



Influence of ‘Lactobacillus serum’ on the growth of *Amaranthus hybridus* and some soil chemical properties under screen house conditions

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Abstract

The potentials of lactic acid bacteria serum, termed as ‘Lactobacillus (LB) serum’, in enhancing soil nutrient availability and supplies for the growth of *Amaranthus hybridus* and some chemical properties of soil were investigated at the screen house of the Department of Soil Science, University of Benin. Three application rates of the serum were adopted, consisting of 3 mL (3,000 L.ha⁻¹), 5 mL (5,000 L.ha⁻¹), and 0 mL (0 L.ha⁻¹), and represented as treatment. *Amaranthus hybridus* was transplanted into pots containing 2 kg of 2 mm sieved air-dried soil. Each treatment was replicated seven times to give a total of 21 pots that were laid out in a Completely Randomized Design (CRD). The treatments were applied twice a week starting from the 2nd week after transplanting. The plant growth indices measured were number of leaves, plant height and plant biomass. The results showed that serum positively influenced the number of leaves and plant biomass (4.333 kg to 4.830 kg) compared to the control (3.901 kg). However, the highest value of the plant biomass was found in the 3 mL (3,000 L.ha⁻¹) treated pots, while the microbial colonies of bacteria in soil after serum application were sustained when compared with the control but at a reduced population for *Bacillus subtilis*. The application of LB. serum slightly improved the soil total organic carbon (320.0 g.kg⁻¹ to 352.0 g.kg⁻¹) and nitrogen (3.102 g.kg⁻¹ to 3.325 g.kg⁻¹) as against, 64.00 g.kg⁻¹, and 0.639 g.kg⁻¹ in the control respectively.

INTRODUCTION

Leafy vegetables are relished by many people in Nigeria because of the plethora of savoury dishes that require their inclusion and the numerous nutritional benefits they offer, which tubers, cereals and other starchy staple foods cannot supply (Mohammed and Sharif, 2011). Cereals and tubers form the bulk of food consumed in the tropics but they are deficient in minerals and vitamins compared to the body requirement to guarantee a good and healthy living (Ogunlade et al., 2011). There is increasing awareness on the value of leafy vegetables in contributing to balanced diet, particularly in areas where animal protein is deficient. Leafy vegetables contribute significantly to the amount

of carotene, vitamin C, protein, minerals, particularly calcium, and low calories (Kwenin et al., 2011) when included in our diet.

Regardless of the use of this crop for the aforementioned purposes, Nigerian farmers have not been able to harness the full production potential of this crop due to the thin layer of organic matter in sub-Saharan African soils that are continuously planted with *Amaranthus hybridus*, which like a number of other vegetables, requires soil with easily decomposable organic matter and adequate nutrient reserve for optimum yield (Olowoake and Ojo, 2014). *A. hybridus* is a heavy feeder, and the use of inorganic fertilizer to increase yield has been found to be effective only within few years, demanding consistent use on a long-term basis (Fonge et al., 2016).

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The hazardous environmental consequences and high cost of inorganic fertilizers make them not only undesirable but also uneconomical and out of reach for the poor small-holder farmers who still dominate the Nigerian agricultural food production space (Shiyam and Binang, 2011). This has led to the increased use of organic manure, a readily available alternative, which proves to be more environmentally friendly, but the mineralization of these organic manure/ materials is slow, thereby resulting in a problem with the supply of nitrogen due to the high demand by the plant.

According to Kang (2019), *Lactobacillus* has been proven to successfully accelerate the rate of decomposition of organic matter and the solubilisation/mineralization of certain soil nutrients to plant required forms. *Lactobacillus* is a genus of naturally occurring gram positive bacteria. *Lactobacillus* helps the decomposition of organic matter, which causes less energy loss from excess heat and gas. In soils, it is thought that the build-up of heat and gas from regular soil decomposition causes harm to the plants, but the fermentation by *Lactobacillus* uses less energy and releases safer by-products. Serum produced from rice wash to culture the abundance of *Lactobacillus* can be used to enhance nutrient supply for the plant growth, and it is considered an easy and relatively cheap mean of improving nutrient supplies in home gardens. The serum is made by rinsing rice in warm water to make the water become rich in carbohydrates when added with whole milk to introduce lactose, which ultimately suppresses the growth of other organisms (Wernli, 2014).

