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COMPARATIVE EFFECT OF BIOFERTILIZER TYPES ON GROWTH AND YIELD OF SELECTED MAIZE CULTIVARS IN ALFISOLS

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Abstract: Two field experiments were conducted at the Teaching and Research Farms of Joseph Ayo Babalola University (JABU) and the Federal University of Technology Akure (FUTA) in 2015 in south west Nigeria, to determine the effect of Azotobacter, Azospirillum and Mycohrriza (biofertilizers) on agronomic parameters of selected cultivars of maize. A 4 x 3 factorial experiment consisted of four cultivars of maize and three biofertilizers was used. The treatments were laid out in Randomized Complete Block Design and replicated three times. Compared to un-inoculated maize plots, Azotobacter, Azospirillium and Mycorrhiza significantly ($P<0.05$) increased plant height, number of leaves, leaf area, grain yield, chlorophyll content, cob diameter, nutrient composition and yield of maize at the two locations. The performance the biofertilizers on plant height, number of leaves and grain yield were in the order of Azotobacter>Azospirillium>Mycorrhiza>-control. Azotobacter and Azospirillium showed significant increase in nutrient uptake and nutritional quality of maize. Azotobacter was the most productive biofertilizer among the treatments.

Keywords: Azotobacter, azospirillum, inoculation, maize cultivars, mycorrhiza

Introduction

The use of various agrochemicals such as mineral fertilizers, pesticides of all kinds and herbicides has pose hazards on man, livestock, aquatic animal and soil beneficial microorganisms. The negative residual effects of long use of mineral fertilizers increasing crop yields has necessitated the fertilizers from organic sources like animal dungs, plant residues, biofertilizers, etc. (Akande, 2002; Olowa and Olowa, 2010).

The aim of agricultural researchers is to obtain optimum crop yield at minimum cost even without the use of additional fertilizers to maintain soil fertility but the increase in agricultural land for various purposes has reduced the available land for crop production, hence, the use of fertilizers to increase crop productivity to feed the population. At present the use of biofertilizers in increasing crop yield at sustainable level has helped the farmers to reduce the cost of procuring mineral fertilizers (Babatunde and Oyatoye, 2005).

Biofertilizer is a substance which contains living microorganisms which when applied to seed, plant surfaces, or soil colonize the rhizosphere or the interior of the plant and promotes growth by increasing the availability of primary nutrients to the host plant (Tijani and Osotimehin, 2007). Biofertilizers have important and long term environmental positive impact thus, negating the adverse effects of chemicals because they are ecological friendly and economically feasible.

In Nigeria, maize is grown in all ecological zones especially in the rainforest and derived savanna zone of Nigeria with a domestic production level of 2.0 million metric tons and domestic demand of about 3.5 million metric tons (Tijani and Osotimehin, 2007). Maize is a staple food of great socio-economic importance and has been in the diet of Nigerian‘s for centuries. It started as a subsistence crop and has gradually become an important crop which now has risen to a commercial crop on which many agro – based industries depend as raw materials (Iken and Amusa, 2004; Nweke, 2004). Many researchers have worked on mineral fertilizers, farm refuse and animal dungs in increasing maize yield in Ondo Southwestern Nigeria (Ayeni *et al*., 2008) but little research work has been done on the effect of biofertilizers on growth and yield of maize. Hence, the objective of this research was to determine the effects of Azotobacter, Azospirillum and Mycohrriza on agronomic parameters of selected cultivars of maize in Ondo Southwestern Nigeria.

Materials and Methods

Description of location and experimental site

This research was carried out at two different locations. The first location was the Teaching and Research Farm of the Joseph Ayo Babalola University, Ikeji-Arakeji, Osun State, Nigeria. The University is located between latitudes $07^0 16$ and $07^0 18$N and longitudes $05^0 09^0$ and $05^0 11$E. General topography of the area is gently undulating with an elevation of about 1150-1250 m above sea level. It is well drained.

