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THE EFFECT OF TWO BIOFERTILIZERS UNDER TWO CROP COMBINATION ON MICROBIAL POPULATION AND EARLY PLANT GROWTH IN SANDY LOAM SOIL

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ABSTRACT

Biofertilizers are becoming increasingly popular in many countries and for many crops, but very few studies on their microbial population and early plant growth in sandy loam soil have been conducted. Therefore, this research evaluated two different biofertilizers: treated Ageratum spp. and Crotoloria spp. in the Soil Science Department Chukwuemeka Odumegwu Ojukwu University, Igbariam Anambra State, Nigeria during 2016 cropping seasons in the growth shelter of the Faculty of Agriculture, using two different test crops (Moringa and Tomato) which was laid out in complete randomized block (CRD). The experiments were conducted in pots with dimension of 17cm × 19cm in length and depth in which the bottoms were uniformly perforated for proper aeration. 10 seeds were planted after which 8 seedlings were thinned down 10 DAP, later, the remaining 2seedlings were harvested 60 DAP to evaluate the biomass production in each stage respectively. Significant biomass and soil microbial population increase due to biofertilizer use were observed in all experimental treatments. The biofertilizer effect on moringa and tomato growth did not significantly differ. Nevertheless, positive effects of the biofertilizers occurred on the biological properties. However, the trends in these results seem to indicate that biofertilizers might be most helpful in rainfed environments. However, for use in these target environments, biofertilizers need to be evaluated under conditions with abiotic stresses typical of such systems such as drought, soil acidity, or low soil fertility.

Keywords: Biofertilizers, Tomato, Moringa, Biomass Production and Microbial Population.

INTRODUCTION

Biofertilizer is defined as products containing active or latent strains of soil microorganisms, either bacteria alone or in combination with algae or fungi that increase the plant availability and uptake of mineral nutrients (Vessey, 2003). Bio-fertilizers enrich the soil with diverse, favorable and agronomical relevant microbes and invertebrates and direct the activities of plant roots, and

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soil organisms to a favorable ecological harmony. The functions of bio-fertilizers are manifold and in most cases beneficial to crop protection, environmental health and enhancement of soil fertility status. All these functions are inter-related, Kohnke (1986). Bio-fertilizers can function as direct and indirect sources of sulphur, nitrogen, phosphorus, potassium, magnesium, calcium and micro-nutrients to the soil microbes and indirectly after mineralization to plant roots. Sugars, carbohydrates, proteins, amino acids, vitamins and fats are important components of bio-fertilizers which constitute sources of energy to various soil organisms thereby controlling their activities. One of the most important contributions of bio-fertilizers to soil conservation and sustainable soil fertility and the ability to detoxify the soil and protect plant roots. During growth, it was observed that certain plant roots excrete toxicants in the soil which inhibited the germination and development of future crops and impaired soil fertility status. In Senegal, Burgo-Leon *et al.,* (1980) reported the incidence of phytotoxicity caused by the exudates of the growing sorghum roots from the flowering stage onwards. The toxicant inhibited germination and seedling establishment of subsequent crops in the field. This frustrating incident was summarily only by the application of farm yard compost (bio-fertilizer). It was finally discovered that it was the bio-fertilizer flora among which were; *Entrobacter cloacae* (bacterium), *Trichodermaviride* (fungus) and *Aspergillus specie* (fungus) which bio-degraded the toxicant and restored the soil productive capability. *Entrobacter cloacae* additionally mediate high nitrogen fixation and production of rooting hormone and realized about a 3-fold increase crop yield. Another spectacular event illustrated the ability of certain bio-fertilizer to detoxify petroleum polluted soil and upgrade its fertility status. Mba (1999) highlighted the bio-detoxification of petroleum polluted soil. The pollutant impaired soil phosphorus availability, inhibited germination of sorghum and soya bean and their seedling establishment and furthermore rendered them vulnerable to serious fungal disease. Application of bio-fertilizer detoxify, eliminated the incidence of fungal disease mediated a 2 – 5 fold increase in soil phosphorus availability, Mba (1999). Though the bio-fertilizer technology is a low cost, eco-friendly technology, several constraints limit the application or implementation of the technology the constraints may be environmental, technological, infrastructural, financial, human resources, unawareness, quality, marketing, etc.

