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Integration of Commercial Microbiological Products into Soil Fertility Practices as a Potential Option for Acclimatization and Growth of TC Banana in Kenya

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Abstract

Tissue culture (TC) banana plantlets at the *in vitro* stage are delicate and devoid of microbes and nutrients that are essential for establishment and subsequent growth. Some microbes are known for function best under certain soil threshold levels of macro and micronutrients and have been associated with growth and performance of TC banana. A green house and field study was conducted to evaluate the effect of combining two commercial biological products [Rhizatech and ECO-T (mycorrhiza and *Trichoderma* based products, respectively)] with various sources of nitrogen and phosphorous including Mavuno, Minjingu phosphate rock, Calcium Ammonium Nitrate (CAN), manure and diammonium phosphate (DAP) on growth and performance of TC banana in Vertisol and Rhodic Ferralsol soil conditions. Tissue culture plants were initially inoculated with Rhizatech and ECO-T at the acclimatization stage and subsequently at the beginning of the potting stage and field establishment. Addition of nutrient sources was also done at the same stages of plant growth by mixing with the soil substrates prior to planting. The performance of plants was significantly (at $p \leq 0.05$) affected by the combinations of nutrient sources depending on the soil type and stage of plant development. The growth of plants in the Vertisol increased with *Trichoderma* combined with either organic manure, DAP or combined with a macro and micro nutrient source (Mavuno) as compared to the sole application of *Trichoderma*. Performance of plants treated with combination of mycorrhiza and either Mavuno and minjigu rock phosphate was con-

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sistently higher in the Rhodic Ferralsol than either mycorrhiza alone or fertilizer alone. This indicates that TC plants could highly benefit from combined application of microbiological products and inorganic and organic fertilizers. However, a prior knowledge of the product's microbial formulation and prevailing soil conditions is essential for optimizing the potential benefits of integrating microbe-based product with inorganic and organic fertilizers.

Keywords

Microbiological Products, Soil Fertility Practices, Integration, Tissue Culture Banana, Growth and Performance

1. Introduction

Tissue culture banana plantlets offer an excellent means for providing pest- and disease-free planting material to farmers [1] [2]. However, tissue culture (TC) plants are fragile, devoid of food reserves and therefore more prone to shock, which may lead to loss of plantlets at establishment. With decline in soil fertility and increase in soilborne pests and diseases, TC plants are likely to succumb to disease and roots may not establish well in low fertility soil environments [3].

Banana requires large amounts of nitrogen and potassium followed by phosphorus, calcium and magnesium to maintain high yields [4] [5]. To fulfill the plant demand for nutritional attributes, it is essential to apply those elements in the soil which mostly come from inorganic chemical sources. The increased use of chemical fertilizer is undesirable because its production is an energetically costly process and considerable pollution is caused through both the production and use of mineral N-fertilizers. This is exacerbated by the relatively low efficiency of their uptake by the plants due to non-extensive root system and may also delete soil organic matter in the long term [6]-[8]. Inoculant biofertilizers are more environmentally sound and their introduction in agricultural production systems could be one of the means to mitigate the onset of global warming as well as the reduction in fertilizer input costs, prevent depletion of organic matter and increase crop yields [9]-[11].

Inoculation of tissue culture banana with biofertilizers such as mycorrhiza (AMF) and *Trichoderma* has been reported to stimulate root growth, nutrient uptake, enhance plant establishment and increase growth, yield and protection against disease and pest infestation [12]-[16]. The growth vigor acquired by biologically inoculated TC banana plants under nursery conditions is expected to give the plants an advantage during field establishment especially in nutritionally and disease challenged soils. However, the functionality of some of the rhizospheric microbes used for constituting biofertilizers such as mycorrhiza (AMF) and *Trichoderma* is greatly influenced by the prevailing soil conditions. Nitrogen (N), phosphorous (P) and other nutrients such as zinc, copper and sulphur levels in the soil affect the functioning of these microorganisms [17]-[21]. Mycorrhizal symbiosis benefits, for example, have been suggested to be optimal at P levels of 50 $mg \cdot kg^{-1}$ [21].

Organic and inorganic fertilizers are used primarily to increase nutrient availability and the type or amount of fertilizer added to soil could directly affect the function performed by the various microbial groups in the soil [22]. Mineral nutrition is essential for growth, sporulation and stimulation of fungal secondary metabolism [18] and combining of mineral and bio fertilizers could greatly benefit both the added microbes and inoculated plant as well as improve soil quality [11] [23]-[25].

Previous studies have focused on the effect of long term fertilization on indigenous microbial diversity and efficiency [26] [27] but little is reported on the effect of combining commercial microbiological strains with fertilizers in nutrient poor soils that have little or no history of fertilization.

Although several formulations of mycorrhiza and *Trichoderma* are available in the market, their efficacy on plant growth is variable depending on the soil nutrient levels. It is therefore essential to add these nutrients in levels that enable optimal functioning of the micro-organisms and consequent promotion of nutrient uptake and plant growth. The integration of microbiological products with fertilizers could address the soil fertility constraint faced by TC banana plantlets, especially under field conditions. This study focused on evaluating the potential of integrating microbiological products with inorganic and organic fertilizers on growth and performance of tissue cultured banana in Vertisol and Rhodic Ferralsol soil conditions under nursery and field conditions.

