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IMPROVED SEEDLING EMERGENCE AND GROWTH OF MAIZE AND BEANS BY *Trichoderma harziunum*

[MEJORAMIENTO DE LA GERMINACIÓN Y CRECIMIENTO DEL MAÍZ Y FRIJOL POR *Trichoderma harzianum*]

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SUMMARY

An indigenous strain of *Trichoderma* spp. was tested for its ability to promote seed germination and growth of maize and bean seedlings grown in the field at Embu District, Kenya. The trial was carried out for three seasons with the following treatments; two types of fertilizers, cow manure, and *Trichoderma* seed coat. Seedlings were counted 14 days after emergence from soil and a sample gently uprooted using a spade. Shoot height, root length, stem and root diameter measurements were taken. *Trichoderma* inoculation significantly increased rate of maize seed germination but had no effect on emergence of bean seedlings. Maize seeds coated with *Trichoderma* inoculum and planted on soils without fertilizer addition recorded the highest germination rate of 82.7% followed by seeds coated with the inoculum and planted in soils treated with manure (82.2%). Combination of the inoculum and fertilizer performed better at improving maize seed germination compared with fertilizers applied singly. This was the case for shoot and root growth. Seeds coated with the inoculum and planted in soils ammended with Triple Superphosphate and Calcium Ammonium Nitrate recorded the greatest shoot and root growth in both maize and beans. Increased growth of shoot and root caused by *Trichoderma* implied that there was beneficial effect of inoculation on plant growth and development since root collar and stem diameters were a measure of survivability of seedlings.

Key words: Growth-promoting effect; *Trichoderma* spp.; fertilizers; inoculum

INTRODUCTION

Seed germination and seedling establishment are determined by several factors including quality of seeds and environmental factors. Within the environment of the seed and seedling are physical, chemical and biological factors that influence growth. The rhizosphere, is relatively rich in nutrients, because as much as 40% of plant photosynthetic products are exudates from roots (Bais *et al*., 2006). Consequently the rhizosphere supports large microbial populations capable of exerting beneficial, neutral, or detrimental effects on plant growth. *Trichoderma* species are free-living fungi that are common in soil and root ecosystems. They have been widely studied for their capacity to enhance plant growth, produce antibiotics, parasitize other fungi, and compete with deleterious plant microorganisms (Adams *et al*., 2007; Chang *et al*., 1986; Harman *et al*., 2004a,; Yedidia *et al*., 2001). The effectiveness of the use of microorganisms as biofertilisers and biocontrols however, is determined by a myriad of factors including virulence of the isolate, environmental factors, time of application, ability to survive in the environments other than their origin and colonize plants roots during certain period of time to control plant pathogens (Kredrics *et al*., 2003; Nemec *et al*., 1996; Stephan *et al*., 2005; Vinale *et al*., 2008) suggesting that augmenting of a local virulent strain would be more successful. This experiment was performed with an indigenous strain of *Trichoderma harziunum* to test whether it has an effect on germination rate and growth of maize and bean seedlings in Embu district, Kenya. Root rots, damping-off before and after seedling emergence, and seed rots of maize and beans caused by *Fusarium*, *Rhizoctonia* and *Macrophomina* have been reported as a major problem in this region (Mwangombe *et al.*, 2007). The desire for a more sustainable approach to agriculture, concerns about the impact of synthetic agrichemicals on human health and the environment, the high frequency of pathogen populations resistant to commonly used fungicides, the increasing cost of soil fumigation, and the continued search for methyl bromide alternatives has led to increased interest in the application of biological control for plant disease management. Hence the search for effect indigenous virulent microbial strains.

MATERIAL AND METHODS

Study site

The study was carried out in Embu District located within the Mount Kenya region bounded by longitudes 37° 18' East and Latitudes 0° S and 0° 28'S. The central point of the study area is transversed by Longitude 37° 28' East and Latitude 0° 20' S. The main land use systems are natural forest (Irangi forest), tea, coffee, mixed small-scale cultivation of food crops, dairy cattle rearing and semi-extensive livestock production.

Preparation and application of formulated *Trichoderma harziunum* inoculum

Indegenous *Trichoderma harziunum* strain initially isolated from the study site (BGBD Project benchmark site in Embu) (Okoth *et al*., 2007) and evaluated in vitro for antagonistic actions against *Rhizoctonia solani*, *Fusarium oxysporum f. sp lycopersici F. oxysporum f. sp phaseoli* and *F. graminearum* was used as a seed coat in this experiment.

