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Influence of Maize Seed Inoculation with Microbial Bio Fertilizers on Morphological and Physiological Parameters of Maize

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ABSTRACT

The exploit of efficient beneficial microorganisms opens up a novel prospect for enhanced crop productivity above and beyond sustaining soil health. At present, the growing demand for sustainable agriculture is driving the use of bio fertilizers composed of beneficial microorganisms. Two separate pot experiments on effect of maize seed inoculation by bio fertilizers (*Trichoderma* and *Bacillus*) at varying levels of concentration on plant growth were conducted. Treatments, T_{0g} (Control), T_{1g} (50%), T_{2g} (100%), T_{4g} (200%) and T_{6g} (300%) were laid out in a randomized complete block design. Seed treatments significantly (P<0.05) affected the co-efficient of variation for plant height, root length, fresh weight and dry weight for the bio fertilizers when compared to the control treatment. Effect of both bio fertilizers was not significant (P>0.05) on number of leaves. For Bacillus based bio fertilizer, T_{2g} gave the best results. T_{1g} obtained maximum root length (42.3cm). The advanced plant growth was as a result of significant (P<0.05) increase of N and P nutrient uptake due to application of bio fertilizers.

Key words: *Bio fertilizer, microorganisms, Trichoderma, Bacillus, inoculation, seed, maize.*

INTRODUCTION

Besides exceeding accomplishments in effectiveness, the extensive exploitation of chemical fertilizers and chemo-synthetic pesticides are accompanied with significant problems associated to the environment and public health, such as contaminations of food, pollutions of air and water bodies, degradation of soil fertility and loss of biodiversity, calling long-term sustainability into question (Matson et al., 1997; Swaminathan, 2006). According to Gyaneshwar et al, (2002) the unbalanced application of chemical fertilizers is liable for decline in soil fertility and environmental degradation. Records from the FAO (Food and Agriculture Organization of the United Nations) point out, that annual yield growth rates for the most important cereal crops, as paddy rice (*Oryza sativa*), wheat (*Triticum aestivum*) and maize (*Zea mays*), have been on the decline globally in recent years, even in numerous of the world's most fertile areas, notwithstanding high agrochemical input intensity (Gruhn et al., 2000; Heisey, 2002; Wiebe, 2003; Swaminathan, 2004). In sub-Saharan Africa, lack of attention for improving agricultural productivity has degenerated to a more complex situation of food insecurity, which has caused economic, environmental and financial losses (Rosegrant et al., 2005; Rukuni, 2002). Therefore, sub-Saharan African nations need to combat food security situation with a scientific, economic and technologically based approach. The exploit of efficient beneficial microorganisms opens up a novel prospect for enhanced crop productivity above and beyond sustaining soil health.

At present, the growing demand for sustainable agriculture is driving the use of biological fertilizers composed of beneficial microorganisms; ranging from bacteria to blue-green algae and fungi. Bio-fertilizers such as *Rhizobium, Azotobacter, Azospirillum, Pseudomonas* and *Bacillus* have very important use in sustainable agriculture due to their environmentally friendliness, cost-effectiveness and superior productivity benefits. Bio fertilizers augments plant nutrition and yield through biological nitrogen fixation, nutrient solubilisation, bio control activities and production of plant growth promoting substances. This is because plants are associated with complex micro biomes, which have a propensity to promote plant growth and stress tolerance, support plant nutrition, and antagonize plant pathogens. The amalgamation of beneficial plant-microbe and micro-biome interactions may perhaps represent a promising sustainable solution to advance agricultural production.

Current scientific advances center on a better understanding of biological processes sustaining soil fertility, healthy plant growth and resource efficiency (Uphoff et al., 2006). This take account of the complex interaction involving plants and microorganisms in their environment, either catalyzing or hampering the working of the whole system (Artursson et al., 2006; Watt et al., 2006; Nadeem et al., 2013), over and above the development of plant own adaptations and defense reactions under diverse abiotic and biotic stresses (Römheld and Neumann, 2006). On the whole, use of bio fertilizers aspires to develop integrated management strategies that make optimal use of the biological potential in agriculture in a manner that, simultaneously, leads to a profitable and continued food production at high quality and yield levels whereas maintaining the natural resource base (Altieri and Rosset, 1995; Roy et al., 2006).