Taking advantage of the known ability of *Lactobacillus* as a soil and plant probiotic can improve the soil conditions. Information on the level of nutrients supplied from accelerated decomposition of (inherent) organic matter that have built up in the soil over time are scanty. Therefore, the objective of this study was to determine the effects of *Lactobacillus* serum (a solution of *Lactobacillus* culture) on the growth and yield of *A. hybridus* and some soil chemical properties.

MATERIALS AND METHODS

Experimental design

Soils were collected from the experimental field (6°24'2" N, 5°37'28" E) of Faculty of Agriculture, University of Benin, Nigeria and potted in perforated plastic buckets at the screen house. As much as 2 kg of 2 mm sieved air-dried soil per bucket was used in this

experiment. To investigate if the rate of application would affect the growth of plants and soil chemical properties, three rates of serum application per pot were adopted, consisting of 3 ml (300 L.ha⁻¹), 5 ml (500 L.ha⁻¹), and 0 ml (0 L.ha⁻¹), which were coded T1, T2 and C, respectively. Each treatment was replicated seven times to give a total number of 21 pots that were laid out in a Completely Randomized Design (CRD).

Preparation of serum/application rates

Serum was prepared according to the method of Wernli (2014) using carbohydrate residue (rice wash). The rice wash was placed in an open container and allowed to remain at room temperature for about a week to attract microbes from the air, then the liquid was extracted, and 10 parts of milk was added by adding 100 ml of whole milk for every 10ml of liquid to introduce lactose to the rice wash. The lactose introduced to the rice wash water prevents other microbes from growing while leaving only *Lactobacillus*. It was then kept in a cotton covered jar at room temperature for five days to allow for fermentation. The curd (a cottage cheese) was separated from the liquid using a sieve. The resulting liquid extract (whey) was used as *Lactobacilli* inoculum (*Lactobacillus* serum). One gram of sugar was added to feed and sustain the *Lactobacilli* for up to six months (Hill, 2019). A ratio of 1:5 of *Lactobacillus* serum (inocula) to water was used in dilution before soil drenching at different rates (0 ml, 3 ml and 5 ml) to the rhizosphere of *A. hybridus* seedling per bucket. The treatments were applied immediately after transplanting a two week old seedling into buckets and repeated twice in a week. Routine management practices were carried out for the seedlings, such as roguing and weeding.

Agronomic parameters measured

Agronomic parameters or plant growth performance indicators such as plant height and number of leaves per plant were measured at 2, 4 and 6 weeks after transplanting. The plant biomass was determined at 62 days after planting. Leaf samples of *A. hybridus* were collected from treatments (T1, T2 and C plants) and tested for total nitrogen, phosphorus, potassium, sulfur, calcium, magnesium and sodium. The plant materials were oven-dried at about 70°C until dry and crispy and then ground to powder. Determination of total nitrogen was done using the micro-kjeldahl method, nitric-perchloric acid digestion was used for sulfur analysis, Perchloric acid digestion (wet oxidation)

of the plant materials was done, and P was determined calorimetrically, while Ca, Mg, K and Na were determined by instrumentation (Atomic Absorption Spectrometry).

Culture and identification of bacteria in soil before and after experiment

Materials for microbial isolation and identification were sterilized in the autoclave. Microbial isolation using a 6-fold serial dilution technique as described by Okhuoya et al., (2012) was done to determine the bacterial in the soil before planting and after treatments. One (1) ml aliquot from dilution factors 1, 3 and 5 was measured into sterile plastic pétri dishes using sterile syringes where 20 ml of growth media (NA) was added and allowed to gel. Culture plates were then transferred to the incubator and incubated at 25 ± 2 °C for bacterial cultures. Microbial isolation and purification were determined from culture plates of dilution factors 3 and 5. Bacterial isolates were sub-cultured and streaked on freshly prepared Pétri plates (containing 20 ml of nutrient agar) to obtain pure cultures. The cultures were viewed macroscopically and macroscopically for several identifiable features and thereafter subjected to biochemical tests for confirmation.