The other alternative method of providing nutrients for plant growth and yield production is use of soil microbes, which have been proved to be advantageous (Adesemoye *et al*., 2008; 2009a, b; Berg, 2009). There are a wide range of microbes in the soil, which are able to act in symbiosis or non-symbiosis association with their host plant (Gray and Smith, 2005).

Furthermore, the environmental issues regarding the use of chemical fertilization is also of significance as excess amount of chemical fertilization results in the pollution of the environment. Chemical fertilization can also decrease the enzyme activities of soil microbes, soil

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pH, and soil structure (Bohme and Bohme, 2006). It is therefore pertinent to apply the optimum amounts of fertilization in the field. Accordingly, it can be favorable that other methods of fertilization be also tested and used to provide necessary nutrients for plant growth and yield production, while keeping the soil structure in good shape and the environment clean.

The excess uses of chemical fertilizers in agriculture are costly with adverse effects on physico-chemical properties of soils. Therefore, in the recent years several organic fertilizers have been introduced that act as natural stimulators for plant growth and development (Khan *et al*., 2009). The knowledge of such natural stimulator or microbial inoculums has long history started with culture of small scale compost production and passes from generation to generation of farmers (Abdul Halim, 2009). A specific group of this kind of fertilizers includes products based on plant growth-promoting microorganisms named biofertilizer or 'microbial inoculants' that are preparation containing live or latent cells of efficient strains of nitrogen fixing, phosphate solubilizing or cellulytic microorganisms. These are used for application of seed, soil or composting areas with the objective to enhance the numbers of such microorganisms and accelerate certain microbial process to augment the extent of the availability of nutrients in a form which can assimilated by plant (Khosro and Yousef, 2012). Such biofertilizers are important components of integrated nutrients management in soil, while play key role in productivity and sustainability of soil. With every passing days, these biofertilizer replacing chemical fertilizers due to cost effectively, ecofriendly and renewable source of plant nutrients. One of the most important effects of compost use is the promotion of soil biology. A great variety of organisms exists within the soil ranging from large, visible organisms to organisms, which can only be viewed under a powerful microscope. These organisms perform a wide range of functions, which are major contributions to what we consider normal and healthy soil. It might be reasonably said that these organisms have essential roles in determining the functioning of the soil system, but this functioning is dependent upon a supply of available carbon. In this context, compost has a stimulation effect on both the microbial community in the compost substrate as well as the soil-born micro biota of soils. However, two fractions of organic matter are responsible for the level of microbial activity in general:

i. Easily degradable organic compounds (labile organic matter pool) may increase microbial activity and biomass temporarily while.
ii. A persistent increase of microbial biomass depends on a constant enhancement of stable organic matter which is particularly promoted by mature compost addition

Due to its multiple positive effects on the physical, chemical and biological soil properties, compost contributes to the stabilization and increase of crop productivity and crop quality (Amlinger *et al*., 2007). Long-term field trials proved that compost has an equalizing effect of annual/seasonal fluctuations regarding water, air and heat balance of soils, the availability of plant nutrients and thus the final crop yields (Amlinger *et al*., 2007). For that reason, a higher yield safety can be expected compared to pure mineral fertilization. Better crop results were often obtained if during the first years higher amounts of compost were applied every 2nd to 3rd year than by applying compost in lower quantities of < 10 Mg (DM) ha- 1 every year (Amlinger *et al*., 2007).

The main objective of the study is to access the effect of Bio fertilizers on soil properties and growth of *Moringa oleifera* and *Solanum esculentum*.

Specific objectives of the study are to:

i. Evaluate the effect of two Biofertilizers: treated *Ageratum spp*. and *Crotolaria spp*. on early plant growth of moringa and tomato.
ii. Evaluate the effect of two Biofertilizer: treatead *Ageratum spp*. and *Crotolaria spp*. on microbial population.