For mass production, the isolate was grown in 1000 ml conical flasks, containing 250g vermiculate, 250g of wheat bran and 250g Czapek-Dox medium autoclaved for 20 minutes at 120°C, on two consecutive days. After 25 days incubation period, contents of the flasks were transferred to plastic plates under sterile conditions, left to air dry then mixed in a blender to become powder and kept in a plastic container at room temperature until ready for use in the field.

The formulation was used as a dry seed treatment where 2g of the formulation was mixed with 1 kg of seeds and treated with 4ml of gum Arabica solution (30g/300ml) as sticker onto the seed. The seeds of maize and beans were surface-sterilized by rinsing thoroughly in sterile distilled water, rolled with the formulation and planted immediately. Seeds treated with sterile distilled water served as control.

Field Trial to test effect of *Trichoderma* on seed germination and growth

Field trials were done in Kibugu and Ndunduri locations in Embu. The experiment was laid out at the ATC 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 500m apart to avoid auto-correlation (Groupe and Theriault Consultants, 1984). Each treatment was a stretch of 5 x 10m. The test crops were maize intercropped with beans. The maize type was hybrid (H516) with spacing of 90 x 30cm 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), 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. The on station experiments were researcher managed while on farm trials were farmer managed.

Seedlings were counted 14 days after emergence. From each hole, one seedling was dug out using a spade and the following parameters measured; plant shoot/stem height was measured from soil surface to apical buds using a ruler. Stem/root caliper width measurement was also recorded.

Table1. 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
T. harziunum	Seed coat
Control	Nil

Triple Superphosphate (TSP) fertilizer (44-52% P_2O_5).
Calcium ammonium nitrate (CAN) contains 27% N
Mavuno is a blend of fertilizers containing 11 nutrients: Nitrogen (N½) 10%, Phosphorous ($P_2 O_5$) 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

Statistical Analysis

Analysis of variance tests were done to establish the effect of *Trichoderma* seed coat and soil amendments on the rate of germination and seedling establishment of maize and beans. Tukey's Honestly Significance Difference (HSD) was used to compare treatment group means (Kindt and Coe, 2005).

RESULTS

Stimulative effect of *Trichoderma* spp. on seed germination

Germination rates of maize and beans planted in soils amended differently are shown in Fig 1 and 2. For maize, the effects were highly significant at p < 0,001 with f- value of 4.716. The highest rate of seedling emergence was 82.7% for seeds treated with *Trichoderma* inoculum followed by 82.2% for seeds coated with the inoculum and planted in soil amended with manure followed by 78.4% for seeds coated with *Trichoderma* and planted in soils amended with Mavuno fertilizer. Plots treated with TSP and CAN without the seed coat recorded the least germination rate (57.7%) followed by control (70.3%).

Treatment of bean seedlings with *Trichoderma* did not influence rate of germination significantly (p = 0.687; f-value = 0.683) though differences were observed with Manure treatment recording the highest germination rate of 74.9% followed by Manure+*Trichoderma* (74.0%) and *Trichoderma* seed coat alone (73.4%) (Figure 2). Control plots and those treated with TSP and CAN scored the least (69.6%).

The effect of soil amendments on bean growth was highly significant at p<0.0001 with f value of 9.023, 3.805, 8.378 and 15.564 for root collar and length, stem diameter and length respectively. Plots treated with TSP/CAN recorded the highest values of root size and stem diameter followed by TSP+CAN+*Trichoderma* , Mavuno+*Trichoderma* and Mavuno (Tables 4,5). Again like in maize the combination treatments of fertilizer with *Trichoderma* performed better than single fertilizer or inoculum application. Though TSP+CAN performed the best for beans Tukey's HSD grouped the performance of TSP+CAN as equal to TSP+CAN+*Trichoderma*, Mavuno+*Trichoderma* and Manure+*Trichoderma.*

Effect of *Trichoderma* and soil fertility amendments on maize and bean growth

All the fertilizers and inoculum had positive effect on maize growth. The variables that were measured were all greater for the treatment compared to the control. This difference was highly significant at p<0.0001 with f value of 13.149, 12.748, 7.612, and 17.115 for root collar diameter, root length, stem diameter and stem length respectively. Effect of *Trichoderma* on maize plant development was obvious with plots treated with TSP and CAN + *Trichoderma* recording the largest root and stem diameter and stem length followed by TSP and Mavuno+ *Trichoderma* fertilizers (Table 2,3) All the fertilizers performed better when combined with *Trichoderma* inoculum than when applied singly. Control plots recorded the smallest size of plants. Among the fertilizers manure performed the least close to control and Tuky's HSD grouped it with control.