2. Materials and Methods

2.1. Source of Tissue Cultured Materials, Soil Properties and Inoculation Process

Tissue culture banana plantlets cv. Gros Michel, were obtained from Jomo Kenyatta University of Agriculture and Technology (JKUAT) Biotechnology laboratory.

The experimental soils (Vertisol and Rhodic Ferralsol) were sampled from two banana growing regions in Kenya namely, Western Kenya (Bondo) and Coastal Kenya (Kilifi) (**Table 1**) at a depth of 0 - 20 cm and used for hardening and potting of tissue culture plantlets. Soil nutrient composition (Nitrogen, Phosphorous, Potassium, Carbon, Magnesium, Calcium, Sodium), Cation Exchange Capacity (CEC), pH and soil texture composition (% Clay, % Sand and % Silt) were determined according to standard procedures [28] [29]. The initial soil characteristics are described in **Table 2**.

2.2. Green House Experiment

A 2 × 2 × 3 factorial experiment comprising of 1) two microbiological products a) Rhizatech b) ECO-T; 2) soil substrates a) Vertisol (sampled from Western Kenya-Bondo) b) Rhodic Ferralsol (sampled from coastal Kenya-Kilifi) and; 3) Fertilizers a) Minjingu phosphate rock (MPR) and CAN as sources of phosphorous (P), Mg, Ca and eight more essential micronutrients (Mwaluko, 1996) and nitrogen (N), b) Mavuno as a source of N, P, potassium (K) and micronutrients such as calcium Ca, Mg, sulphur (S) and other essential micronutrients including boron (B), manganese (Mn), zinc (Zn), molybdate (Mo) and copper (Cu) c) manure and diammonium phosphate (DAP) as a source of P, N and carbon (C). Three replicates consisting of 16 plants per replicate were considered per treatment. Control experimental units (without product addition) were also included with three replicates per soil. The experimental units were arranged in a completely randomized design under greenhouse conditions. The microbiological products are described in **Table 3**. Application of products was done as recom-

Table 1. Description of soils used for hardening and potting of tissue culture banana.

Site of soil collection	Description of soil
Western Kenya (Bondo)	Vertisol: dark montmorillonite with swelling properties, heavy cracking when dry; poorly drained, leaching of weathering minerals limited, high in Ca and Mg, pH. Low hydraulic conductivity; N, P and micronutrient deficient; no P-fixation [33].
Coastal Kenya (Kilifi)	Rhodic Ferralsol: developed on limestone, reddish brown to weak red top soil, weak to moderate texture silty clay texture with low nutrient status, especially low in N and P. Deeply weathered soil, moderately fertile, texture loamy sandy with slightly acid pH [33].

Modified from [30].

Table 2. Initial soil characteristics (0 - 20 cm) of Rhodic Ferralsol and Vertisol, used for establishing tissue culture banana under greenhouse conditions.

Soil property*	Units	Rhodic Ferralsol	Vertisol
pH (H_2O)		6.9	5.87
Olsen P	($mg{\cdot}P{\cdot}kg^{-1}$)	7	3
K	($cmolc{\cdot}kg^{-1}$)	0.6	0.82
Ca	($cmolc{\cdot}kg^{-1}$)	4	25.99
Mg	($cmolc{\cdot}kg^{-1}$)	1.7	12.53
Na	($cmolc{\cdot}kg^{-1}$)	0.1	0.19
ECEC	($cmolc{\cdot}kg^{-1}$)	7	52.5
N	(%)	0.1	0.25
C	(%)	1	3.67
Clay	(%)	19.7	54.7
Sand	(%)	76.3	20.3
Silt	(%)	4.0	25

Modified from [30].

Table 3. Description of microbe-based commercial products used in the experiments.

Product	Manufacturer	Composition	Asserted non-active ingredients	Dose	Mode of application
ECO-T	Plant Health Products (Pty) Ltd., South Africa Distributed by Lachlan Ltd., Kenya	Spores of *Trichoderma* harzianum strain Rifai KRL AG2 (4.8%)	Kaolin clay (95.2%)	0.25 g per 3 m^2	Dry powder applied to soil
Rhizatech	Dudutech (K) Ltd., Kenya	Spores and mycelial fragments of arbuscular mycorrhizal fungi species: *Glomus mosseae*, *G. etunicatum*, *G. intraradices* and *G. aggregatum*). (50 propagules/cm^3)	Not specified	60 $kg{\cdot}ha^{-1}$	Granules applied to soil

CFU, colony forming units. PHC Colonize AG coded as PHC Colonize in the manuscript (modified from [30]).

mended by the manufacturers. Application rates per plant corresponded to the recommended rate in the field, converted using the medium planting density for banana (2500 plants·ha^{-1}). The area of pots used for establishing plants was considered when determining the exact amount of inoculum to be added per pot. The mode of application of products is described in **Table 3**. ECO-T was applied at 1.25 g/L, and Rhizatech at 2 g/plant. Three replicates consisting of 10 plants per replicate were considered per treatment. Fertilizer application rates followed the recommended fertilizer rates and were applied at the hardening, potting and at field establishment by mixing with the soil substrate. The areas of pot used for establishing plants were considered when determining the exact amount of microbiological inoculum to be added per pot.

