que resulta en bajos niveles de fósforo y posibles deficiencias de micronutrientes. Aun cuando no se encontraron diferencias en la colonización de las raíces por AMF con la aplicación de las diversas prácticas de mejora de la fertilidad del suelo, si se observaron diferencias en la producción. El fríjol respondió mejor a la inoculación con AMF en cuanto a producción. La combinación de la inoculación con AMF con fertilizantes orgánicos e inorgánicos resulta en una mayor producción en comparación con el empleó de AMF solo. Así, la utilización de AMF nativos tiene el potencial de ser un método de bajo impacto ambiental para mejorar la fertilidad del suelo y mejorar la producción de maíz y frijol en sistemas de producción de pequeña escala, sin embargo debe considerarse la condición de los nutrientes del suelo de cada lugar, las diferentes prácticas de mejora de fertilidad del suelo y los cultivos dónde serán empleados.

Palabras clave: Hongos arbusculares micorrizicos; prácticas de mejora de la fertilidad del suelo; especies nativas; inoculación; producción de cultivos; intensidad de colonización.

INTRODUCTION

Plants form symbiotic association with mycorrhizae fungi and it is most widespread symbiosis in a natural ecosystem (Mozafar *et al*., 2000; Jansa *et al*., 2003). In agriculture, the arbuscular mycorrhizal fungi (AMF) of phylum Glomeromycota (Schussler *et al*., 2001), are most important. AMF forms association with over 80% of all crop plants (Cardoso and Kuyper, 2006; Sjöberg., 2005). The AM association has received attention as part of an increasingly popular paradigm that considers an active and diverse soil biological community as essential for increasing the sustainability of agricultural systems (Cardoso and Kuyper, 2006).

The role of AMF in enhancing plant growth and yield of crops has been previously reported (Bolan, 1991). AM fungi benefits the host plants with an increase in biomass and growth which may be caused by the host plant's increased ability to acquire essential nutrients and water (Ruiz-Lozano and Azcon, 1995).

Majority of AMF-crop interaction experiments have been conducted in the greenhouse, (e.g. Xavier and Germida, 1997) which does not explain AMF relevance to the field situation, although, a number of field experiments showed a positive results such as increased nutrient uptake and yield or reduced disease severity (Vosatka, 1995; Torres- Barragan *et al*., 1996; Koch *et al*., 1997; Kahiluoto and Vestberg, 1998; Al-Karaki, 2002; Al-Karaki *et al*., 2004; Mohammad *et al*., 2004; Douds *et al*., 2005). Maize is the most important agricultural commodity in Kenya contributing more than 25% of agricultural employment and 20% of total agricultural production (Government of Kenya, 2001) and providing about 40% of the populations' caloric requirements (Wekesa *et al*., 2003; Pingali 2001). The common bean is one of the most important edible pulse in the world (Debouck, 1990) and it is the second most important crop after maize as a staple crop in eastern and central Africa (MOALDM, 1996; Mwaniki, 2000). The productivity of maize and beans has been attributed to (i) declining soil fertility and (ii) increase in world fertilizer prices (Ariga *et al*. 2006; Omamo, 2003; Xu, *et al*. 2006; Bationo, 2008). The AMF symbiosis has potential to address some of the maize and beans production constraints. There are however conflicting results on the effect on AMF inoculation on maize and bean yield which necessitates more field studies for different ecological zones.

Field inoculation attempts have focussed the use of exotic strains, disregarding the potential of the existing naturally occurring strains. This could be cited as one possible reason for failure in field inoculation attempts. Native species have been cited as more adapted to the soil environment than introduced strains and as a result may out-compete the added AMF (Izaguirre-Mayoral *et al*., 2000; Klironomos, 2003). In the current study, we evaluate the effect of indigenous species on the *ex-situ* colonisation and performance of maize and bean. The study is conducted with support from the Below Ground Biodiversity (BGBD) GEF funded project at selected research sites, where the potential yield of maize is 4,000 kg per hactare, while the yield obtained by the farmers is on the average only 900 kg per hectare (Muya *et al*., 2009).

MATERIAL AND METHODS

Study Site

Figure 1: Location of Embu (red) and Taita (green) districts (Experimental sites)

The study was conducted in Taita Hills of Taita Taveta District and Mount Kenya region of Embu District. The benchmark sites have high biodiversity, as they are known to contain a number of endemic plant and animal species and are designated among the twenty-five globally recognized biodiversity “hotspots” (TSBF-CIAT BGBD GEF-UNEP Project, 2002). The communities in both study areas are mainly smallholder subsistence farmers.