Trichoderma increased lateral root growth of both maize and beans (Fig 3). The increase was more pronounced in seedlings grown in plots treated with combination of fertilizer.

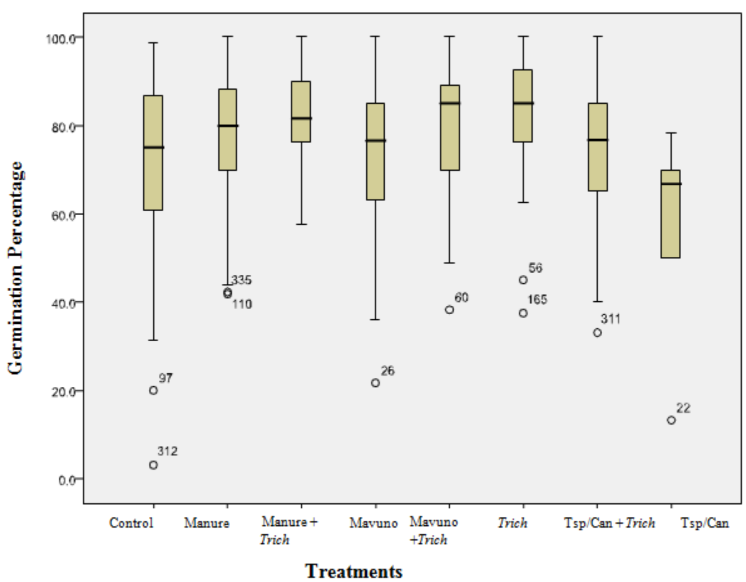

Figure 1. Improvement on germination of maize seeds by *Trichoderma* spp. at fourteen days after emergence.

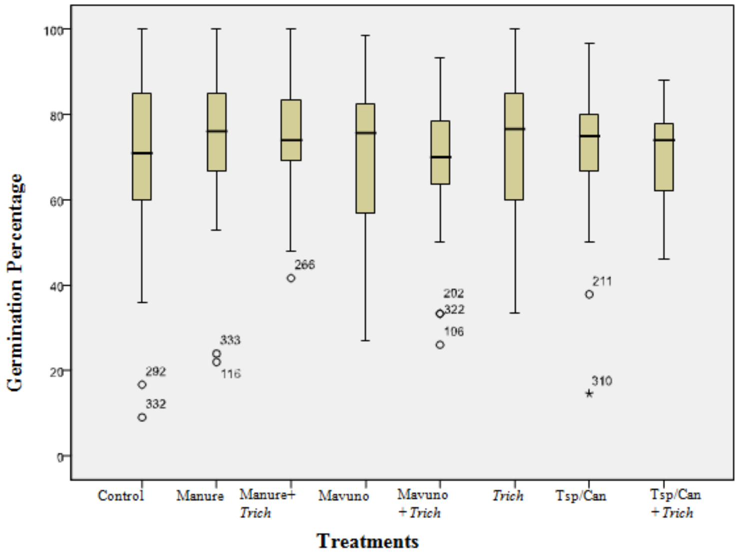

Figure 2. Improvement on germination of bean seeds by *Trichoderma* spp. at fourteen days after emergence.

Table 2. Effect of *Trichoderma* and soil amendment on maize root growth

		Root Collar (mm)		Root Length (mm)	
	N	Mean	Std. Error of Mean	Mean	Std. Error of Mean
Control	54	0.141 A*	0.0047	9.7772 A*	0.13859
Manure	54	0.149 A	0.0036	10.0396 AB	0.10349
Manure + *Trichoderma*	43	0.163 ABC	0.0053	10.9602 BCD	0.20445
Mavuno	54	0.178 BC	0.0053	11.2876 CD	0.22263
Mavuno + *Trichoderma*	47	0.181 C	0.0065	11.8902 D	0.41049
Trichoderma	45	0.155 ABC	0.0051	10.3016 ABC	0.20504
TSP+CAN	54	0.186 CD	0.0052	11.6926 D	0.16897
TSP+CAN + *Trichoderma*	15	0.211 D	0.0112	11.2933 CD	0.35706
Total	366	0.167	0.0021	10.8583	0.08963

*Figures followed by the same letter are not significantly different according to Tukey's Honestly Significant Difference (HSD) test

Table 3. Effect of *Trichoderma* and soil amendment on maize stem growth.