The present studies seek to determine the best possible level of application of the bio fertilizers to realize optimum growth for sustainable maize production.

RESEARCH METHODOLOGY

Site Description

The experiment was conducted at Africa University Farm located at 18°53’70.3” South and 32°36’27.9” East and at an altitude of 1131m. The mean annual precipitation is approximately 800-1000 mm with most of rain falling between December and February. The average summer temperature is 27ºC and winter temperature is about 7ºC. The medium used in the pot experiment was a sandy loam soil. The soil at AU farm is a red sandy clay loam, Fersiallitic 5E soil under Zimbabwe soil classification system (Nyamapfene, 1991).

Experimental Design, Treatments And Establishment

A pot experimental study was conducted to determine the effect of a bio fertilizer on growth and development of maize crop. The experimental treatments were laid out in a Randomized Complete Block Design (RCBD) with three replications. Maize grains were surface sterilized by immersing in 70% ethanol for 2 min and then in 0.2% sodium hypochlorite (NaoCl) for 3 min. Seeds were washed several times with sterile distilled water. The microbial bio fertilizers selected for this study contained *Trichoderma harziunum* at a concentration of not less than $1.0x10^8$ cfu/g and the other *Bacillus oryzicola* YC7007 at a concentration of not than $1.0x10^{10}$ cfu/g.

The concentration treatments for the bio fertilizers were applied to the seeds as follows: T_{0g} = *Control*, T_{1g} = *50%*, T_{2g} = *100%*, T_{4g} = *200% and* T_{6g} = *300%*.

Ten seeds were placed into a plastic pot, each filled with about 5 kg of sandy loam based garden soil and replicated three times in a randomized block design. The seeds were sown at 2 to 3 cm depth in each pot and when seedlings growth was 10 old days, the seedling density was reduced to 6 seedlings per pot. The pots were watered every 72 h with equal amount of water. When the developed plants had reached 45 days old, 4 plants from each treatment were carefully uprooted.

Data collection;

Data were recorded on: Plant height, number of leaves, fresh weight and dry matter of shoots and roots, and root length.

Statistical Analyses Of Experimental Data

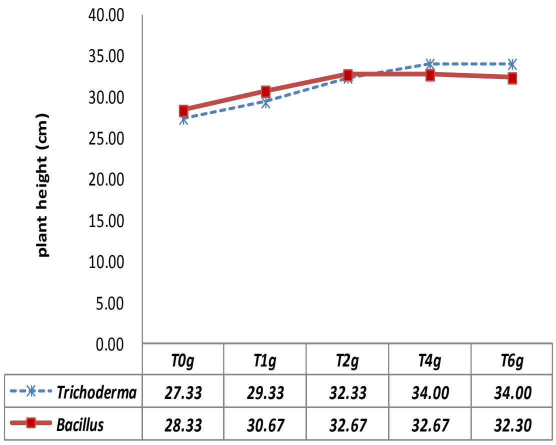
Data collected was statistically analyzed using the GenStat Analysis of Variance (ANOVA) software. Differences between means were determined using the Least Significant Difference (LSD) test at P=0.05 level.

RESULTS

Effect of Bio fertilizer Concentration

Plant Height

Effect of concentration of the *Trichoderma* based-bio fertilizer on plant height was significant ($P<0.05$) (Figure 1).



	T0g	T1g	T2g	T4g	T6g
Trichoderma	27.33	29.33	32.33	34.00	34.00
Bacillus	28.33	30.67	32.67	32.67	32.30

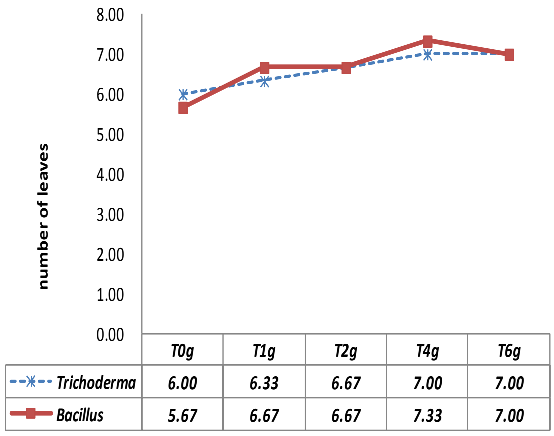
Concentration treatments: T0g = Control, T1g = 50%, T2g = 100%, T4g = 200% and T6g = 300%.