2.3. Inoculation Process

Inoculation of plantlets with products, soil sterilization (Vertisol and Rhodic Ferralsol soils), acclimatization and maintenance under high humidity conditions was done according to standard procedures [30]. The inoculation strategy applied for initial inoculation of deflasked plantlets at the acclimatization stage and subsequently at the potting (8 weeks after deflasking) and field establishment stages (22 weeks after deflasking) is shown in **Figure 1**.

2.4. Study Site Description and Experimental Design under Field Conditions

The experiment was carried out at two sites with similar soil conditions as those used for hardening and potting of plants at under green house nursery conditions. Tissue cultured banana plants established under green house conditions were used as planting materials and established in two agro ecological zones (Western Kenya in Bondo and Coastal Kenya in Kilifi). The experimental design followed a multilocational complete block design in four farms per study site. Each farm was considered as a replicate. Diammonium phosphate (DAP) and Minjigu rock phosphate fertilizers, used as sources of phosphorous were applied at the rate of 50 kg per hectare. Calcium Ammonium Nitrate (CAN) used as a source of nitrogen was applied at the rate of 120 kg per hectare. Mavuno fertilizer which was used as a source of macro and micro nutrients was applied at the manufacturers recommended rate of 185 kg per hectare. High quality farm yard manure was considered as a source of organic nitrogen and carbon and was applied at 6 tonnes per hectare. Application rates per plant corresponded to the recommended rate in the field, converted using the medium planting density for banana (2500 plants·ha^{-1}).

2.5. Planting and Crop Management

Medium planting density of 2500 plants per hectare was adopted hence the plants were spaced at 2 m by 2 m and established in 60 × 60 × 60 cm planting pits. Guard row plants were established three meters away from the experimental plants only in edge plots, hence surrounding the entire farm. The inner 2 m × 2 m inner mats were monitored and evaluated up to the on-set of flowering of the mother plant. Fertilizer was mixed with top soil and incorporated around the banana plant in the pit followed by topping with sub-soil before planting. Hand weeding was done when needed avoiding the use of tools that could mutilate superficial banana roots. Desuckering of plants was carried out in order to have a 1-2-3 system (mother, first ratoon, second ratoon), preferably with a circular movement of plants, so that original planting density was maintained. Deleafing (leaves having <50% of

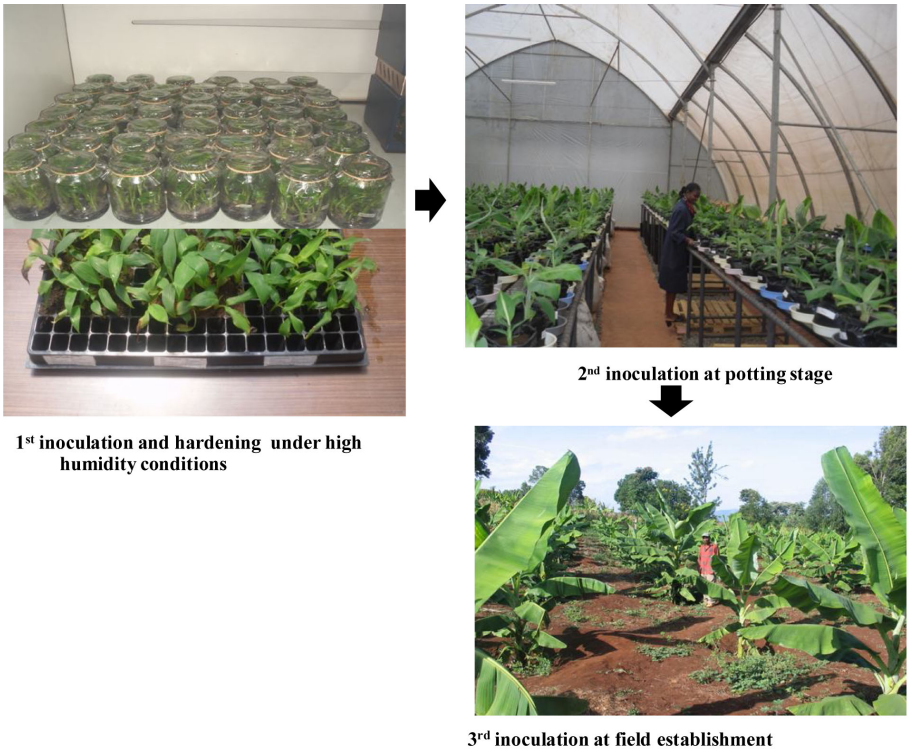

Figure 1. Strategy for inoculating micropropagated banana plantlets.

functional leaf surface area) and watering during periods of severe drought stress were also part of the management practices carried out.