		Stem Diameter (mm)		Stem Length	
	N	Mean	Std. Error of Mean	Mean	Std. Error of Mean
Control	54	0.3361 A*	0.02759	13.3083 A*	0.43342
Manure	54	0.3452 A	0.02673	14.7543 AB	0.36347
Manure + *Trichoderma*	43	0.4634 A	0.04537	15.0556 AB	0.49529
Mavuno	54	0.4752 A	0.04781	16.7970 C	0.46677
Mavuno + *Trichoderma*	47	0.4709 A	0.03879	16.6426 C	0.46008
Trichoderma	45	0.3577 A	0.02486	14.4721 AB	0.49372
TSP+CAN	54	0.4215 A	0.03228	17.8037 C	0.41972
TSP+CAN + *Trichoderma*	15	0.8131 A	0.09243	20.8220 D	0.68270
Total	366	.4250	0.01429	15.7841	0.18765

*Figures followed by the same letter are not significantly different according to Tukey's Honestly Significant Difference (HSD) test

Table 4. Effect of *Trichoderma* and soil amendment on bean root growth.

		Collar Diameter (mm)		Length (mm)	
Treatment	N	Mean	Std. Error of Mean	Mean (mm)	Std. Error of Mean
Control	53	0.1121 A*	0.00108	9.493 A*	0.1469
Manure	53	0.1161 ABC	0.00131	9.656 AB	0.1692
Manure + *Trichoderma*	41	0.1204 ABCD	0.00215	9.874 AB	0.2012
Mavuno	54	0.1230 BC	0.00167	10.237 AB	0.1303
Mavuno + *Trichoderma*	47	0.1237 CD	0.00253	10.366 B	0.2401
Trichoderma	45	0.1145 AB	0.00146	9.854 AB	0.1641
TSP+CAN	51	0.1292 D	0.00263	10.462 B	0.1952
TSP+CAN + *Trichoderma*	17	0.1239 CD	0.00374	10.083 AB	0.3100
Total	361	0.1200	0.00076	9.995	0.0677

*Figures followed by the same letter are not significantly different according to Tukey's Honestly Significant Difference (HSD) test

Table 5. Effect of *Trichoderma* and soil amendment on bean stem growth.

		Stem diameter (mm)		Stem length (mm)	
	N	Mean	Std. Error of Mean	Mean	Std. Error of Mean
Control	53	0.1749 A*	0.00420	9.2908 A*	0.32655
Manure	53	0.2031 AB	0.00703	11.0440 A	0.26542
Manure + *Trichoderma*	41	0.2130 B	0.00802	12.4066 AC	0.29130
Mavuno	54	0.2219 B	0.00723	12.5981 C	0.28116
Mavuno + *Trichoderma*	47	0.2271 B	0.00761	12.2851 BC	0.29777
Trichoderma	45	0.2043 AB	0.00706	11.6207 BC	0.16732
TSP+CAN	51	0.2342 B	0.00596	11.6357 BC	0.32226
TSP+CAN + *Trichoderma*	17	0.2321 B	0.00716	12.9388 C	0.35476
Total	361	.2119	.00263	11.5801	.11872

*Figures followed by the same letter are not significantly different according to Tukey's Honestly Significant Difference (HSD) test