Embu District is in the Eastern Province of Kenya (latitude: 03º 30′ S, longitude: 37º 30′ E, and altitude 1480 m above sea level). The area receives a total annual rainfall of between 1200 and 1500 mm in two rainy seasons, ‘long rains’ (March to June) and ‘short rains’ (mid-October to December). Mean monthly temperature ranges between 14ºC and 19.5ºC.

The second benchmark site was in Taita Hills (lat 3°25´; long 38°20´), situated in Taita-Taveta District in South-Eastern Kenya (Coast Province) at an altitude of 2228 m above sea level). The land use in Taita Hills is dominated by intensive agriculture. The climate of the area is under the influence of Inter-Tropical Convergence Zone (ITCZ) and receives an average annual rainfall of 1500 mm in the highlands and 250 mm in the lowlands and the mean monthly temperature ranges between 17.4ºC and 34.5ºC. The soils in Taita Taveta benchmark site are classified as Plinthic Lixisols, Plinthic Acrisols, Dystric Cambisols and Chromic Luvisols, while those from Embu are Rhodic Nitisols, Humic Nitisols, Humic Acrisols, Haplic Acrisols and Umbric Andosols (Muya *et al*., 2009). Soils at Embu have high soil pH which results in low phosphorus levels and possible micronutrients deficiencies (Table 1).

Table 1 Average soil nutrients and pH measurements at Embu and Taita experimental sites.

Soil parameters	Embu	Taita
Soil pH	4.3	4.9
Nitrogen (%)	0.3	0.2
Organic Carbon(%)	2.3	2.1
Phosphorus (PPM)	12.1	41.8
Potassium (%)	0.5	0.5
Calcium (%)	3.8	2.0
Iron (Fe) (PPM)	37.0	58.1

Experimental site layout

A total of 60 plots with eight (8) treatments replicated five (5) times separated by 1m wide strips were established under randomized block design at the Agricultural training Centre (ATC). Similar treatments were applied at Taita and Embu comprising of one bio-inoculant treatments (AMF species) and three fertilizer practices (manure, MAVUNO and TSP plus CAN), two crops (beans and maize), and a control. Plots measuring 3m x 3m divided by 1m pathway were demarcated and blocks were randomized. MAVUNO is a multi-nutrient fertilizer that contains micronutrients.

Inoculation with mycorrhizae

The source of mycorrhizae inoculant used was derived from indigenous species from the two respective experimental sites. This was applied as in mixed form with each site receiving AMF inoculum that had been cultured one and a half years in sorghum then transferred to leek (*Allium porrum* L) four weeks before planting to generate infective mycelia and infected root fragments that are more infective. The cultures were initiated and maintained at the National Museums of Kenya and inoculum produced as by Munro *et al.,* (1999). The inoculum consisting of indigenous species was applied as a thick slurry coating of the seeds in a crude state comprising of spores, mycelia and infected root fragment at planting. Leek was used in culturing and bulking up of the inoculum. AMF spore abundance in trap culture soils and infective propagules was measured by MPN per 20g of air dried soil. The mean spore abundance in soils from the trap culture was 64.28 per 50g soil sample.

The Most Probable Number Method (MPN)

AMF spore abundance from field and trap culture soils and AMF infective propagules was determined by MPN. The Most Probable Number (MPN) technique uses a baiting plant (Porter, 1979). The presence or absence of an infective propagule in a dilution of inoculum was shown by presence or absence of any AMF root among the roots which had grown in that dilution. A tenfold series dilutions of each inoculum (test soil) were prepared with sterile river sand as the diluent. To make a tenfold dilution 30 ml of un-sterile soil was mixed with 270 ml of sterile sand to make 10 -1 dilution. From the 10 -1 dilution 30 ml were taken and mixed with 270 ml of sterile soil to make the next dilution. Three dilutions were made and every time the subsequent dilution was made by mixing 30 ml from the previous dilution with sterile sand. The soils were thoroughly mixed and transferred into pots of volume of 50 ml. The essence of using a small volume of soil is to make sure that the soil is thoroughly exploited by roots, so that in theory, infection will occur if only a single propagule is present (Porter, 1979). A tenfold series of soil dilution up to 10-4 replicated five times was established with leek (*Allum sepa*) as the test plant.