The study area is situated in the humid tropical forest zone of Nigeria. It has an annual average rainfall of between 1500-1800 mm annually. The vegetation of the area has been considerably modified as a result of continuous cropping. Tree species such as *Albeziazygia* and others are visible. The rest of the site contained scattered trees and shrubs. The second experimental site was located at the Teaching and Research Farm (Crop section) of the Federal University of Technology, Akure. Akure lies between Latitude 5^o N of the equator and within the tropical rain forest belt, with an average annual rainfall of about 1613 mm per annum and an annual mean temperature of about 27^0C.

In order to ascertain the fertility status of the soil before planting and after harvesting, random soil samples were collected from the soil prior to planting and after harvesting for physical and chemical analyses between 0 – 20 cm depth. The composite samples were air- dried crushed and sieved through 2 and 0.5 mm meshes. The soil pH was determined using the pH meter in a 1:2.5 soil water ratio, total phosphorus was determined by Bray 1 method. The cations were extracted with ammonium acetate. Calcium (Ca) and magnesium (Mg) were determined using the atomic absorption spectrophotometer (AAS) and sodium (Na) and potassium (K) by flame emission photometry. The organic carbon content was determined according to Walkley and Black, with the organic carbon content multiplied with a factor 1.724 to obtain the soil organic matter content.

The land was manually cleared, pegged and made into beds of 4m x 4m size. A 4 x 3 factorial experiment comprising maize cultivar TZEE-Y, TZE-Y, TZEE-W and TZE-W., and three different types of biofertilizers (Azotobacter, Azospirillium and Mycorrhiza) were fitted into Randomized Complete Block Design (RCBD) and replicated three times.

Sources of maize cultivars and biofertilizers

The four varieties of maize seeds and inoculi which were Arbscuclar Mycorrhiza (AMF), Azotobacter, Azospirillum were acquired from International Institute of Tropical Agriculture, Ibadan (IITA). The maize varieties planted are the newly- bred improved high yielding varieties commonly grown by farmers, for example tropical zea extra- yellow population striga resistance cycle C5(TZEE-YPOP STR- C5).

Seeds inoculation

Seed inoculation with Azotobacter and Azospirillum was carried out by using 10% sugar solution carrier. The sugar solution was prepared by adding 100 g of sugar in 1 litre of water and boiled. After cooling the solution, maize seeds were put in the solution pot and taken out and the inoculants were thoroughly mixed with the maize seeds. These seeds were kept in shade before planting. The inoculation was done twenty four (24) h before planting. Ten (10) g of Mycorrhiza was applied to plots treated with Mycorrhiza at planting this is because the Mycorrhiza has already been inoculated with the soil.

Maize seeds were planted at (1) seed per hole with spacing of 25 x 75 cm. The plots were weeded with hand held hoe at three weeks interval.

Data collection

Five (5) plants per sub-plot were randomly selected from the trial plot from the two (2) middle rows at 50% tasseling. Data were collected on Plant height, leaf length and number of leaves at 2, 4, 6, 8 and 10 weeks. Plant height was determined in centimeter (cm) by measuring the plant from the base of the plant to the apex. The number of leaves were done through visual counting. The total grain yield from the two middle rows from each plot at maturity were harvested, dehusked, shelled, threshed and oven-dried till 14% moisture content was obtained.

The weight of 1000 Seeds was oven-dried to 14% moisture content and converted to (Kg).

The fresh weight of dehusked cob was measured to the nearest gram (g) and converted to Kilogram (Kg) from the five randomly selected plant samples from the middle rows. This was used to compute the score for each plot.

At maturity (75 days) after planting, maize cobs were harvested from the tagged sample at the center rows. The ears were removed and cobs length was measured from base to the top. The diameter of each cob was measured at the middle point using a veneer caliper. The cobs were oven-dried at 100^0C for 48 h to a constant weight. The cobs were shelled to separate the rachis and grains. The grains were oven-dried again at 65^0C for about 10 min to 13% moisture content and weighed to determine the grain weight. The 13% grain moisture content was confirmed with the use of grain moisture tester (Microprocessor).