2.6. Assessment of Growth Parameters

Plant growth parameters such as shoot height (from base), length and width of the second leaf from the sword shoot, girth (at two centimeters from the base) of the stem, and number of functional leaves were recorded every two weeks under greenhouse conditions and every two months under field conditions up to the on-set of flowering stage. Only treatments with a high number of surviving plants under green house conditions were considered for field establishment.

2.7. Data Analysis

To assess the effects of products, soils, fertilizers and the interaction between soils, products and fertilizers, data were subjected to analyses of variance (ANOVA) using the mixed procedure of SAS [31]. Means found to be significant at $p \leq 0.05$ were separated using Tukey's HSD test.

3. Results

3.1. Integration of Microbiological Products with Fertilizers on Growth and Performance of Tissue Cultured Plants

The effect of combining products with various fertilizers was evident in both Vertisol and Rhodic Ferralsol. Highly significant ($p \leq 0.001$) differences between treatments were observed on all plant growth parameters evaluated in both soils (**Table 4** and **Table 5**). In the Rhodic Ferralsol, the highest plant height was observed on plants treated with combined application of Rhizatech and Mavuno with an increase of 136% and 116.5% compared to the non-treated control and sole application of Rhizatech, respectively (**Table 4**). The highest number of leaves (10), which was statistically different from all other treatments, was observed with the combined application of minjigu rock phosphate and CAN (MRPCAN). Plants treated with combined application of ECO-T

Table 4. Growth of tissue cultured banana plants treated with microbiological products and different fertilizers in Rhodic Ferralsol, 22 weeks after deflasking.

Product	Fertilizer	Height	NOL	Girth	LL	LW
None	None	8.0a	7.7a	1.7a	15.6a	5.6a
ECOT	None	10.9b	7.8a	1.6a	19.8b	7.1abc
Rhizatech	None	8.7a	8.2ab	1.6a	16.9a	5.7a
None	MRPCAN	11.8b	9.1c	1.8a	19.5b	6.8ab
ECOT	MRPCAN	11.0b	8.7bc	2.6b	20.1b	7.1abc
Rhizatech	MRPCAN	13.7c	9.9d	1. 9ab	24.6c	8.7cd
None	Manure + DAP	13.6c	7.7a	1.84ab	19.2b	6.9abc
ECOT	Manure + DAP	16.8e	8.6bc	2.1ab	26.7d	9.1d
Rhizatech	Manure + DAP	15.3d	8.9bc	2.0ab	23.6c	6.9abc
None	Mavuno	18.4f	8.7bc	2.2ab	24.3c	8.7cd
ECO-T	Mavuno	16.6de	7.8a	2.1ab	23.8	8.5bcd
Rhizatech	Mavuno	18.9f	8.4abc	2.1ab	25.0cd	11.1e
SED						
Product		0.197	0.112	0.115	0.314	0.275
Fertilizer		0.227	0.129	0.133	0.363	0.318
Product × Fertilizer		0.393	0.224	0.23	0.628	0.551
p value						
Product		<0.001	<0.001	0.147	<0.001	<0.001
Fertilizer		<0.001	<0.001	<0.001	<0.001	<0.001
Product × Fertilizer		<0.001	<0.001	0.068	<0.001	<0.001

Means of 16 plants replicated three times. Within the same column, means followed by the same letter are not significantly different (Tukey's HSD test) at $p > 0.05$. NOL, number of leaves; LL, leaf length; LW, leaf width. Leaf length and leaf width measured in centimeters.

Table 5. Shoot and root growth of tissue cultured banana plants treated with microbiological products and different fertilizers in Rhodic Ferralsol, 22 weeks after deflasking.

Product	Fertilizer	SDWT (g)	RDWT (g)	NOSR
None	None	2.1	1.8ab	14.3a
ECOT	None	4.3	2.1ab	19.3ab
Rhizatech	None	2.5	1.2a	14.7ab
None	MRPCAN	5.4	3.4ab	21ab
ECO-T	MRPCAN	3.3	3.5ab	19ab
Rhizatech	MRPCAN	3.7	2.8ab	18ab
None	Mavuno	7.3	6.3ab	24ab
ECO-T	Mavuno	6.5	5.1ab	24.3ab
Rhizatech	Mavuno	4.9	2.7ab	18ab
None	Manure + DAP	3.4	4.5ab	27.3b
ECO-T	Manure + DAP	5.9	6.1b	20ab
Rhizatech	Manure + DAP	6.1	5.6ab	24.7ab
SED				
Product		0.740	0.643	1.547
Fertilizer		0.855	0.743	1.786
Product × Fertilizer		1.481	1.287	3.093
p value				
Product		0.598	0.178	0.201
Fertilizer		0.006	<0.001	0.001
Product × Fertilizer		0.147	0.343	0.113