Figure 1 Effect of bio fertilizer concentration on plant height

Plant height increased numerically with increase in the concentration of the bio fertilizer. However, T_{0g} and T_{1g} recorded the shortest plants but were not statistically ($P>0.05$) different from each other. The tallest plants were recorded from T_{2g}, T_{4g} and T_{6g}. These were not statistically ($P>0.05$) different from each other. The mean plant height was 31.4cm.

Effect of *Bacillus* on plant height was also significant with concentration T_{0g} and T_{1g} recording the shortest plant as well. The trend showed that the tallest plants were recorded from T_{2g}, T_{4g} and T_{6g} and these were not statistically ($P>0.05$) different from each other. The mean plant height was 31.3cm.

Leaf number



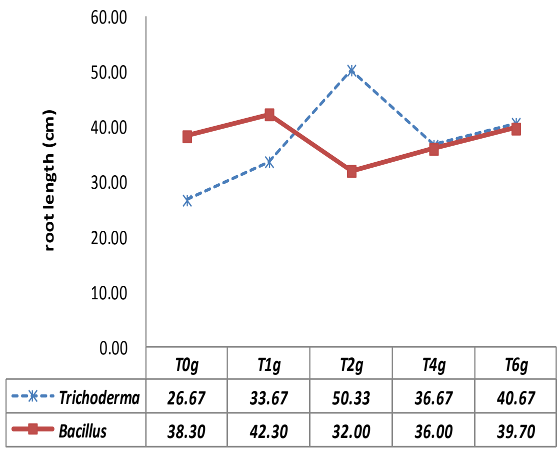
	T0g	T1g	T2g	T4g	T6g
Trichoderma	6.00	6.33	6.67	7.00	7.00
Bacillus	5.67	6.67	6.67	7.33	7.00

Concentration treatments: T0g = Control, T1g = 50%, T2g = 100%, T4g = 200% and T6g = 300%.

Figure 2 Effect of bio fertilizer concentration on number of leaves

Data pertaining to effect of bio fertilizer concentration on leaf number is shown in Figure 2. With regards to concentration of both *Trichoderma* and *Bacillus* based-bio fertilizer the number of leaves was not significantly ($P>0.05$) different.

Root length



	T0g	T1g	T2g	T4g	T6g
Trichoderma	26.67	33.67	50.33	36.67	40.67
Bacillus	38.30	42.30	32.00	36.00	39.70

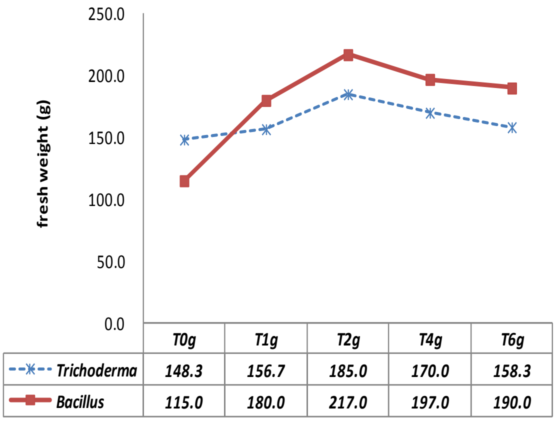
Concentration treatments: T0g = Control, T1g = 50%, T2g = 100%, T4g = 200% and T6g = 300%.

Figure 3 Effect of bio fertilizer concentration on root length

Root length was significant ($P<0.05$) for both *Trichoderma* and *Bacillus* bio fertilizer (Figure 3). For *Trichoderma* the trend was that from T0g the length significantly increased up to T_{2g} then began to decrease beyond this point. The highest root length was recorded at T_{2g}. It was interesting to note that results for T_{1g} and T_{4g} were not significantly ($P>0.05$) differently from each other. Mean root length recorded was 37.6cm.

For *Bacillus*, the highest root length was recorded at T_{1g}. however, results for T_{1g} was not statistically different from results at T_{0g}, T_{4g} and T_{6g}. The mean root length recorded was 37.7g.