The leaf area was measured following the procedure of Stewart and Dwyer (1999), Elings (2000) by multiplying the length of a leaf by its widest width by alpha, where alpha is 0.743 (L x W x 0.743).

Statistical analysis

The data collected were subjected to Analysis of Variance (ANOVA) at 5% probability level. Means were separated using Duncan's Multiple Range Test (DMRT). GenStat Statistical package was used for the analysis.

Result and Discussion

The characteristics of the soils used for the conduct of the experiment are shown in Table 1. The two soils were sandy loam. According to Akinrinade and Obigbesan (2002), the soils were slightly acidic, low in OM, total N, available P, exchangeable K, Ca and Mg (FUTA) and CEC. Hence, the two soils need additional input as source of fertilizer for optimum maize production. Though both JABU and FUTA soils were deficient in plant nutrients yet the nutrients status of JABU were higher than FUTA.

Table 1: Physical and chemical properties of the soil before planting

Properties	Values	
	JABU	**FUTA**
Textural	Sandy loam	Sandy loam
Sand (%)	64.00	60.00
Silt (%)	24.00	20.00
Clay(%)	12.00	20.00
PH (H_20)	5.90	5.46
Soil organic matter (%)	0.80	2.35
Total N (%)	0.07	0.14
Available P (Bray-1) (mg/kg)	3.44	0.24
Exchangeable cations (cmol/kg)		
Potassium (cmol/100g of soil)	0.15	0.25
Sodium (cmol/100g of soil)	0.17	0.42
Calcium (cmol/100g of soil)	0.08	2.51
Magnesium (cmol/100g of soil)	0.35	1.43
Exch. Acidity (cmol/100g of soil)	1.36	0.30
ECEC (cmol/kg)	2.83	3.98

The result in Table 2 showed that the response of the four maize cultivars on plant height differ from one another. Compared to un-inoculated maize cultivars (except TZEE-W), TZEE-W inoculated with myccorhiza, TZEE-W inoculated with Azotobacter and TZEE-W inoculated with azospirillum significantly increased plant height at JABU and FUTA two weeks after planting (2WAP). At 4 WAP, all the maize cultivars inoculated with Mycorrhiza, TZE-W inoculated with Azotobacter and TZEE-Y inoculated with azospirillum significantly increased plant height compared to un-inoculated TZEE-W. At 6 WAP, TZE-W inoculated with myccorhiza, TZEE-Y inoculated with Azotobacter and TZEY inoculated with azospirillum significantly increased maize height compared to un-inoculatedTZEE-Y at JABU and FUTA. At 8WAP, in JABU, TZE-W inoculated with myccorhiza, TZE-W inoculated with Azotobacter, TZEW-W and TZEE-Y inoculated with azospirillum significantly increased maize plant height compared to un-inoculated TZE-Y. At 10WAP, in JABU and FUTA, all the treatments inoculated with Mycorrhiza, Azotobacter and azospirillum significantly increased the plant height compared to un-inoculated TZE-Y. It was observed that un-inoculated TZE-W compared favourably with all the un-inoculated maize cultivars with biofertilizer types at 4, 6 and 10 WAP. Un-inoculated TZEE-Y performed favourably with all the inoculated cultivars at 4 and 8 WAP at both sites. Un-inoculated TZEE-W compared favourably with all the inoculated maize cultivars at 2 and 4WAP.