Means of 3 replicates. Within the same column, means followed by the same letter are not significantly different (Tukey's HSD test) at $p > 0.05$. SDWT, shoot dry weight; RDWT, root dry weight; NOSR, number of secondary roots.

and MRPCAN recorded the highest increase in girth of 55% compared to the non-treated control, 44% and 63% compared to the application of MRPCAN alone and ECO-T alone. Leaf length was statistically different between most treatments with the longest leaf length observed on plants treated with combined application of ECO-T and manure and DAP. The combined application of ECO-T and manure and DAP increased leaf length by 71.3%, 39.2% and 34.8% compared to the non-treated control, addition of manure and DAP alone and sole application of ECO-T respectively. Combining of Rhizatech with fertilizers also significantly affected leaf length recording an increase of 45.8% (Rhizatech plus MRPCAN), 40.8% (Rhizatech plus manure plus DAP) and 48.3% (Rhizatech plus Mavuno) above the sole fertilizer applications. Effect of combining Rhizatech and Mavuno on leaf width was statistically different from all other treatments recording an increase of 100%, 35.9% and 96% compared to the non-treated control and sole application of Mavuno and Rhizatech respectively. Other treatments with effects on leaf length statistically different from the non-treated control include Rhizatech plus MRPCAN, ECO-T plus manure plus DAP, Mavuno alone, ECO-T plus Mavuno and Rhizatech plus Mavuno increasing leaf length by 55.7%, 62.6%, 55.6%, 52.7% and 100% respectively (**Table 4**).

Plants treated with manure alone in the Rhodic Ferralsol had significantly higher number of secondary roots (27) compared to the plants that received neither fertilizer nor product (un-treated control) and plants treated with Rhizatech (**Table 5**). Effects of all other treatments were statistically the same as the untreated control and Rhizatech. Combined application of fertilizer and products had no significant effects on shoot dry weights (SDWT) in the Rhodic Ferralsol. The highest SDWT (6.07 g) in the Rhodic Ferralsol were observed on plants treated with a combination of Rhizatech plus manure plus DAP and the least on untreated control plants. Plants treated with Rhizatech alone had significantly lower root dry weights (RDWT) (1.22 g) than plants treated with ECO-T plus manure plus DAP (6.11 g) and Mavuno alone (6.27 g) and statistically the same as all other treatments.

3.2. Growth of Tissue Cultured Banana Treated with Microbiological Products and Different Fertilizers in a Vertisol Soil

Under green house conditions, plant growth (height, girth, no. of leaves, leaf length and leaf width) was significantly affected by sole and combined application of products and fertilizers (**Table 6**). Effect of combined application of ECO-T plus manure plus DAP on plant height was statistically different from all other treatments increasing height by 110.6%, 33.7% and 51.5% compared to the non-treated control and sole application of manure plus DAP and ECO-T, respectively. Similar observations were made on plant girth, with combined application of ECO-T plus manure plus DAP increasing plant girth by 22.6%, 9.3% and 83.9% compared to the non-treated control and sole application of manure plus DAP and ECO-T respectively. Significant differences between treatments were observed on number of leaves with the highest number of leaves (11) observed on plants treated with sole application of MRPCAN. Most treatments negatively affected number of leaves up to a magnitude of 9.5%.

Leaf length of plants treated with ECO-T and manure plus DAP was statistically different from all other treatments. An increase of 51.6%, 25.4% and 25.9% above the non-treated control and sole application of manure plus DAP and ECO-T, respectively was observed. Combined application of Rhizatech and Mavuno, ECO-T plus manure plus DAP, Rhizatech plus manure plus DAP, Rhizatech plus MRPCAN had similar effects but statistically higher leaf length than non-treated control, sole application of Rhizatech and combined application of ECO-T and MRPCAN (**Table 6**).

Combined application of fertilizer and products had no significant effects on shoot dry weights (SDWT) in the Vertisol (**Table 7**). The highest SDWT was observed with addition of Mavuno alone (13 g) and the least with application of Rhizatech plus MPRCAN) (3.87 g). The highest RDWT was observed with combined application of ECO-T plus manure plus DAP (7.45) and the least on the un-treated control plants. The number of secondary roots on plants treated with Mavuno fertilizer alone (31) was statistically different from plants treated with ECO-T (20) and mijingu rock phosphate plus CAN (MPRCAN) (20) but statistically the same as all other treatments (**Table 7**).

3.3. Growth and Performance of Tissue Culture Banana Plants Treated with Microbiological Products and Different Fertilizers in Rhodic Ferralsol under Field Conditions

Combining of microbiological products with fertilizers in Rhodic Ferralsol significantly ($p < 0.001$) increased

Table 6. Growth of tissue cultured banana plants treated with microbiological products and different fertilizers in a Vertisol, 22 weeks after deflasking.