Morphological and biochemical characteristics of bacteria

The identification of morphological and biochemical characteristics of bacteria was done in accordance to the standard presented by Bridson, (2006) and Willey et al. (2008).

Gram stain: The smears of the isolates were made on glass slides using a wire loop and were heat-fixed and allowed to cool. The smears were stained with crystal violet stain for a minute before washing off immediately with potable water. The smears were then covered with Lugol's iodine for 30–60 seconds and immediately washed off with water. The smears were rapidly decolorized with acetone or alcohol and washed rapidly with clean water after five seconds. The smears were stained with safranin for 60 seconds and immediately washed off. The stained smears were then allowed to air-dry after which a few drops of oil immersion were dropped on the smears and were viewed under the optical microscope using the 100× objective lens. The Gram-positive organisms were viewed as purple cells, while the Gram-negative organisms were viewed as pink or red cells. The cell morphology was taken into consideration, and cells which stains

Gram positive (purple) with a rod-like structure were considered to be of interest in this study.

Growth on differential agar: Differential media, consisting of *Bacillus cereus* agar (with appropriate supplement), Mannitol salt agar and Deman Rogosa Sharpe (MRS) agar were prepared according to the manufacturer's instruction and used for culture of bacteria isolates and observation of growth characteristic after which other biochemical test were carried out to further confirm the identity of the bacterial isolates. Blood agar plates were used to confirm beta hemolytic activity of *S. pyogenes*.

Spore staining technique: On a clean glass slide, two drops of water were added, and the bacterial culture was smeared on the drops of water. The slide was allowed to air dry and heat-fixed on the slide with the smear facing up. It was thereafter allowed to cool, and the smear was covered with an absorbent paper. The slide was placed over a staining rack containing beaker/water bath of steaming water. The absorbent paper was flooded with malachite green and allowed to steam for 3–5 minutes. The paper on the slide was gently removed, and the slide was rinsed (allowing the water to flow over the smear). It was then counter stained for a minute with safranin and rinsed on both sides to remove the safranin. The slide was allowed to dry and viewed under the microscope (×100).

Biochemical tests: These tests were conducted to determine the ability of the bacterial isolates to produce enzymes, such as catalase, oxidase, and urease. Other biochemical tests were carried out to determine the ability of the bacteria to utilize either sugar or substrate sources.

Catalase (Hydrogen peroxide; H₂O₂) test: A drop of H₂O₂ (3%) was placed on a grease free slide to which a loopful of the bacteria isolate was applied. Positive catalase activity was shown by effervescence, while no effervescence indicated absence of the enzyme.

Oxidase test: A whatman filter paper was soaked with a solution of 1% tetramethylphenylene diamine hydrochloride. A 24-hour culture of the test isolate(s) was smeared onto the impregnated filter paper. The presence of a purple color indicated a positive result.

Test for urea hydrolysis (Urease test): This was performed to show the ability of some bacteria to form an alkaline product (ammonia) by splitting urea under the influence or action of the enzyme urease. Urea was added to urease agar base before it was inoculated with the test organism in a slant. At an optimum temperature of 37°C, incubation was done for 24–48

hrs. The development of an intense pink/red color was indicative of a positive results, while negative results showed no color.

Indole formation test: A filter paper was saturated with 1% paradimethylaminocinnamaldehyde reagent, and a loop was used to remove a colony of the culture to be tested from the agar surface and robbed on the surface of the filter paper saturated with the reagent. Positive result was confirmed when a blue color developed within 30 seconds. Most indole-producing organisms turn blue within 30 seconds to one minute. The development of a slightly pink coloration or none at all is indicative of a negative result.

Citrate utilization test (Simon Citrate Agar (SCA) slant): Using a test tube, the medium was prepared as a slant to culture the bacteria isolates to be tested and allowed to stand for 24 hours in an incubator. Development of a blue color indicates a positive reaction to citrate, while no color change, or if the green color of the medium is retained, indicates a negative reaction.