Compared to un-inoculated maize cultivars, TZE-Y inoculated with Mycorrhiza, TZE-W inoculated with Azotobacter and TZEE-Y inoculated with Azotobacter significantly increased the leaf area of maize cultivars (Table 3). Only TZE-Y inoculated with Azospirillum and TZE-Y inoculated with Azotobacter significantly increased the root biomass while all the treatments significantly increased total biomass and cob diameter. All the treatments recorded higher increase in cob weight compared to un-inoculated TZE-W and TZEE-W at JABU (Table 3). At FUTA, the increase in agronomic parameters as influenced by the biofertilizer types was not consistent (Table 4). Compared to control, TZE-Y inoculated with Myccorhiza and TZEE-Y inoculated with Azotobacter significantly increased the leaf area and chlorophyll content of maize leaves (except Azospirillum). Maize TZE cultivar inoculated with Azotobacter recorded the highest increase in leaf area, chlorophyll content of the leaves, maize cob diameter and cob length (Table 4).

Figure 1 shows the effect of Azospirillium, Azotobacter and Mycorrhiza inoculations on the agronomic parameters of the four improved cultivars of maize studied in this research. Maize TZEE-Y inoculated with Azotobacter in JABU had the highest grain weight of 366.67 followed by TZE-Y inoculated with Azotobacter The TZEE-Y in FUTA also was with highest 1000 grain weight 343.77 under control condition. The lowest 1000 grain weight 165.10 in FUTA was obtained in TZE-Y with Mycorrhiza inoculation and TZE-Y in JABU 188.00 under control conditions.

Table 2: Effects of biofertilizers on the plant height (cm) of four maize cultivars at JABU and FUTA

		WEEKS AFTER PLANTING									
		2		4		6		8		10	
Treatments	Cultivar	JABU	FUTA	JABU	FUTA	JABU	FUTA	JABU	FUTA	JABU	FUTA
Azospiriliu	TZEE-W	18.95a	17.10a	24.92b	23.07b	68.40c	66.55b	125.07b	123.22b	149.99b	148.14c
	TZEE-Y	11.57c	9.72bc	33.00a	31.15a	86.33b	84.48ab	167.13a	165.28a	191.53a	189.68a
	TZE-W	12.42b	10.57b	26.68b	24.83b	95.25a	93.40a	144.23ab	142.38ab	153.07b	151.22c
	TZE-Y	12.18b	10.33b	16.13bc	14.28c	83.13b	81.28ab	167.07a	165.22a	189.65ab	187.80b
Azotobacte	TZEE-W	25.93a	29.08a	27.60b	25.75b	41.00c	50.15b	159.63b	157.78ab	176.53ab	171.52bc
	TZEE-Y	14.10bc	14.24b	26.53bc	24.68b	81.13a	79.28a	145.73c	143.88b	179.20a	177.35b
	TZE-W	13.66bc	11.81bc	31.67a	29.82a	48.34c	64.55ab	178.00a	176.15a	174.60ab	180.24a
	TZE-Y	16.67b	13.82b	27.33b	25.48b	68.13b	66.28ab	157.73b	155.88ab	181.33a	179.48a
Mycorrhiza	TZEE-W	20.30a	18.45a	26.47a	24.62a	52.00b	39.15b	141.91b	140.06ab	173.37b	174.68a
	TZEE-Y	16.09b	12.26b	24.20ab	22.35ab	52.42b	50.57ab	137.03c	135.18b	176.57ab	174.72a
	TZE-W	12.48c	10.64bc	26.77a	24.92a	66.40a	46.49ab	169.81a	167.96a	182.00a	172.75ab
	TZE-Y	12.81c	10.96bc	22.95ab	21.10ab	55.57b	53.72a	146.67b	144.82ab	175.60ab	173.75ab
Control	TZEE-W	16.22a	16.81a	21.59b	19.74b	81.93a	80.08a	124.73c	122.88b	138.31d	136.46d
	TZEE-Y	9.97b	8.12b	31.33a	29.48a	63.47b	61.62b	149.73a	147.88a	172.47b	170.62b
	TZE-W	9.60b	6.94bc	25.31ab	23.46ab	82.67a	80.82a	122.11d	120.26b	175.13a	173.28a
	TZE-Y	9.34b	7.49b	31.67a	29.82a	71.27ab	69.42ab	144.07b	142.22ab	156.77c	154.92c