Maize and bean establishment

At Embu benchmark site Hybrid 513 (maize) and Mwitemania (GLP 92) beans were planted being the common varieties in use by local communities at the beginning of the project. At Taita benchmark site Hybrid 513 and Mwezi moja (GLP-1004), maize and bean varieties were planted respectively. Each plot had row spacing of beans inter-cropped with maize at 45cm apart with interspacing of 30cm with 2 seeds per hole. Each plot had intra row spacing of maize planted at 90x30cm with 2 seeds per hole (seed rate; 20-25 kg/ha). Each plot (3x3m) had 4 rows of maize with 10 holes per row (plant density of 4x10x2= 80 seeds per plot). Wet planting was done at the depth of 2.5-4cm. Thinning was carried out and single plant per hole was retained: a total of 40 plants per plot were allowed to grow to maturity.

Each plot (3x3m) had 3 rows of beans with 10 holes per row; the plant density of 3x10x2= 60 plants per plot. Thinning was carried out and single plant per hole was retained, a total of 30 plants per plot are allowed to grow to maturity. A maize and bean guard row around the farms was also established.

Amendments with manure and fertilizers

Cow manure was broadcasted at 9 kg per plot (equivalent of 40-60 tons ha^{-1}) and maize-bean intercrop planted as described. TSP plus CAN, the Triple Super Phosphate (TSP) was broadcasted at 0.8 kg per plot (200kg ha^{-1}) and Calcium Ammonium Nitrate (CAN) was applied at 0.5 kg per plot (150-200 kg ha^{-1}). The control treatment had no application of AMF or fertilizer while MAVUNO fertilizer (N (N1/2) 10%, Phosphorus (P_2O_5) 26%, Potassium (K_2O) 10%, Sulphur (SO_4) 4%, Calcium (CaO) 10% , Magnesium (MgO) 4% and Micronutrients (Zinc, Copper, Molybdenum, Boron and Manganese) was spread evenly at a rate of 0.9 kg /plot (40 kg ha^{-1}). AMF Inoculum was applied as seed coating (20g ml^{-1}).

Crop Management

Common farming practices were accorded to the plots and these include, land preparation, planting of crops with onset of rains, gapping so as to replace dead seedlings 14 days after germination, weeding and thinning so as to uproot excess plantlets.

Assessment of AMF colonisation

Five (5) plants per plot were randomly selected and roots obtained for AMF assessment. This was carried out 30 days after planting; during thinning time. The roots were stored in plastic bottles in 70% v/v ethanol preservation until assessment was done. The roots were processed and stained according to procedures by Koske and Gemma (1989). Slides were examined under the compound microscope and the intensity of AMF colonization (arbuscules, coils, vesicles, internal and external hyphae) recorded. The intensity of AMF colonization was recorded as the percentage cover of AMF colonization in each root fragment as described in McGonigle *et al*. (1990). AMF colonization is an indicator of AMF functioning and total AMF colonization of roots in this study was distinguished to arbuscules, coiled hyphae and vesicles making it possible to determine quality of colonization.

Data analysis

Significant differences of mycorrhizal colonisation and crop yield among the 8 treatments were tested using ANOVA followed by post Tukey test ($P \leq 0.05$). The data were analysed using SPSS 18th edition program for Windows (SPSS IBM, New York, U.S.A). The significance differences were tested based on the means of 5 replicates.

RESULTS

Effect of soil fertility amendment practices on AM colonisation of maize roots

Use of the various soil fertility amendments showed no significant difference ($P \leq 0.05$) on the colonisation of maize root by AMF and this was true even for individual features of AM infection; hyphae, arbuscules and vesicles infection at the two experimental sites; Embu and Taita (Table 2). However, higher coiled hyphae and total colonisation intensity was recorded from Embu experimental site compared to Taita while the highest root colonisation by arbuscules and vesicles was recorded from Taita site (Figure 2).

Effect of soil fertility amendment practices on AM % colonisation on bean roots

Use of the various soil fertility amendments showed no significant difference ($P \leq 0.05$) on the total root colonisation and on individual features of AM colonisation (Table 3). At experimental site level, there was no significant difference ($P \leq 0.05$) recorded for all AMF features at Embu but a significant difference ($P \leq 0.05$) was recorded for arbuscules colonisation at Taita while hyphae, vesicles and colonisation intensity showed no significant difference.