Product	Fertilizer	Height (cm)	Girth (cm)	NOL	LL (cm)	LW (cm)
None	None	11.7a	2.1bcd	10.7bcd	23.6a	9.1a
ECO-T	None	16.3c	1.4a	10.8cd	28.5cd	10.9bcde
Rhizatech	None	13.7b	2.1bcd	10.6abcd	25.2ab	9.4a
None	MRP + CAN	15.1bc	2.0bc	11.2d	26.8bc	9.7ab
ECO-T	MRP + CAN	15.6c	2.4def	10.1ab	26.1b	9.2a
Rhizatech	MRP + CAN	19.0d	2.3cde	10.5abc	29.1d	11.4de
None	Manure + DAP	18.5d	2.4def	10.5abc	28.6cd	10.1abc
ECO-T	Manure + DAP	24.7g	2.6f	10.5abc	35.8f	11.6e
Rhizatech	Manure + DAP	22.4f	2.5ef	10.5abc	33.1e	11.5de
None	Mavuno	19.7de	1.9bc	10.4abc	28.4cd	10.3abcd
ECO-T	Mavuno	20.6e	1.8ab	10.0a	29.7d	11.1cde
Rhizatech	Mavuno	22.5f	2.5ef	10.4abc	33.5e	11.8e
SED						
Product		0.219	0.059	0.099	0.059	0.187
Fertilizer		0.253	0.068	0.115	0.068	0.215
Product × Fertilizer		0.439	0.117	0.199	0.117	0.373
p value						
Product		<0.001	<0.001	0.006	<0.001	<0.001
Fertilizer		<0.001	<0.001	0.001	<0.001	<0.001
Product X Fertilizer		<0.001	<0.001	<0.001	<0.001	<0.001

Means of 16 plants replicated three times. Within the same column, Means followed by the same letter are not significantly different (Tukey's HSD test) at $p > 0.05$. NOL, number of leaves; LL, leaf length; LW, leaf width; MRPCAN, Minjigu Rock Phosphate + Calcium Ammonium Nitrate.

Table 7. Shoot and root growth of tissue cultured banana plants treated with microbiological products and different fertilizers in a Vertisol, 22 weeks after deflasking.

Product	Fertilizer	SDWT(g)	RDWT(g)	NOSR
None	None	4.2	2.6	2.6
ECOT	None	6.2	4.1	4.1
Rhizatech	None	7.4	3.9	3.9
None	MRPCAN	4.8	3.4	3.5
ECO-T	MRPCAN	7.1	6.6	6.6
Rhizatech	MRPCAN	3.9	3.8	3.8
None	Mavuno	9.2	6.8	6.8
ECO-T	Mavuno	7.7	6.5	6.5
Rhizatech	Mavuno	8.7	5.8	5.8
None	Manure+DAP	5.9	7.1	7.1
ECO-T	Manure+DAP	4.6	7.4	7.4
Rhizatech	Manure+DAP	5.6	5.6	5.6
SED				
Product		1.023	0.676	1.483
Fertilizer		1.181	0.781	1.712
Product X Fertilizer		2.045	1.352	2.966
P value				
Product		0.926	0.11	0.233
Fertilizer		0.041	0.001	0.003
Product X Fertilizer		0.458	0.47	0.023

Means of 3 replicates. Within the same column, means followed by the same letter are not significantly different (Tukey's HSD test) at $p > 0.05$. SWDT, shoot dry weight; RDWT, root dry weight; NOSR, number of secondary roots; MRPCAN, Minjigu Rock Phosphate + Calcium Ammonium Nitrate.

growth of banana plants (**Table 8**). Plants treated with either fertilizer alone or inorganic fertilizer plus products performed better than plants treated with products only. Combination of ECO-T with manure plus DAP increased plant height by 33.8% and 31.2% above the sole application of manure plus DAP and ECO-T respectively. The effect of combining Rhizatech and MRPCAN on plant height was statistically the same as that of combining ECO-T and manure plus DAP. An increase of 26.5% and 59.7% above sole application of MRPCAN and Rhizatech respectively was recorded with the combined application of Rhizatech and MRPCAN. Plants treated with Rhizatech alone had statistically lower heights than all other treatments.

Similar to the observations on plant height, plants treated with the combination of Rhizatech and MRPCAN increased girth by 14% and 27%, respectively compared to sole application of MRPCAN and manure plus DAP, respectively. The highest number of leaves (12) was observed on the plants treated with Rhizatech or ECO-T alone and this was statistically different from MRPCAN and ECO-T plus Mavuno-treated plants, which had the least number of leaves (10). Leaf length was significantly increased by combined application of Rhizatech and MRPCAN (32.7%) and ECO-T plus manure and DAP (21%). All treatments had statistically the same effects on leaf width. The trend of plant growth in the Rhodic Ferralsol is shown in **Figure 2**.

3.4. Growth and Performance of Tissue Cultured Plants Treated with Microbiological Products and Different Fertilizers in Vertisol under Field Conditions

Plant height in the Vertisol was significantly ($p = 0.021$) affected by the combined application of commercial products with either inorganic or organic fertilizers in (**Table 9**). Combining of ECO-T with macro and micronutrients (Mavuno) increased growth of plants by 13.5% compared to the sole addition of Mavuno and by 2% compared to the addition of ECO-T alone. Combined application of Rhizatech and with P and inorganic N increased plant height by 11.8% compared to sole addition of P and inorganic N and reduced plant height by 7% compared to sole addition of Rhizatech. The trend of plant growth in the Vertisol is shown in **Figure 3**.