Sugar fermentation and production of gases using Triple sugar iron agar (TSI): TSI (Oxoid) was prepared following manufacturer's instruction, and the prepared media was placed in a test tube and kept in a slant position for it to solidify. The slant and butt of the medium was inoculated with the test bacterium using a sterile loop, and it was incubated at 35–37°C for 18–24 hours. The results were read on the basis of acid or alkaline production in the slant or butt region of the tube, and gas production was confirmed by the presence of crack or air bubbles in the slant or but region. More so, production of hydrogen sulfide was confirmed by the blackening of the medium. A prepared laboratory chat was used for result interpretation in line with microbiological standard protocol as well as other biochemical tests carried out on the isolates to confirm or ascertain their identity.

Soil chemical properties

The chemical properties of the soils after treatments with the serum were also considered. Organic carbon content of the soil was determined by Nelson and Sommers (1982) chromic acid wet oxidation method. The total Nitrogen was determined using the micro kjeldahl digestion method (Bremner and Mulvaney, 1982), and the available Phosphorous was determined by the method of Bray and Kurtz (1945). Exchangeable Cations (Ca^{2+} , Na^{2+} , K^{2+} , Mg^{2+}) were extracted with 1 N ammonium acetate solution, and the concentration

of Ca^{2+} and Mg^{2+} in the soil were determined by Atomic Absorption Spectrophotometer (AAS). Meanwhile, while potassium (K) and sodium (Na) were determined with flame photometer (Thomas, 1982). Exchangeable acidity was determined by the KCL volumetric procedure. The soil pH was determined in 1:1 soil to water ratio using an electrode pH meter. Electrical Conductivity (EC) was also determined by inserting an electrical conductivity meter into the above suspension, and Exchangeable Cation Exchange (ECEC) was determined by summation method (Maclean, 1972).

Statistical analysis

Data generated from the plant parameters were analyzed using ANOVA (Analysis of Variance) using the GENSTAT software 12th edition, and then continued with Duncan's Multiple Range Test at $\alpha=5\%$ level of probability.

RESULTS AND DISCUSSION

Effect of serum on the growth of *Amaranthus hybridus*, nutrient uptake and soil bacteria

Based on Table 1a, the chemical composition of the serum consists of 2.01 mg.L⁻¹ (N), 9.95 mg.L⁻¹ (P) and 15,568 mg.L⁻¹ (K) coupled with a high amount (23,520 mg.L⁻¹) of total organic carbon. Its richness in NPK is enough to captivate the interest of researchers towards the experimentation and use of lactobacillus serum as a plant growth promoting bacteria (PGPB). This finding agrees with the work of Singh et al. (2014), who reported that some bacterial inoculants possess qualities that can make them used as biofertilizer.

The plants treated with serum showed higher performances in some respects than the control (Table 2). A significant difference was observed in the number of leaves of the plants from week 6–8, in which the highest number of leaves were observed in T1. Although the physical appearance of the plants in the field indicated variations in plant height (cm), they were not statistically significant ($p \geq 0.05$). The higher number of leaves in T1 and T2 can be attributed to the significant level of nitrogen in T1 (3.325 g.kg⁻¹) and T2 (3.102 g.kg⁻¹) soils compared with the content (0.639 g.kg⁻¹) in the control (Alejandro et al., 2017). The plant biomass was also higher in T1 and T2 at the end of 62 days after transplanting. Statistically, the biomass of T1 (4.830 g) was better than the T2 (4.333 g) at $\alpha=5\%$ level of probability. This result therefore suggest that

Table 1a. Some physical and chemical properties of serum

Parameters	Units	Value
pH		6.25
N	mg.L ⁻¹	2.01
P	mg.L ⁻¹	9.95
K	mg.L ⁻¹	155.68
Ca	mg.L ⁻¹	192.38
Mg	mg.L ⁻¹	87.52
S	mg.L ⁻¹	12.04
Na	mg.L ⁻¹	44.92
TOC	mg.L ⁻¹	23,520