Table 3: Effects of application of biofertilizers on growth parameters of the four maize cultivars at JABU

Treatments	LFAREA	RTBIOM	COBLENT	TBMAS	COBDIAM	CHLORO	COBWT	STMBIOM
TZEE-Y Azospi	502.43d	0.06c	15.19ab	0.48cd	5.04ab	0.48e	0.18a	0.46b
TZE-Y Azospi	534.55d	0.10a	17.30a	0.71a	5.02ab	0.95e	0.15a	0.54b
TZEE-W Azospi	536.88cd	0.06c	14.58b	0.47c	5.02ab	0.82e	0.16a	0.41b
TZE-W Azospi	496.33d	0.07ab	16.77a	0.52b	5.21a	2.85a	0.16a	0.37b
TZEE-Y Azoto	664.77a	0.07ab	17.26a	0.55b	5.32a	2.31ab	0.17a	0.42b
TZE-Y Azoto	556.41c	0.11a	19.07a	0.62ab	5.28a	2.59a	0.19a	0.58b
TZEE-W Azoto	595.97b	0.06c	16.94c	0.47c	5.30a	1.23d	0.17a	0.44b
TZE-W Azoto	630.3ab	0.06c	18.15a	0.49b	5.39a	2.07ab	0.17a	0.44b
TZEE-Y Myco	500.00d	0.09b	16.00c	0.44c	5.00a	1.82b	0.17a	0.56b
TZE-Y Myco	558.27c	0.07ab	15.97c	0.39cd	4.64bc	1.66c	0.11b	0.38b
TZEE -W Myco	552.00c	0.06c	16.92c	0.45c	4.32c	1.66c	0.13b	0.49b
TZE-W Myco	590.01b	0.09b	16.00a	0.50b	5.23a	1.70bc	0.16a	0.40b
TZEE-Y Control	506.93d	0.04c	13.48d	0.44c	3.94d	0.62e	0.17a	2.50a
TZE-Y Control	634.97b	0.07ab	12.76d	0.34cd	3.94d	0.68e	0.15a	0.37b
TZEE-W Control	558.96c	0.05c	12.99d	0.28d	4.00d	0.70e	0.11b	0.28b
TZE-W Control	506.83d	0.06c	15.36c	0.42c	4.38cd	1.55c	0.11b	0.28b

Means followed by the different alphabets within the same column and within the same treatment differed significantly
Where, LFAREA=Leaf Area (cm),RTBIOM=Root Biomass (g),COBLENT=Cob length (cm)'TBMAS=Total Biomass (g),COBDIAM=Cob Diameter(cm),CHLORO=Chlorophyll Content(mg/ml), COBWT=Cob Weight(g), STMBIOM=Stem Biomass(g).

Table 4: Effects of biofertilizers application on growth parameters of the four maize cultivars at FUTA