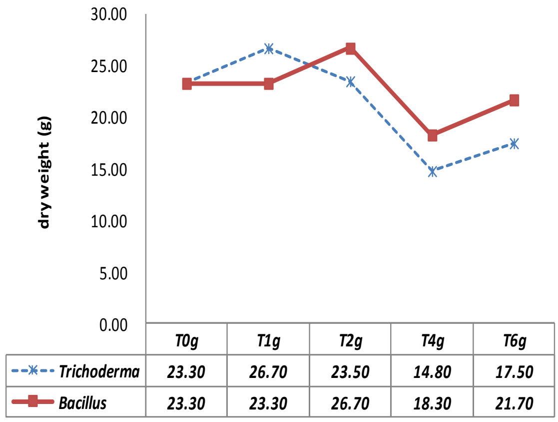
Fresh weight



Concentration treatments: T0g = Control, T1g = 50%, T2g = 100%, T4g = 200% and T6g = 300%.
Figure 4 Effect of bio fertilizer concentration on fresh weight

Results on effect of *Trichoderma* based-bio fertilizer concentration on fresh weight was not significant ($P>0.05$) (Figure 4). However, results for fresh weight were statistically ($P<0.05$) different for *Bacillus* based-bio fertilizer. As the concentration increased from T_{0g} to T_{6g} the fresh weight increased to a peak at T_{2g} (217.0g) then started to decline with increase in concentration. The mean fresh weight recorded was 180.0g.

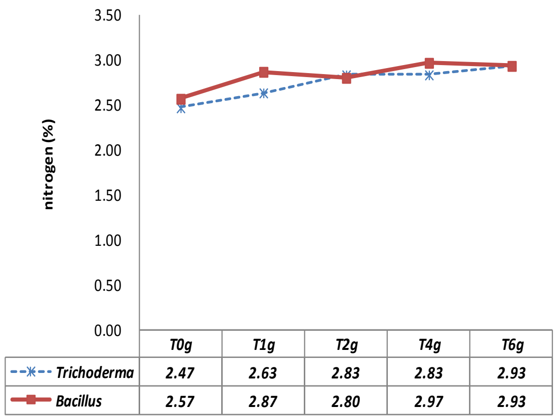
Dry weight



Concentration treatments: T0g = Control, T1g = 50%, T2g = 100%, T4g = 200% and T6g = 300%.
Figure 5 Effect of bio fertilizer concentration on dry weight

Results on effect of *Trichoderma* based-bio fertilizer concentration on dry weight was not significant ($P>0.05$) (Figure 5). On the other hand, effect of *Bacillus* based-bio fertilizer concentration on dry weight was significant ($P<0.05$). From T_{0g} to T_{2g} dry weight was increasing numerically but statistically the means were non-significant ($P>0.05$) from each other. From T_{4g} to T_{6g} the dry weight was declining numerically. The mean dry weight recorded was 22.7g.

Nitrogen (N)

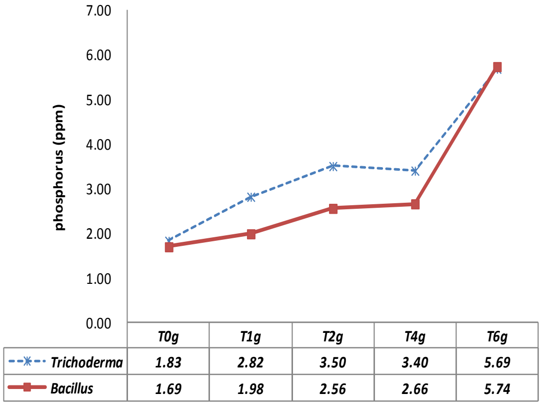


Concentration treatments: T0g = Control, T1g = 50%, T2g = 100%, T4g = 200% and T6g = 300%.
Figure 6 Effect of bio fertilizer concentration on nitrogen content

Data pertaining to effect of *Trichoderma* based-bio fertilizer on tissue N content was significant ($P<0.05$) among the treatments (Figure 6). Treatment T_{1g} was not significant ($P>0.05$) from the control but all other treatments were significant. The highest (2.93%) tissue N content was recorded from T_{6g}. The mean N content recorded was 2.74%.

Effect of Bacillus concentration on tissue N was also significant (P<0.05) among treatments. All treatment means were significantly (P<0.05) higher than the control. The highest N content (2.97%) was recorded from T_{4g}. the mean N content recorded was 2.83%.

Phosphorus (P)



Concentration treatments: T0g = Control, T1g = 50%, T2g = 100%, T4g = 200% and T6g = 300%.