As observed from maize crop, similar results were obtained from bean crop whereby among the different AMF colonisation features, coiled hyphae were the most common followed by vesicles and arbuscules respectively.

As similarly observed from maize crop, higher total and coiled hyphae colonisation intensity was recorded from Embu experimental site compared to Taita while the highest root colonisation by arbuscules and vesicles was recorded from Taita site (Figure 2).

Table 2: AMF % colonisation intensity of maize roots under application of various soil fertility amendment practices at Embu and Taita districts in Kenya.

Treatment	Arbuscules	Hyphae	Vesicles	Total Colonisation
AMF	1.55	50.78	5.27	57.6
AMF + Manure	2.20	34.86	11.05	48.11
AMF + Mavuno	0.81	45.64	4.95	51.4
AMF + TSP plus CAN	1.93	55.69	12.69	70.31
Control	1.21	48.60	12.84	62.65
Manure	2.73	57.93	8.10	68.76
MAVUNO	2.33	62.01	6.49	70.83
TSP plus CAN	5.15	52.05	8.95	66.15
Significance ($P \leq 0.05$)	0.714	0.380	0.549	0.415

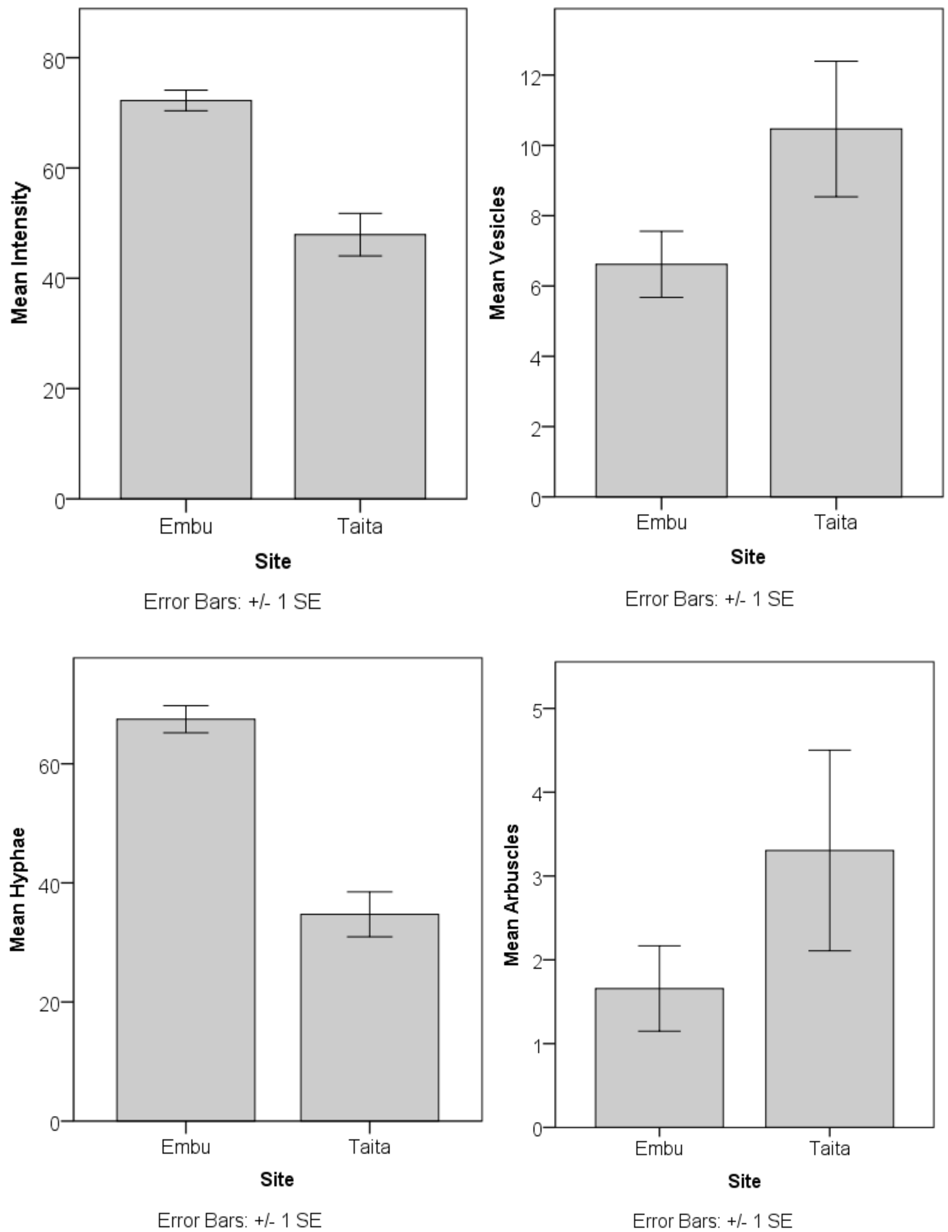

Figure 2: Comparison of Embu and Taita districts maize roots percentage colonisation by AMF arbuscules, coiled hyphae, vesicles and intensity of infection under application of various soil fertility amendment practices.

Table 3: AMF colonisation intensity of bean roots under application of various soil fertility amendment practices at Embu and Taita districts in Kenya.

Treatment	Arbuscules	Hyphae	Vesicles	Colonisation intensity
AMF	2.72	32.83	5.27	40.82
AMF + Manure	2.52	35.67	11.52	49.71
AMF + MAVUNO	1.44	50.71	7.88	60.03
Control	1.21	48.60	12.84	62.65
Manure	0.66	64.59	12.54	77.79
MAVUNO	3.81	41.34	6.99	52.14
TSP plus CAN	4.19	62.29	7.68	74.16
Significance ($P \leq 0.05$)	0.134	0.209	0.66	0.143