Treatments	LFAREA	RTBIOM	STMBIOM	COBWT	CHLORO	COBDIAM	TBIOM	COBLENT
TZEE-Y Azospi	473.83d	0.05b	0.39b	0.15b	0.05e	3.92ab	0.38c	12.97b
TZE-Y Azospi	505.95c	0.09a	0.48b	0.12b	0.52d	3.90b	0.61a	15.08a
TZEE-W Azospi	508.28c	0.05b	0.34b	0.10b	0.39d	3.90b	0.37c	12.36b
TZE-W Azospi	467.73d	0.06b	0.30b	0.13b	2.42a	4.09a	0.42b	14.55a
TZEE-Y Azoto	636.17a	0.04bc	0.35b	0.14b	1.88b	4.20a	0.45b	15.04a
TZE-Y Azoto	527.81bc	0.10a	0.51b	0.16b	2.16a	4.16a	0.52ab	16.85a
TZEE-W Azoto	567.37b	0.05b	0.38b	0.14b	0.80d	4.18a	0.37c	14.72a
TZE-W Azoto	601.70ab	0.05b	0.38b	0.14b	1.64ab	4.27a	0.39c	15.93a
TZEE-Y Myco	561.00b	0.05b	0.39b	0.14b	1.20c	3.22c	0.37c	12.04b
TZE-Y Myco	519.67c	0.06b	0.32b	0.13b	1.23bc	3.52c	0.29cd	13.75b
TZEE-W Myco	515.12c	0.03c	0.38b	0.14b	1.25b	3.91b	0.30c	13.9ab
TZE-W Myco	500.00c	0.03c	0.37b	0.14b	1.56c	4.00a	0.40b	14.00ab
TZEE-Y control	478.33d	0.03c	2.44a	0.14b	0.19d	2.82d	0.34c	11.26b
TZE-Y Control	606.37ab	0.06b	0.31b	0.12b	0.25d	2.82d	0.24d	10.54b
TZEE-W control	530.36b	0.03c	0.22b	0.10b	0.27d	2.86d	0.18d	10.77b
TZE-W Control	478.23d	0.05b	0.21b	1.43a	1.12cd	3.26c	0.33c	13.14b

Means followed by the different alphabets within the same column and within the same treatment differed significantly.
Where, LFAREA=Leaf Area (cm),RTBIOM=Root Biomass (g),COBLENT=Cob length (cm)'TBMAS=Total Biomass (g),COBDIAM=Cob Diameter(cm),CHLORO=Chlorophyll Content(mg/ml), COBWT=Cob Weight(g), STMBIOM=Biomass(g).