Figure 7 Effect of bio fertilizer concentration on phosphorus content

Results for effect of Trichoderma on tissue P recorded show significant (P<0.05) diffences between treatments (Figure 7). All treatments with the bio fertilizer were significant (P<0.05) from the control. Treatment T_{2g} and T_{4g} were however not significant from each other and the highest (5.69 ppm) recorded tissue P content was from T_{6g}. The mean P content recorded was 3.45 ppm.

Results from *Bacillus* treated samples almost followed the same trend as that *Trichoderma* treatments. All treatment means were statistically higher than the control. The tissue P content was increasing with increase in the concentration of the bio fertilizer applied. The highest tissue P content was 5.74 ppm. The mean P content recorded was 2.93 ppm.

DISCUSSION

The improved root length observed from this study as a result of treatment with bio fertilizers is in agreement with Glick, (1995) and Welbaum et al. (2004) who attributed the improvement to the ability of PGPR to excite the plant growth and their importance for viable agriculture. For that reason, inoculation can compensate the insufficiency in nutrients and increase the development of the plant through production of growth regulator stimulating root development. A large root system allows a better absorption of water and nutrients from the soil (Wu et al., 2005). Gamalero & Glick (2011) indicated that the promotion of plant growth by soil bacteria is due to provision of nutrients which are not sufficiently available in the soil. *Bacillus* can improve the uptake and availability of micronutrients in the soil (Adeleke et al., 2010; Ahsan et al., 2012; Esitken et al., 2010). In other studies as well, PGPR promoted the plant growth through several mechanisms such as the nitrogen fixation (N_2) (Whipps, 2001; Dobbelaere et al., 2003; Cakmakci et al., 2006; Orhan et al., 2006). The improved plant growth is attributed to separate accumulation of N, P and K in the soil as a result of the bio fertilizers, thereby maintaining soil nutrient balance (Adesemoye et al., 2008; Egamberdiyeva, 2007). Results for plant uptake of P and N in this study can confirm this assertion because increase in nutrient may also be enhanced by bacterial activity of the PGPR group that used as bio fertilizer. The use of *Trichoderma* sp. and *Bacillus* sp., as bio fertilizer had a positive significant contribution in improving the nutrient absorption of maize seedlings. The availability of N compounds in the soil increased and easily be absorbed by plant roots. Dissolved P was also made available for the plants as explained by Vessey, (2003). According to Wu et al. (2005) application of bio fertilizer involves not only increases rate of nutrient absorption at the plant level such as N, P, and K, but also maintains organic compound content and N total in the soil.

The increase in both fresh weight and dry weight with application of the bio fertilizers compared to the treatment without might be ascribed to the improvement in biological yield resulting from taller plants. Bio fertilization might have augmented in area of the leaves, thus the photosynthetic rate resulting in further accumulation of carbohydrate in the leaves and thus increase in whole plant weight.

Plant roots are important organ in the absorption of nutrient and water. Like Budi et al., 1999 root length in this experiment was improved probably because in the plant rhizospehere, there was better establishment of micro-organisms which finally improved the phosphorus concentration. Increase in root length might be attributed to the higher absorption of nutrients, improved cell division, cell elongation and thus concomitant increase in metabolic activity (Torrey, 1950; Vessey, 2003). The soil status might have improved from the biological activity of the microorganisms to become ready to serve the plant root zone for growth of the plant. Positive effects of the application of bio fertilizers were also reported by Patel et al., (1998) and Vivek et al., (2001).

CONCLUSION

This study was aimed at determining an optimum level of application of the bio fertilizers in maize plant growth. The study results revealed significant improved in plant growth as a result of application of the bio fertilizers. The two bio fertilizers had noticeable effect on all growth parameters under study i.e.; plant height, root length, fresh weight and dry weight; except number of leaves. *Bacillus* bio fertilizer gave better results using the rate of 2g per plant station. For areas which are water stressed, bacillus can be applied at a rate of 1g to obtain maximum root length. The enhanced plant growth and increase of plant nutrient uptake was as a result of bio fertilizers used. Further research on *Trichoderma* and other bio fertilizers is recommended. On the other hand, the resource-poor farmers who cultivate on nutrient-poor sub-Saharan African soil can use bio fertilizers as efficient technology to increase maize growth and possibly better yield and profitability

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