MATERIALS AND METHODS

Site Description

The soil sample for the planting were collected beside Faculty of Agricultural building complex of Chukwuemeka Odumegwu Ojukwu University, Igbariam Campus at the depth of 5cm – 15cm, the soil was sieved thoroughly to remove unwanted materials. The vegetation ranges from light rain forest to savanna. Dense vegetal cover with high trees is prominent around stream and the shaley lowlands while savanna vegetation and isolated trees are prominent on sandy highland. Anambra State is located at latitude 5°40 and 6°46N, longitude 6°40 and 7°20E. The annual rainfall of the area varies from 1800mm to about 2500mm, the temperature of the area vary from 21° to over 25° while relative humidity is from 60% to over 85%. The soil sample collected was taken to the Soil Science Department of Chukwuemeka Odumegwu Ojukwu University, where it was used for experiment.

Treatment Arrangement and Allocation

Ageratum conyzoid and *Crotolaria spp*. was be harvested green and freshly chopped into pieces, mixed with fresh pig dropping at 50:50 weight – weight incubated airtight for 2weeks, after which the compost was aerated for 3 – 4 days. 4kg of the sieved subsoil was weighed out in 36 plastic pots. The dimension of the pot is 17cm × 19cm in length and depth. The bottom side of the pot was uniformly perforated to allow adequate aeration and drainage. The weighed soil was thoroughly mixed with 350g of treated bio-fertilizer and watered with 500ml of water at initial

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planting after which the subsequent watering was depend on evaporation rate in the growth shelter. 10 seeds were planted in each pot. 10 days after planting, 8 plants were thinned down leaving behind 2 plants with equal spacing in each pot till 12 weeks after for the final harvest for biomass weight production. The experiment was set up in Complete Randomized Design (CRD), with 6 treatments replicated 3 times to give a total of 18 pots. Table 1 below shows the treatment combination that was imposed to the soil.

Table 1: Treatment Application Detail

Treatment Code	Treatment Combination
F_{Ctr} + T	Soil + Tomato
F_{Ctr} + M	Soil + *Moringa oleifera*
F_{Ag} + T	*Ageratum conyzoid* + Tomato
F_{Ag} + M	*Ageratum conyzoid* + *Moringa oleifera*
F_{Crot} + T	Crotolaria + Tomato
F_{Crot} + M +	Crotolaria + *Moringa oleifera*

Key: Ct = Control, Ag = Ageratum Biofertilizer, T = Tomato, Crot = Crotalaria Biofertilizer, M = Moringa

Data Collection

Agronomic Data were collected on percentage of seed germination and plant biomass production, while biological data were collected on the total microbial population using Bunt and Rovira medium (Bunt *et al*., 1955). All data generated from the study was then subjected to ANOVA using SPSS version 20, and the mean difference of the effects of the biofertilizers on soil properties and plant biomass was separated using Duncan Multiple Range Test method and compared using the least significant difference ($LSD_{0.05}$) as described by Obi (2002).

RESULTS

Table 2 shows some physicochemical properties of Igbariam soil at the start of the experiment. The soil was found to be infertile for crop production, acidic, low buffer capacity, low pH and electrical conductivity with low cation exchange capacity and high exchange acidity. Due to all these deficiencies the soil of this class needs serious improvement which can be actualized by application of biofertilizer and biopesticides. The result of the study in Table 2 shows chemical and biological properties of the biofertilizers used at the start of the experiment. The table shows that the biofertilizer itself has optimum chemical and biological properties in order to address the infertility nature of the experimental soil for crop production and increase in biological activities.

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Table 2: Some physicochemical properties of the Igbariam soil

Soil Properties	Values
Sand (%)	80.80±6.12
Silt (%)	3.20±0.15
Clay (%)	16.0±3.63
Textural Class	Sandy Loam
Dispersion Ratio (%)	36.00±6.00
Aeration Porosity (v/v)	11.08±2.32
Total Porosity (v/v)	22.60±4.32
Bulk Density (g/cm^3)	1.98±0.01
Field Capacity (v/v)	25.10±5.40
Plant Available Water (%)	16.53±2.22
pH (H_2O)	4.10±1.00
pH (KCL)	3.43±0.56
Base Saturation (%)	40.0±1.05
Buffer Capacity (meq/100)	0.23±0.00
Cation Exchage Capacity (meq/100g soil)	8.16±0.04
Soluble Cation (Cmol/kg)	0.025±0.00
Exchangeable Acidity (meq/100g soil)	3.25±0.01
Electrical Conductivity (µs/cm)	10.00±1.00