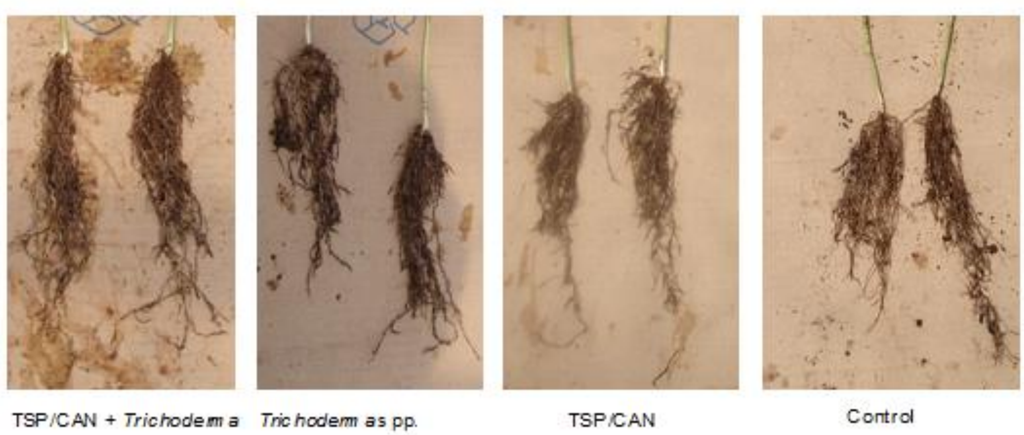

Figure 3. Effect of *Trichoderma* on root growth of bean seedlings

DISCUSSION

Trichoderma increased the rate of germination of maize and seedling growth of both maize and beans. The increased root length and collar diameter, stem length and diameter by *Trichoderma* treatment are measures of seedling's survivability and illustrate the direct effect of the fungus on the plants. A number of mechanisms for plant growth promotion by *Trichoderma* have been proposed (Harman *et a.l*, 2004a; Jaleed *et al.*, 1988). These include production of antibiotics, parasitization of other fungi, and competition with deleterious plant microorganisms. Until recently, these traits were considered to be the basis for how *Trichoderma* exerted beneficial effects on plant growth and development. However it is becoming increasingly clear that certain strains have substantial direct influence on plant development and crop productivity (Harman, 2006).

Combination of *Trichoderma* inoculum and fertilizers performed better compared with single application of either fertilizer or inoculum showing that when soil fertility was increased, the level of increased maize and beans growth induced by *Trichoderma* spp. was enhanced. Addition of fertilizer could have provided substrate for proliferation of the fungus thereby increasing its overall performance. Windham *et al*., (1986) also reported that when soil fertility was increased, the level of increased tomato growth induced by *Trichoderma* spp. was enhanced. Manure effect on seedling growth was low compared to other fertilizers probably due to its quality and the short period of running the experiment. Manure has been reported to have several effects when added to the soil system which include, immediate supply of nutrients like nitrogen as ammonium, phosphorus, potassium, and micronutrients that can be used directly by plants and microorganisms; delayed supply of nutrients that are part of organic (carbon-containing) compounds; lowered pH; salt and ammonia toxicity; improved soil structure; enhanced biological activity. The immediate supply of nutrients by manure could explain the improved effect of *Trichoderma* on germination when soils were treated with the fertilizer. The delayed supply of nutrients from manure explains the low effect of the fertilizer on eventual growth parameters of the seedlings. Gopichand *et al*., (2006) working on *Curcuma aromatica,* and Zane and Basil (2004) working on *Pennisetum americanum* also reported such delayed effects on nutrient release from manure. Eghball *et al.,* (2004) also reported residual effects of manure and compost applications on corn production and soil properties.

Trichoderma promoted growth of primary root length and root branching in maize and beans by inducing lateral root growth. In plants auxins have been demonstrated to initiate lateral root growth (Casimiro *et al.*,2001) and the observed effects of *Trichoderma* in promoting lateral root development is similar to in vitro experiments performed by Hexon *et al.,* (2009) that showed that *Trichoderma* spp. produced indole-3-acetic acid (IAA) that promoted lateral root formation in *Arabidopsis thaliana.* The root system is important for plant fitness because it provides anchorage, contributes to water use efficiency, and facilitates the acquisition of mineral nutrients from the soil (Lopez-Bucio *et al.,* 2005a). Increased root size resulted into increased shoot size which translates into increased shoot biomass production indicating a beneficial effect of inoculation on plant growth and development. The positive influence of *Trichoderma* on root system architecture would therefore relate to increased yield of plants. *Trichoderma* enhanced root biomass production and increased root hair development has also been reported by Bjorkman *et al.*, 1998; Harman *et al.,* 2004b.

CONCLUSION

We conclude that the *Trichoderma* spp. isolate tested increased the rate of seed germination and shoot and root growth of maize and bean seedlings. Addition of fertilizers enhanced *Trichoderma* activities. The development of this isolate as a successful biostimulant is dependent upon understanding of the complex interactions of this organism and plants in the soil ecosystem.

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