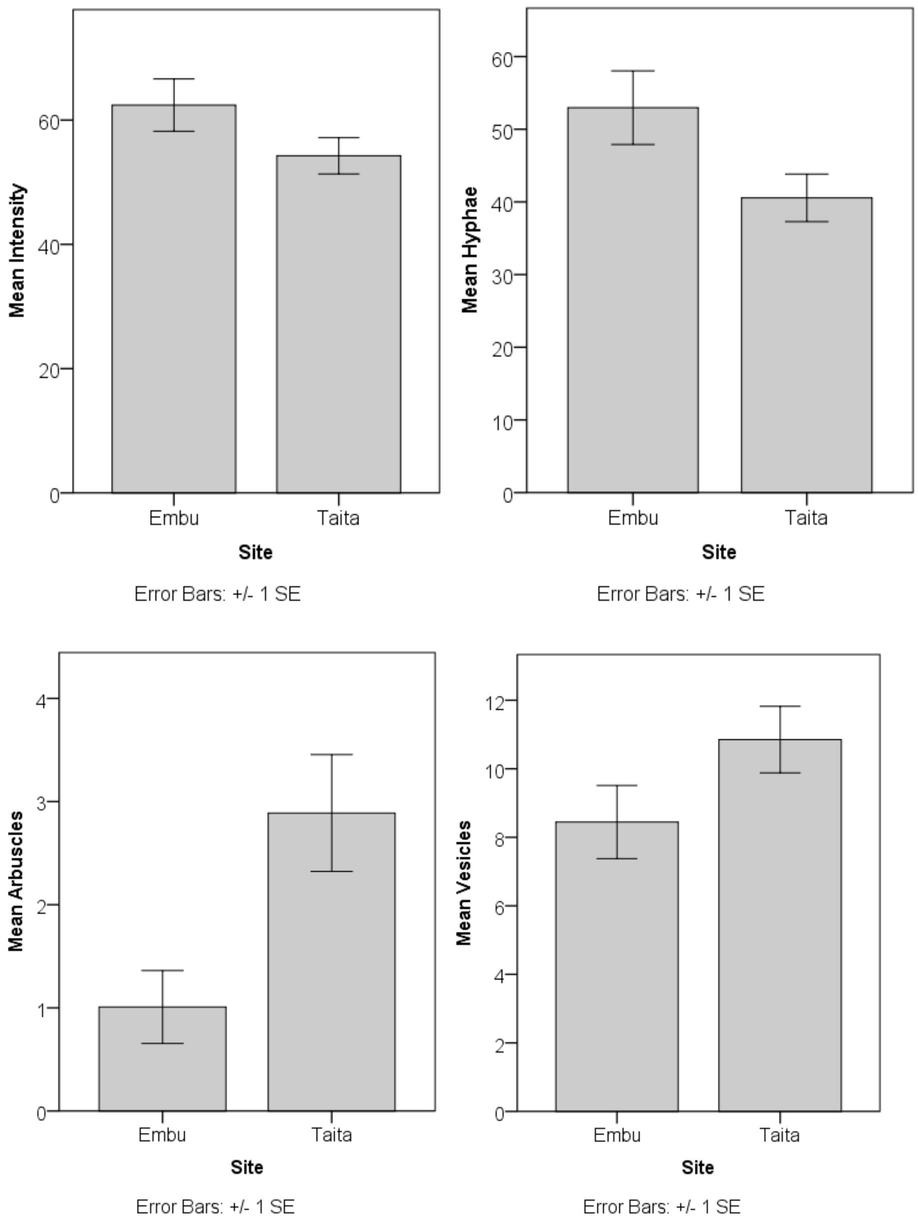

Figure 3: Comparison between Embu and Taita experimental sites bean roots percentage colonisation by AMF arbuscules, coiled hyphae, vesicles and intensity of infection under application of various soil fertility amendment practices.

Effect of soil fertility amendment practices on maize performance

Application of the various soil fertility amendments showed no significant difference ($P \leq 0.05$) on the total weight of maize stovers (biomass) for the two sites and seasons combined. However individually, each site resulted to significance difference ($P \leq 0.05$) on the total biomass production with application of different soil fertility amendment practices. Also, analysis at season's level resulted to significance difference ($P \leq 0.05$) on the total biomass production with application of different soil fertility amendment practices. The highest biomass production was recorded from treatment under MAVUNO with value of 7.1 tons ha^{-1} at Taita experimental site and lowest value of 3.8 tons ha^{-1} was recorded at the same site from plots under application of AMF plus manure. Application of AMF, alone did not enhance maize yields compared to control treatment while application of AMF combined with TSP plus CAN increased maize production by 34.04 % compared to TSP plus CAN applied singly at Embu experimental site. However at Taita site, a decrease of 6.25% was recorded with use of AMF combined with TSP plus CAN compared to TSP plus CAN applied singly (Table 4 and Figure 4). AMF applied in combination with MAVUNO also resulted in a 2.9% reduction of maize yield at Embu experimental site and 29.58% at Taita compared to application of MAVUNO singly. Application of manure singly or in combination with AMF did not improve maize yields in the experiments.