Plant girth was enhanced by application of Rhizatech plus MRPCAN by 5.1%, 37.2% and 16.1% compared to sole application of Rhizatech, MRPCAN and manure plus DAP respectively. This was followed by combined application of ECO-T plus Mavuno that increased plant girth by 4.8%, 13.8% and 15.8% respectively compared to the sole application of ECO-T, Mavuno or manure plus DAP respectively.

Leaf width was more affected by combined application of Rhizatech and MRPCAN and ECO-T plus manure plus DAP than leaf width (the effect of the two treatments was statistically the same). Combined application of Rhizatech plus MRPCAN increased leaf length by 17%, 35% and 20.8% compared to the sole application of Rhizatech, RPCAN and manure plus DAP respectively. ECO-T plus Mavuno increased leaf length by 14%, 13.6% and 17% compared to the sole application of ECO-T, Mavuno and manure plus DAP.

Table 8. Growth of tissue cultured banana plants treated with microbiological products and different fertilizers, 12 months after field establishment in a Rhodic Ferralsol.

Product	Fertilizer	Height (cm)	Leaf length (cm)	Leaf width (cm)	No. of leaves	Girth (cm)
Rhizatech	None	166.5a	182.6a	63.9	12.bc	18.76
ECO-T	None	205.1ab	204.7ab	63.9	12c	20.8
None	Manure + DAP	206.6ab	203ab	65.1	11abc	18.2
ECO-T	Mavuno	226.7bc	217.8ab	65.1	10a	21.4
None	MRP + CAN	235.8bc	219.2ab	66.4	10a	20.6
None	Mavuno	245.3bc	230.6bc	72.8	11abc	20.9
Rhizatech	MRP + CAN	261.6c	242.4bc	74.5	11abc	23.5
ECO-T	Manure + DAP	273.7c	247.9bc	71.6	116abc	23.2
p value						
Product		0.116	0.193	0.685	0.206	0.160
Fertilizer		0.171	0.496	0.359	0.022	0.287
Product × Fertilizer		0.001	0.019	0.111	0.001	0.146

Means of four replicates. Within the same column, means followed by the same letter are not significantly different (Tukey's HSD test) at $p > 0.05$. MRPCAN, Minjigu Rock Phosphate + Calcium Ammonium Nitrate.

Table 9. Growth and performance of tissue cultured plants treated with microbiological products and different fertilizers in Vertisol, 12 months after field establishment.

Product	Fertilizer	Height (cm)	Leaf length (cm)	Leaf width (cm)	No. of leaves	Girth (cm)
Rhizatech	None	168.6ab	159.9ab	50.4a	6.6abc	20.6a
EcoT	None	207.3ab	195.8b	62.3b	7.8c	20.1a
None	MRP + CAN	156.7a	140.8a	50.8a	6ab	15.8b
None	Manure + DAP	186.5ab	173ab	58.8ab	6.3abc	18.7a
None	Mavuno	186.2ab	165.6ab	58.6ab	6.1abc	19a
Rhizatech	MRP + CAN	156.7ab	179.5ab	58.9ab	5.5a	17.1ab
Rhizatech	Mavuno	183.1ab	167.6ab	58.9ab	6.7abc	17. ab
ECO-T	Mavuno	211.3b	195.1b	63.1b	7.1bc	21.6a
P value						
Product		0.4	0.3097	0.8957	0.2189	0.169
Fertilizer		0.6896	0.6638	0.592	0.9501	0.7413
Product × Fertilizer		0.0157	<0.0001	0.105	0.003	<0.0001

Means of four replicates. Within the same column, means followed by the same letter are not significantly different (Tukey's HSD test) at $p > 0.05$. MRPCAN, Minjigu Rock Phosphate + Calcium Ammonium Nitrate.

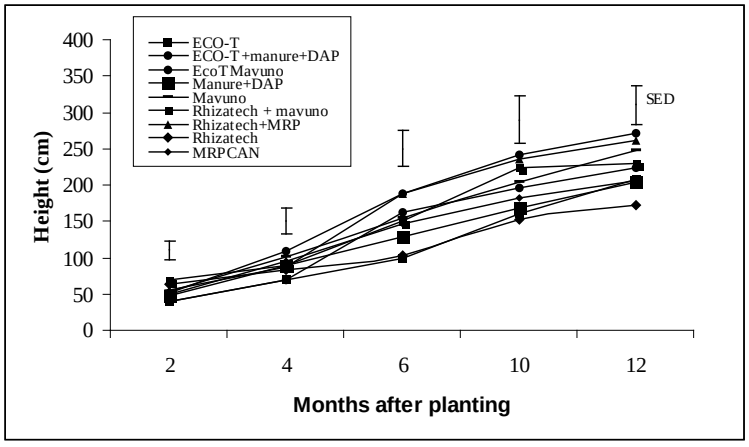

Figure 2. Growth of tissue cultured plants treated with microbiological products and different fertilizers in Rhodic Ferralsol. SED, standard error of difference between means at 12 months after field establishment.