Table 1b. Bacteria species and population in soil before experiment

Species	Population (colony forming units × 10 ³)
<i>Bacillus subtilis</i>	5.2
<i>Klebsiella spp.</i>	2.6
<i>Micrococcus</i>	2.2
<i>Staphylococcus aureus</i>	1.6

Table 2. Effects of serum on the growth of *A. hybridus*

Treatments	Plant height (cm) (4 WAP)	No. of leaves (4 WAP)	Plant height (cm) (6 WAP)	No. of leaves (6 WAP)	Plant height (cm) (8 WAP)	No. of leaves (8 WAP)	Plant biomass (g) (62 DAP)
Control (0 ml)	5.371 a	7 a	19.74 a	25 a	27.80 a	39 a	3.901 c
T ₁ (3 ml)	6.600 a	7 a	23.43 a	40 b	32.36 a	53 a	4.830 a
T ₂ (5 ml)	5.500 a	7 a	21.46 a	32 ab	32.00 a	48 a	4.333 b
Mean	5.82	7	21.5	32	30.7	47	4.35

Remarks: Means followed by the same letters are not significantly different according to Duncan's Multiple Range Test (DMRT) at α=5%.

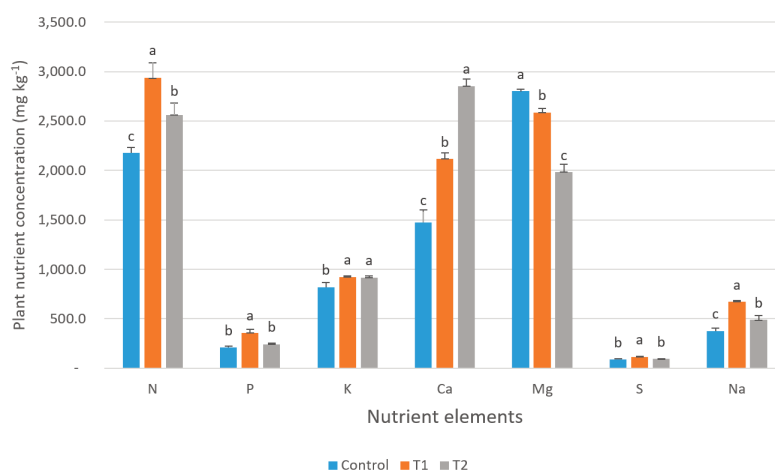


Figure 1. Nutrient properties of plant (*A. hybridus*) tissue

Remarks: Bars followed by the same letters are not significantly different according to Duncan's Multiple Range Test (DMRT) at α=5%.

Table 3. Effects of treatments on soil bacteria population after experiment

Sample	Isolates	Population (colony forming units × 10 ³)
Control (0 ml)	<i>Klebsiella spp.</i>	1.3
	<i>Micrococcus</i>	2.0
	<i>Staphylococcus aureus</i>	2.2
T ₁ (3 ml)	<i>Bacillus subtilis</i>	3.8
	<i>Klebsiella spp.</i>	2.4
	<i>Micrococcus</i>	2.0
	<i>Staphylococcus aureus</i>	2.0
	<i>Staphylococcus pyogenes</i>	1.8
	<i>Lactobacillus</i>	5.4
	<i>Bacillus cereus</i>	1.8
T ₂ (5 ml)	<i>Bacillus subtilis</i>	4.2
	<i>Klebsiella spp.</i>	2.4
	<i>Micrococcus</i>	2.2
	<i>Lactobacillus</i>	6.2

Table 4. Physical and chemical properties of soils after experiment

Treatments	Control (0 ml)	T ₁ (3 ml)	T ₂ (5 ml)
pH	5.380 a	5.450 a	5.460 a
TOC (g.kg ⁻¹)	64.000 c	352.000 a	320.000 b
N (g.kg ⁻¹)	0.639 c	3.325 a	3.102 b
P (mg.kg ⁻¹)	68.760 a	74.640 a	70.480 a
S	52.190 a	25.690 b	30.950 b
K	0.089 a	0.066 b	0.081 a
Ca (Cmol.kg ⁻¹)	6.413 a	6.253 a	6.733 a
Mg	0.810 b	0.648 b	1.459 a
Na	0.243 a	0.165 c	0.183 b
EA	0.200 a	0.200 a	0.100 b
ECEC (Cmol.kg ⁻¹)	7.750 b	7.340 b	9.030 a

Remarks: Means followed by the same letters are not significantly different according to Duncan’s Multiple Range Test (DMRT) at α=5%.

excessive rate of lactobacillus serum application above the optimal water requirement by *A. hybridus* can also cause the plant not to attain maximum production.