Higher maize biomass yield was recorded from Taita experimental site compared to Embu and the difference was significant at $P \geq 0.05$. A 44.1 % increase in biomass yield at Taita was recorded compared to Embu.

Effect of soil fertility amendment practices on bean yield

Application of different soil fertility amendment practices resulted in significant difference ($P \geq 0.05$) on bean yield; bean grain, bean litter and straw (Table 5). From individual site there was no significance difference ($P \leq 0.05$) recorded from Embu site on the total biomass production with application of different soil fertility amendment practices, contrary, a significant difference was recorded from Taita experimental site for both the litter and grain weight.

At Taita experimental site, the highest mean bean production (biomass) was observed from MAVUNO and the combination of CAN and TSP treatments with values of 1.67 tons Ha^{-1} and 1.61 tons Ha^{-1} respectively. The lowest value of 0.58 tons Ha^{-1} was recorded from plots under control treatment (Table 5). AMF inoculation applied singly increased bean yield (grain, straw and litter weight) by 60.34% compared to the control. Application of AMF in combination with TSP plus CAN and MAVUNO reduced bean yield compared to individual fertilizer only treatments by 42.23% and 44.31% respectively. An increase in yield of 25.84% was however observed when AMF was combined with manure compared to manure alone treatment (Table 5).

As similarly observed under maize crop, higher bean biomass yield was recorded from Taita experimental site compared to Embu and the difference was significant at $P \geq 0.05$. A 39.3 % increase in biomass yield at Taita experimental site was recorded compared to Embu.

Table 4 Effect of treatments on weight of stover and weight of cobs in maize crop.