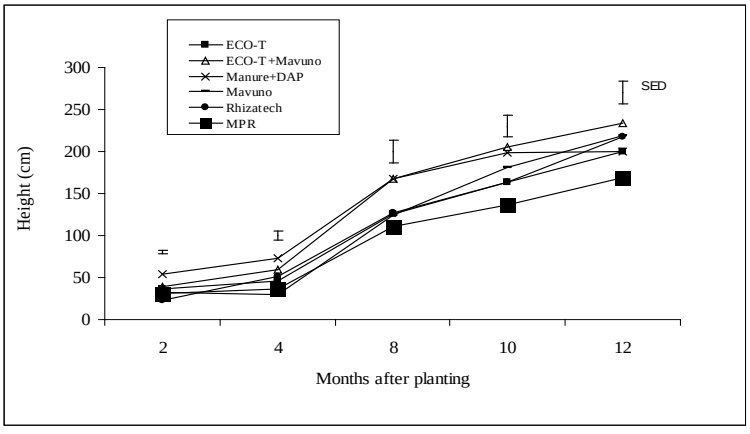

Figure 3. Growth of tissue cultured plants treated with microbiological products and different fertilizers in a Vertisol. SED, standard error of difference between means at at 12 months after field establishment.

4. Discussion

The performance of tissue cultured banana plants treated with combined application of fertilizers and commercial microbiological products was variable and depended on prevailing soil conditions. Results indicate preference for certain sources of inorganic or organic nutrients by the *Trichoderma* based product (ECO-T) and the mycorrhiza-based product (Rhizatech). The positive effects of combining *Trichoderma* with organic and inorganic fertilizers were more evident in the Vertisol than that in the Rhodic Ferralsol suggesting a preference for soils with higher nitrogen levels *i.e.* 0.25% in the Vertisol versus 0.1% in the Rhodic Ferralsol. The performance of plants was greatly enhanced with *Trichoderma* combined with either organic manure inorganic phosphate diammonium phosphate) or combined with a macro and micro nutrient source (Mavuno) as compared to the sole application of *Trichoderma*. This indicates that organic and inorganic amendments could enhance the proliferation and efficacy of *Trichoderma* and subsequently enhance plant growth. Higher amounts of *Trichoderma* in soils with organic amendments have been reported [17]. The enhanced performance of plants when inoculated with *Trichoderma* combined with mineral nutrients (macro and micro nutrients) could be attributed to availability of nutrients for plant uptake as well as increased proliferation of *Trichoderma* populations with a consequent result of improved plant growth. Mineral nutrition is essential for growth, sporulation and stimulation of fungal secondary metabolism [18] [20] as well high total N availability [20]. A positive correlation between soil nitrate levels and cellulose production has also been reported [32]. Performance of plants treated with combination of mycorrhiza and either Mavuno (macro and micro nutrients) and minjigu rock phosphate was consistently better in the Rhodic Ferralsol than either mycorrhiza alone or fertilizer alone. This could suggest that the efficacy of mycorrhiza is enhanced by addition of mineral nutrients to nutrient poor soils, as is the case with the Rhodic Ferralsol [33]. This could also suggest an improved fertilizer use when mycorrhiza is combined with mineral fertilizer. This finding is in agreement with the finding of other authors who have reported that arbuscular mycorrhizal symbiosis provides numerous services to crops [34], including efficient use of fertilizer and soil nutrients [23], increased N-fixation in legumes [35], improving plant access to mineral nutrients, including N, P, K, Ca, Mg, Fe, Cu, Zn, and Mn [36]. Evaluation of the effect of biofertilizers combined with different soil amendments on potted rice plants has been reported to enhance 100-grain weight in the NPK fertilizer amendment [37].

Amendment of both the Vertisol and Rhodic Ferralsol soils with minjigu rock phosphate alone had the lowest effect on plant growth enhancement suggesting minimal solubilization and utilization of rock phosphate by plants. However, plant growth was greatly enhanced with combined application of minjigu rock phosphate and mycorrhiza (Rhizatech) indicating a positive effect of mycorrhiza in the added phosphate (P) solubilization and acquisition of by plants with a consequent increase in plant growth. This finding conforms with the reports of other authors, who have shown that mycorrhiza play an important role in effecting the availability of soil P to plant roots, and increasing P mobilization in soil [38] [39].

5. Conclusion

In conclusion, it is evident that tissue culture banana plants could greatly benefit from combined application of commercial microbiological and fertilizers. Combinations of mycorrhiza and inorganic macro and micro nutrients or rock phosphate and the combined application of *Trichoderma* and organic sources could greatly enhance plant growth in nutrient poor soils as was observed in the Rhodic Ferralsol and Vertisol. Knowledge of product formulations and prevailing soil conditions is essential prior to inoculation and amendment of the soils.

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