Nutrient concentrations of *A. hybridus* tissues are presented in Figure 1. The nitrogen content was greater in T1 (2,934.7 mg.kg⁻¹) followed by T2 (2,558.7 mg.kg⁻¹), and the least value was found in the control (2,176.0 mg.kg⁻¹). Nitrogen is highly leachable (Agbemin, 2020), and the higher rate of serum application must have resulted in the leaching and reduction in the amount of N present in plants treated with T2. A similar trend in results were also observed in P, K, S and Na contents

in the plants. While the calcium content in the plants treated with T2 (5 ml serum) was the highest, the magnesium content in plants harvested from the pots at the end of the experiment was observed to decrease with increasing rates of serum application. This result suggests that lactobacillus serum has the ability of increasing Ca uptake and limits magnesium uptake by *A. hybridus*. The result, however, showed that except for magnesium, the serum had positive influence on nutrient uptake ability by the plants with its N, P, K and S content resulting from the various treatment being in the following order of increase: T1 > T2 > C. This

Table 5. Cultural, morphological and biochemical characteristics of bacterial isolates

Morphological							
Elevation	Flat	Flat	Raised	Raised	Raised	Flat	Flat
Margin	Undulate	Undulate	Smooth	Undulate	Undulate	Round	Entire
Color	Cream	Cream	Cream	Yellow	Cream	Cream	Cream
Shape	Irregular	Irregular	Irregular	Irregular	Circular	Regular	Circular
Size	Large	Large	Small	Small	Small	Large	Small
Gr. diff. agar	BCA	BCA	MSA	MSA	Blood agar	MRS	EMB
Colour	Blue	Straw	Yellow	Pink	Beta haemolytic	Pale straw/whitish	Pink
Staining							
Gram stain	+	+	+	+	+	+	-
cell type	Rod	Rod	Cocci	Cocci	Cocci	Rod	Rod
Arrangement	Disperse	Disperse	Clusters	Tetrads	Tetrads	Single Rods/Pairs	Disperse
Color	Purple	Purple	Purple	Purple	Purple	Purple	Pink
Spore staining	+	+	-	-	-	-	-
Biochemical							
KOH test	-	-	-	-	-	-	-
Catalase	+	+	+	+	-	-	+
Indole	-	-	-	-	-	-	-
Citrate	+	+	+	+	-	-	+
Oxidase	-	-	-	+	-	-	-
Urease	-	-	+	+	-	-	+
Glucose	+	+	+	-	+	+	+
Sucrose	+	+	+	-	+	+	+
Lactose	+	+	+	-	+	+	+
Mannitol	-	+	-	-	-	+	-
Gas formation	-	-	-	-	-	-	+
H ₂ S formation	-	-	-	-	-	-	-
Identity	<i>Bacillus cereus</i>	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Micrococcus luteus</i>	<i>Streptococcus pyogenes</i>	<i>Lactobacillus acidophilus</i>	<i>Klebsiella pneumoniae</i>

result is similar to the findings of Islam and Hussain (2012), who also reported that probiotic bacteria applied to soil or crop can increase nutrient acquisition ability of plants.

The bacteria isolates (Table 3) present in the soils at the end of the experiment showed that two of the bacteria isolates (*Lacobacillus* and *Bacillus subtilis*) were found to be present in the serum soils treated with T1 and T2 and absent in the control (Table 4). However, the *Lactobacillus* from the serum seems to have a suppressive effect on the *Bacillus subtilis*, which was observed to have reduced from a population of 5.2×10^3 colony forming units (cfu) before experiment

to 3.8×10^3 and 4.2×10^3 cfu in T1 and T2, respectively. The relative abundance of *Lacobacillus* over other species perhaps made it the microbe responsible for the notable response of *A. hybridus* in terms of nutrient content and growth in accordance with the report of Alejandro et al. (2017).