Treatment	Biomass production at Embu (Tons Ha^{-1})	Biomass production at Taita (Tons Ha^{-1})
AMF	4.9 ± 0.5	4.6 ± 0.4
AMF + Manure	6.3 ± 0.2	3.8 ± 0.4
AMF + MAVUNO	6.7 ± 0.5	5.0 ± 0.7
AMF + TSP plus CAN	6.3 ± 0.5	6.4 ± 0.4
Control	5.6 ± 0.6	5.1 ± 0.5
Manure	6.6 ± 0.4	5.4 ± 0.8
MAVUNO	6.9 ± 0.4	7.1 ± 0.9
TSP plus CAN	4.7 ± 0.3	6.8 ± 0.6
Significance ($P \geq 0.05$)	0.09	0.03

Data are means of 8 replicates.

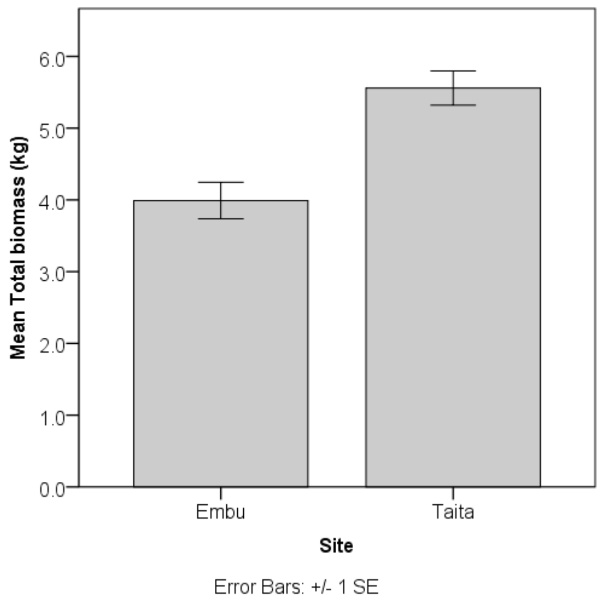

Figure 4: Bar graph showing maize yield for Embu and Taita the experimental sites; the highest biomass production was recorded from the Taita site showing a 44.1 % increase compared to Embu site.

Table 5: Effect of treatments on the bean production (total biomass) at Embu and Taita experimental sites.

Treatment	Bean yield (Tons Ha^{-1}) (Embu)	Bean yield (Tons Ha^{-1}) (Taita)
AMF	0.7 ± 0.3	0.9 ± 0.15
AMF + Manure	1.0 ± 0.2	1.12 ± 0.14
AMF + MAVUNO	1.0 ± 0.22	0.93 ± 0.13
AMF + CAN plus TSP	0.94 ± 0.24	0.93 ± 0.13
Control	0.73 ± 0.26	0.58 ± 0.07
Manure	0.95 ± 0.24	0.89 ± 0.10
MAVUNO	1.03 ± 0.22	1.67 ± 0.43
CAN plus TSP	0.99 ± 0.23	1.61 ± 0.22
P value	0.923	0.003

Data are means of 8 replicates.

DISCUSSION

Soil fertility amendment practices (SFAP) were studied to determine their effect on maize and been colonisation by AMF in an on station experiment. The results showed that SFAP had no significant ($P \geq 0.05$) effect on root colonisation of maize and bean by AMF. Such results were unexpected, considering the well-established negative effects of mineral nutrients on mycorrhizal functioning (Verbruggen and Kiers, 2010). This can be attributed to short experimental period of the current study which was two cropping seasons and equally important the timing of root sampling. However, a number of distinct patterns were observed taking into account of the two cropping seasons and experimental sites. First, for both crops, application of manure and inorganic fertilizers simulated AMF regeneration as highest level of coiled hyphae was recorded. This can be explained by the fact that improved nutrients availability facilitates growth of root and AMF mycelia, resulting in high AMF colonization (Karunasinghe *et al*., 2009). Also, organic nutrients increase the abundance of soil organisms by providing organic matter and micronutrients for organisms such as fungal mycorrhiza (Pimentel *et al*., 2005), which aid plants in absorbing nutrients and can drastically reduce external inputs such as fertilizer, at the cost of decreased yield (Mäder *et al.*, 2010). Positive effects of organic inputs on AMF colonization have been shown before (Galván *et al*., 2009; Gosling *et al*., 2010). Secondly, coiled hyphae which may serve similar function as arbuscules were the most common AM features recorded compared to arbuscules and vesicles. Coiled hyphae are the early stage of arbuscules and the high number recorded can be explained by the fact that root sampling were carried out at the early stages of plant growth. Dominance by arbuscules and coiled hyphae is also indicative of symbiotic strategy by the fungi and that the study area is still under low external nutrient inputs (Verbruggen and Kiers, 2010). Thirdly, there was high vesicle colonization under control treatment which is an indication of less benefit accrued to the host and also possibility of stress on the plant. This is supported by the fact that presence of vesicles depicts a stage of preparation for fungi entering into senescence as they generally serve as storage structures and when old as reproductive structures.