Table 3: The Chemical and Biological Properties of the biofertilizers used for the trial

Properties	*Ageratum spp.*	*Crotolaria spp.*
pH (H2O)	7.00	6.95
Organic Carbon (%)	5.26	5.09
Total Nitrogen (%)	0.53	0.50
Available Phosphorus (ppm)	0.05	0.05
Microbial Population	1.33×10^5	1.67×10^5

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Table 3 shows the germination percentage and dry biomass weight at 10 DAP and 60 DAP respectively. From the result, the germination percentage did not differ significantly; this is evidence that the plant does not necessarily need fertilizer for its germination. Unlike the germination rate, the biomass production of the biofertilizer treated plant differs significantly when compared to the control plant. This study could be explained that the biofertilizers enhanced the soil properties as well as increased the plant biomass production at 10 DAPS and 60 DAP respectively. Organic additions to soil have long been considered important in maintaining the quality of both natural and managed soils, principally because of their role in providing nutrients to the soil.

Table 3: Germination Percentage and Dry Biomass weight at 10 DAP and 60 DAP respectively

Treatment	Germination Percentage (%)	Dry Biomass @ 10 DAP (g)	Dry Biomass @ 60 DAP (g)
Ctr+T	100.00 ± 0.00^{e}	0.08 ± 0.01^{a}	0.14 ± 0.04^{a}
Ctr+M	90.00 ± 10.00^{c}	2.12 ± 0.10^{c}	3.64 ± 0.56^{d}
Ag+T	93.33 ± 5.77^{d}	0.50 ± 0.20^{b}	1.00 ± 0.61^{c}
Ag+M	73.33 ± 28.87^{b}	3.99 ± 2.75^{d}	6.06±4.66e
Crot+T	90.00 ± 0.00^{c}	0.55 ± 0.09^{b}	0.74 ± 0.20^{b}
Crot+M	66.67 ± 5.77^{a}	3.08 ± 0.39^{d}	7.96 ± 1.49^{f}
$LSD_{0.05}$	**NS**	NS	**

** = Significant at P < 0.05, NS = not significant.

Key: Ct = Control, Ag = Ageratum Biofertilizer, T = Tomato, Crot = Crotalaria Biofertilizer, M = Moringa

Table 4 shows microbial population of the soil after harvesting. The result in the table shows that there is statistical and significant difference between the microbial populations of the biofertilizer treated soil when compared with the control soil. This is evidence that one of the most important effects of biofertilizer use is the promotion of soil biology. A great variety of organisms exists within the soil ranging from large, visible organisms to organisms, which can only be viewed under a powerful microscope. These organisms perform a wide range of functions, which are major contributions to what we consider normal and healthy soil. It might be reasonably said that these organisms have essential roles in determining the functioning of the soil system, but this functioning is dependent upon a supply of available carbon. In this context, biofertilizer has a stimulation effect on both the microbial community in the biofertilizer substrate as well as the soil-born micro biota of soils. As reported by Brown and Cotton, (2011), the application of

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compost has increased microbial population (565000.00±30413.81) in comparison to the control soils (34800).

Table 4: Microbial population of the Soil after harvesting

Treatment	Microbial Population
Ctr+T	500000.00±13228.76[b]
Ctr+M	510000.00±10000.00[a]
Ag+T	558000.00±2000.00[e]
Ag+M	565000.00±30413.81[f]
Crot+T	542000.00±2000.00[d]
Crot+M	546000.00±2000.00[c]
$LSD_{0.05}$	**

** = Significant at P < 0.05, NS = not significant.

Key: Ct = Control, Ag = Ageratum Biofertilizer, T = Tomato, Crot = Crotalaria Biofertilizer, M = Moringa