Effect of 'Lactobacillus serum' on some soil chemical properties

The effects of 'Lactobacillus serum' application on the chemical properties of the soils are shown in Table 4. However, its effect on soils cannot be determined based on the amount of nutrients in the soil alone but

as a composite of the amount in the soil and plant tissues collectively because *A. hybridus* would have absorbed some nutrients into its biomass. The pH levels of the treated soils (T1-5.45 and T2-5.46) were observed to have slight increase in value than the control soil (C-5.36). However, these values (C- 5.36, T1-5.45 and T2-5.46) were still within the classification range of strongly acidic soil (Burt, 2014) and were not significantly different ($p \geq 0.05$) at the end of the experiment. This result contradicts the findings of Rashid et al. (2004), who reported a decrease in pH due to the presence of some bacteria in a media. The total organic carbon (TOC) was found to be higher in the treated soils (320–352 g.kg⁻¹) compared with the control (64 g.kg⁻¹). The increase in TOC implies an increase in organic matter in correspondence to the application of Lactobacillus serum (Kang, 2019). The higher amount of N in treatments (T1 and T2 soils) commensurate with that of TOC. This result conforms to the findings of Alejandro et al. (2017), who also reported the application of probiotic microbes in agricultural soils to be capable of stimulating and facilitating some biological processes leading to the release of nutrients in an inorganic form for plant uptake. The soil available phosphorus, although not significantly different at $\alpha=5\%$ level of probability, was slightly higher in T1 (74.64 mg.kg⁻¹) followed by T2 (70.48 mg.kg⁻¹), and the least was found in C (68.76 mg.kg⁻¹). This result justifies the reason why the uptake of P by *A. hybridus* was observed to be greater in T1 plants (Figure 1). Whereas, soil sulfur and potassium were higher in control (52.19 and 0.089 cmol.kg⁻¹) than in T1 (25.69 and 0.006 cmol.kg⁻¹) and T2 (30.95 and 0.081 cmol.kg⁻¹). The probiotic bacteria in the serum must be responsible for mineralization and availability of these major nutrients (N and P), which is in accordance with the report of Islam and Hussain (2012). Magnesium (Mg) was the highest in T2 soils, while in C and T1, the Mg content in *A. hybridus* tissue decreased with increase in treatment (Figure 1). The soil calcium level was not significantly ($p \geq 0.05$) different. This result aligns with the findings of Ogbemudia et al. (2020), who observed no significant changes in the soil calcium level after applying *Bacillus thuringensis* to okra (*Abelmoschus esculentus* L.). Whereas, a significant result was obtained in the ECEC at the end of the experiment in T2 soils (9.03 cmol.kg⁻¹) over T1 (7.34 cmol.kg⁻¹) and Control (7.75 cmol.kg⁻¹) soils.

CONCLUSIONS

The application of lactobacillus serum (LBS) to *Amaranthus hybridus* grown on experimental potted soils resulted in higher number of leaves and plant total biomass without a significant ($p \geq 0.05$) increase in plant height. It also had a positive effect on the nutrient (N, P, K and S) content of the plant but not to be capable of enhancing magnesium (Mg) uptake since the content of Mg in the *A. hybridus* tissues were observed to reduce with increasing rates of serum. LBS application was also proven to have enhanced the soil total organic carbon (TOC), nitrogen (N), phosphorus (P) and ECEC of the experimental soil, while the soil calcium (Ca) level was not significantly ($p \geq 0.05$) altered. *Lactobacillus* and *bacillus subtilis* were the most dominant soil bacteria present in the inoculated soils after experiment. However, the *Bacillus subtilis* was observed to have reduced in population when compared with its population figure present in the initial soil test. This may suggest that the application of LBS could be considered useful in suppressing the presence of *Bacillus subtilis* in soils when necessary.

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