Lastly, when AMF was applied in combination with CAN plus TSP an increase in maize yield was observed but this increment was site specific with a higher increase recorded from Embu site which had low level of phosphorus at 12.1 ppm. Under lower P levels, Embu site, there was higher AMF activity and this is similar observation as that made by Allison and Goldberg (2002). The effects of AMF are manifested only in conditions of optimum P levels, hence where soil conditions have extremely low P level AMF will not be effective and in conditions of extremely high P AMF will also not be effective (Picone, 2002). In the latter condition (of extremely high P), AMF could also be detrimental with carbon reallocated to the fungi from the plant a factor that might account for decline in grain yield in the crop in cases where its function in P uptake is no longer required. However, under optimum P conditions, the carbon relocation is offset by increased uptake of phosphorus by the crop as a result of mycorrhizal contribution (Grigera *et al*., 2006). This may explain the differences in AMF response at the two sites to inorganic fertilizer application. The study maintains that high phosphorus levels and acidity has a negative influence on AMF colonisation of crops and this is in agreement with study by Jefwa *et al.* (2009) at Embu, Kenya.

Application of the various soil fertility amendments showed significant difference ($P \leq 0.05$) on the total weight of maize stovers (biomass) for individual sites. Fertilizer use remained the best intervention increasing yield up to 59.6% with application of MAVUNO. Use of organic and inorganic sources of nutrients is associated with C availability and balanced nutrient supply which improves crop yield (Ayeni and Adetunji., 2010). The recorded value of 1237.4kg per acre is however still below the potential yield of 4000kg per acre which implies presence of other growth constraints such as low precipitation. From both experimental sites, high maize and bean yield under MAVUNO (macro- and micronutrients and secondary nutrients) was recorded compared to other soil fertility amendment practices. Trace elements such as zinc, manganese and copper are increasingly being recognized as essential when aiming for better yields (White and Zasoski, 1999; Mann *et al*., 2002; Rashid and Ryan, 2004; He *et al*., 2005). This study highlights the importance of micronutrients to crop performance in tropic agricultural systems where micronutrient deficiencies, especially those of zinc (Zn) are common (Cordoso and Kuyper, 2006). AMF combined with TSP plus CAN increased maize production at Embu experimental site but this was not observed at Taita. This can be attributed to variation in P levels, where, the effect of inorganic fertilizer application was positive in Embu with an extremely low available P, it was negative in Taita where soils have higher P which is likely to inhibit AMF colonization. This is in agreement with Hu *et al*. (2009), whereby they found that high P supply capacity of soil is detrimental to AMF activities and that mycorrhizal plants are more dependent on AM in P-poor soils. Application of AMF, alone did not enhance maize yields compared to control treatment from both experimental sites.

In bean production, effect of application of different soil fertility amendment practices was found to be site specific with significant difference ($P \leq 0.05$) recorded from Taita and non from Embu experimental site. Highest bean yield was recorded from MAVUNO followed by CAN plus TSP treatment with the lowest yield obtained from control treatment. AMF inoculation applied singly increased bean yield (grain, straw and litter weight) by 60.34% compared to the control treatment.

CONCLUSION

Even though no significant difference was observed from root colonisation by AMF with application of the different SFAP, significant difference was observed at the crop yield and this suggest on poor timing of root sampling. Bean crop was more responsive to AMF inoculation than maize with higher yield from bean crop and lower maize crop yield recorded under use of AMF inoculant compared to control treatment. Combination of AMF inoculant with other organic and inorganic fertilizers resulted in higher crop yield compared to AMF applied singly. Thus, utilisation of indigenous AMF species has potential to constitute an environmentally friendly method of soil fertility amendment over time to improve maize and bean production potential of small-scale holders but consideration should be done on the local soil nutrients conditions, other soil fertility amendment practices in use and the targeted crop. Lastly, an evaluation on the effect of AMF inoculation on the spore abundance in the soil needs to be carried out as this would explain whether this lead to increased AMF diversity in the soil.

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