DISCUSSION

Biofertilizer effect on soil biological properties

One of the most important effects of biofertilizers use is the promotion of soil biology. A great variety of organisms exists within the soil ranging from large, visible organisms to organisms, which can only be viewed under a powerful microscope. These organisms perform a wide range of functions, which are major contributions to what we consider normal and healthy soil. It might be reasonably said that these organisms have essential roles in determining the functioning of the soil system, but this functioning is dependent upon a supply of available carbon. In this context, compost has a stimulation effect on both the microbial community in the biofertilizer substrate as well as the soil-born micro biota of soils. As reported by Brown and Cotton, (2011), the application of biofertilizers has increased microbial activity in comparison to the control soils. They observed microbial activity was 2.23 times greater in the biofertilizers amended soils as compared to the control soils, because organic matter found in biofertilizers provides food for microorganisms. Paul (2003) had conducted an experiment on long-term effects of biofertilizers and mineral fertilizers on soil biological activity and observed microbial activity was enhanced in biofertilizers treated field plots. In his trial, soil fertility was enhanced in the organic plots compared to the conventional plots as indicated by a higher microbial biomass, earthworm biomass and enhanced mycorrhizal root colonization. Moreover, the functional diversity of soil microorganisms and their efficiency to metabolize organic carbon sources was increased in the organically fertilized systems with highest values in the biofertilizers soils.

Biofertilizers effect on crop productivity

Due to its multiple positive effects on the physical, chemical and biological soil properties, biofertilizer contributes to the stabilization and increase of crop productivity and crop quality (Tayebeh *et al*., 2010 and Amlinger *et al*., 2007). Long-term field trials proved that biofertilizers has an equalizing effect of annual/seasonal fluctuations regarding water, air and heat balance of soils, the availability of plant nutrients and thus the final crop yields (Amlinger *et al*., 2007). However, crop yields after biofertilizers application were mostly lower when compared to mineral fertilization (Agegnehu *et al*., 2014 and Amlinger *et al*., 2007), at least during the first years. This can be explained by the slow release of nutrients (especially N) during mineralization of compost. Mohammed *et al*. (2004) has compared the use of composted organic wastes as alternative to synthetic fertilizers for enhancing crop productivity and agricultural sustainability in two season (wet and dry). This was an indication that additional application suppressed the grain production probably due to lush green vegetative growth that was observed during the growing season (Mohammed *et al*., 2004). Moreover, biofertilizer increases available form of nutrients for plant in soil and then increases growth and nutrient uptake by plant that results in plant stem height and dry weight (Soheil *et al*., 2012). Gamal (2009) also reported that application of 5 t/ha biofertilizers increased sorghum grain yield by 45% as compared to no biofertilizers plots, while the grain yield was higher at biofertilized plots (10 t/ha) by 19% than no biofertilized plots in different sites. Biofertilizers do not only improve the growth and productivity of crops in terms of quantity but it could be also proved that quality of agricultural products is influenced in a positive way (Mehammed *et al*., 2012). Gemal (2009) observed that the quality of corn crop was improved as the result of increasing biofertilizers application rate. Tayebeh *et al*. (2010) also observed that biofertilizers had a significant effect on seed protein and the maximum amount of seed protein was observed in 60mg biofertilizer/ha treatment.

CONCLUSION

The results showed significant increases in plant growth and biomass production for all the treatment tested during the research seasons but the most consistent results were achieved by the *Ageratum spp.*-based biofertilizer. In most cases, the observed plant growth and biomass increases were not huge (0.2 to 0.5 $t \cdot ha^{-1}$) but could provide substantial income gains given the relatively low costs of all biofertilizers tested. The positive effect of the tested biofertilizers on soil biological properties was tremendous. The results achieved can already be used to develop better advice for farmers on biofertilizer use in moringa and tomato production, but several important questions remain. In particular, biofertilizers need to be evaluated under conditions with abiotic stresses typical for most low- to medium-input systems (e.g., under drought or low

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soil fertility) and with a range of germplasm because their effect might depend also on the variety used.

RECOMMENDATION

More upstream-oriented research would be needed to better understand the actual mechanisms involved, which in turn could also contribute to making the best use of biofertilizers in moringa and tomato production.

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