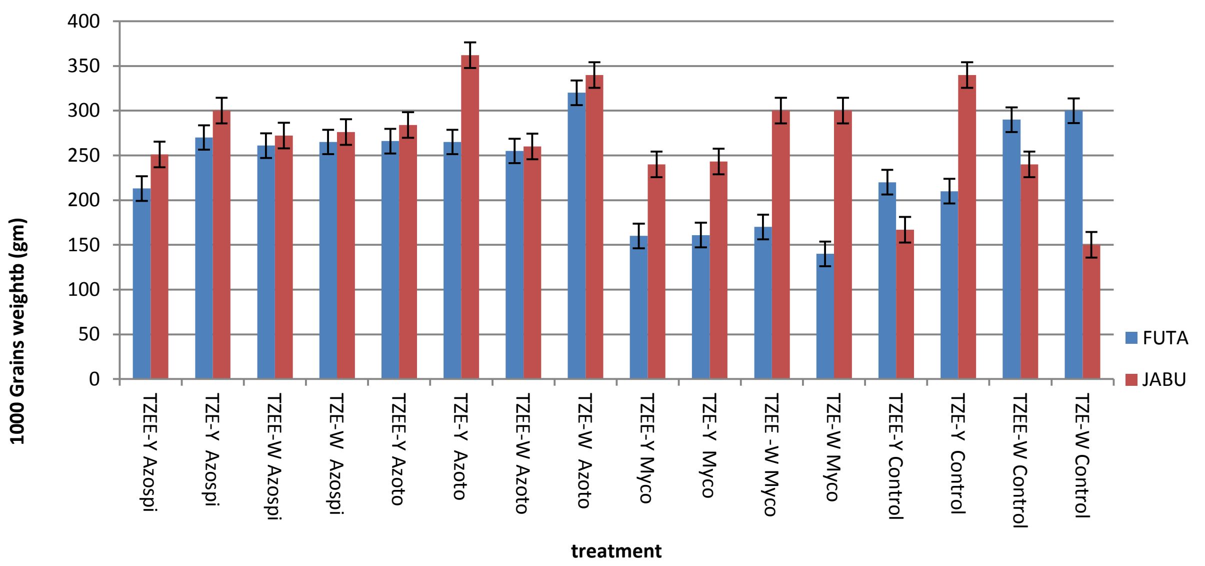

Fig. 1: Effect of biofertilizers on grain yield

The findings from the experiment showed that maize varieties used in the experiment responded differently to Azotobacter, Mycorrhiza and Azospirillum inoculation. The Azotobacter, Mycorrhizal and Azosprillum inoculated plants had better growth, development and yield performances over the un-inoculated control plants. These results agreed with that of Peng *et al*. (2013) who concluded that *Azotobacter chroococcum* inoculated with maize seeds was not only economically most efficient at lower doses of N but also helped in promoting growth and yield of maize. Biari *et al*. (2008) also found positive effect of Azotobacter application on maize grain yield increase at organic field condition.

The plant height was significantly influenced by the treatments. However, the highest plant height was recorded in TZEE-Y both in FUTA and JABU but JABU was the tallest under Azotobacter treatments among the cultivars while TZE-W cultivar treated with Mycorrhiza at the experimental site of FUTA recorded the least plant height. The increase in plant height was in conformity with the results of Amruthesh *et al.* (2003) and Hameeda *et al.* (2008) who also observed, increase in maize height due to biofertilizers application.

Among the different growth parameters, significant increase in the plant height was observed till the end of crop growth period (10WAP). Increase in plant height in the plants inoculated with biofertilizers might be due to the application of the biofertilizers. The increase in growth of maize could be attributed to the nutrient use efficiency in the presence of organic fertilizer. Many studies have shown that the organic materials release nutrients slowly and may reduce the leaching losses, particularly N (Ayeni *et al*., 2015; Ayeni *et al*., 2018).

The inoculation of TZEE-Y with Azospirillium resulted in the production of significantly higher number of maize leaves in JABU. This might be due to the positive effect of biofertilizers on better root development which resulted in more nutrient uptake. These microorganisms also produce vitamins and plant growth promoting substances for the improvement of plant growth. Organic Biofertilizers not only slowly release nutrients but also prevent the losses of nutrients by leaching (Arshad *et al*. 2004; Anup Das *et al*., 2010). The maize leaf length obtained was also greater in TZEE-Y at JABU, followed by TZE-Y with Azospirillium inoculation. These results are in accordance with the work of (Shaharoona *et al.* 2006) who reported such increase in yield attributes of maize due to *Pseudomonas* inoculation.

The inoculation TZEE-Y with Azotobacter recorded the highest yield in JABU and followed by TZE-Y also in JABU compared to other inoculations. This might be due to more availability of nutrients due to the beneficial effects of inoculation with *Azotobacter* which enhanced plant growth. It might also be due to production of amino acids (IAA), vitamins and growth promoting substances like indole acetic acid and gibberellic acid secreted by these introduced beneficial microorganisms which resulted in enhanced nutrient uptake, translocation and synthesis of photosynthate assimilates which resulted increased plant growth characters and in obtaining economically profitable yield (Dutta and Singh, 2002; Suke *et al*., 2011). Appreciable 1000 grain weight was recorded in TZEE-Y with Azotobacter treatments at JABU. This might be due to the ability of biofertilizers to transport of major nutrients like N besides secreting plant growth promoting substances such as IAA and gibberellins. (.Abou-el-Seoud and Abdel-Megeed, 2012). However, among all the treatments, Mycorrhiza recorded less significant effect on agronomic parameters.

Conclusion

Two field experiments were conducted in Southwestern Nigeria to compare the effect of biofertilizer types on agronomic parameters of selected maize cultivars. Application of biofertilizers resulted in better performance on growth parameters of the selected maize cultivars. However, the cultivars inoculated with biofertilizers had higher plant height most especially Azotobacter and azospirillum compared un-inoculated maize. It was shown that all maize cultivars inoculated with Azotobacter and azospirillum showed better performance in terms of plant growth (Plant height, plant diameter, number of leaf and leaf length) over the un-inoculated (control) maize. Therefore application of Azotobacter and azospirillum had positive effect on the growth of maize regardless of the cultivars.

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