



Biofertilizer potentials of *Rhizobium leguminosarum* on two common tropical vegetable plants *Talinum triangulare* (waterleaf) and *Telfairia occidentalis* (pumpkin)

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ABSTRACT

This study explored the biofertilizer capabilities of *Rhizobium leguminosarum* on two tropical vegetables, *Talinum triangulare* (waterleaf) and *Telfairia occidentalis* (pumpkin). The microorganisms were isolated, characterized, and inoculated onto activated charcoal carriers at varying concentrations for each vegetable, with controls using sterile activated charcoal and N.P.K fertilizer. After 28 days, growth indices including plant biomass, height, and mean leaf area were evaluated. Results showed significant growth improvements with *Rhizobium leguminosarum*, ranging from 107.77% to 286.78% for waterleaf and 166.52% to 358.26% for pumpkin, surpassing N.P.K fertilizer. Leaf areas also increased significantly, with *Rhizobium*-inoculated vegetables outperforming chemically fertilized ones. While the impact on plant height was relatively lower, this research highlights the potential of *Rhizobium leguminosarum* as a fertilizer for enhancing waterleaf and pumpkin growth. Future studies should expand on these findings in different vegetable types and environmental conditions to validate real-world field applications.

Keywords: Biofertilizer capabilities; *Rhizobium leguminosarum*; Tropical vegetables; Growth indices; Plant biomass; Real-world validation

Introduction

Agriculture stands as the cornerstone of the economies of many developing nations, none more so than in countries like Nigeria, where 45-60% of the labor force is engaged in agricultural activities, contributing significantly to the gross national product (The World Bank, 2007). Vegetables, serving as both essential food sources and industrial raw materials, play a pivotal role in driving economic interests. However, a grave concern in today's world is the pervasive soil pollution and contamination, largely exacerbated by the widespread use of chemical fertilizers and pesticides, resulting in profound harm to the environment (Petsas and Vagi, 2019). Addressing this pressing issue, bio-fertilizers have emerged as an environmentally friendly alternative that is gaining global recognition. These bio-fertilizers consist of living microorganisms, including bacteria, fungi, and cyanobacteria (blue-green algae) (Itelima *et al.* 2018), known for their unique ability to enhance soil nutrient quality. The most remarkable aspect of this relationship is symbiosis, in which

both plants and microorganisms derive mutual benefits (Youssef and Eissa 2014).

Bio-fertilizers represent substances containing live microorganisms that colonize the rhizosphere, seed surfaces, or plant interiors, thereby enhancing plant growth by increasing the supply or availability of primary nutrients to the host plant (Hashmi and Bareliya, 2017). Bio-fertilizers operate through natural processes such as nitrogen fixation, phosphorus solubilization, and the synthesis of growth-promoting substances. The adoption of bio-fertilizers holds the promise of reducing the dependence on chemical fertilizers and pesticides, offering an eco-friendly and cost-effective approach to agriculture (Kumar *et al.* 2018). Bio-fertilizers, by establishing symbiotic associations with plant roots, adeptly convert complex organic matter into simpler compounds for plant absorption, all while preserving the soil's natural habitat (Mahdi *et al.* 2010).

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In this context, the artificial cultivation of efficient microorganism cultures plays a pivotal role in accelerating soil microbial processes (Hayat *et al.*, 2010). The integration of bio-fertilizers into holistic nutrient management practices complements the use of chemical fertilizers, proving to be both cost-effective and sustainable for agriculture (Hayat *et al.* 2010). The versatility of microorganisms as bio-fertilizers extends across various crop types, encompassing legumes, cereals, root crops, and tuber crops (Mohammed *et al.* 2009; Mahdi *et al.* 2010).

Microbial bio-fertilizers are gaining prominence due to their numerous advantages over chemical fertilizers. They contribute to the restoration of the soil's natural nutrient cycle, enrich organic matter content, and promote sustainable plant growth. Notably, certain essential plant nutrients, such as phosphate, often remain scarce due to immobilization by mineral elements like iron, aluminum, and calcium, resulting in the inefficient use of chemical phosphate fertilizers (Mahdi *et al.* 2010). Research findings have indicated that less than 20% of added phosphate as chemical fertilizer is absorbed by plants, while the rest is either immobilized or leached out, leading to wastage and ecological pollution. Consequently, the use of phosphate-solubilizing microorganisms as bio-fertilizers is gaining prominence as a more sustainable alternative to chemical phosphate fertilizers (Mohammed *et al.*, 2009).

Some scholars (Sullivan, 2001) have defined bio-fertilizers as microbial inoculants that represent agricultural soil amendments. These beneficial microorganisms establish symbiotic relationships with target crops, benefiting both parties. Beyond phosphate solubilization and immobilization, bio-fertilizer microorganisms enhance soil quality by increasing the availability of other primary nutrients. For instance, *Azotobacter* and *Rhizobium* species are known to fix nitrogen in the soil. While *Rhizobium* is highly regarded for its efficient bio-fertilizer capabilities due to its symbiotic nitrogen fixation, *Azotobacter* are recognized for their additional role in improving soil aggregation through the production of slime.

In light of the remarkable achievements and the environmental benefits associated with microbial bio-fertilizers, this project aims to evaluate the potential of selected bacterial species, specifically *Rhizobium leguminosarum*, as bio-fertilizers and assess their impact on the growth patterns of two common tropical vegetable plants, *Talinum triangulare* and *Telfairia occidentalis*. The project's objectives encompass the isolation and identification of *Rhizobium* species, molecular characterization of the isolates, testing the bio-fertilizer potential of the two isolates using the two plant seedlings,

and assessing the growth performances of the two bio-fertilized vegetables based on plant height, biomass, and leaf area over an eight-week period. Comparative analyses will be conducted between the bio-fertilized plants and control groups (non-bio-fertilized plants), as well as among different test organisms.

Materials and methods

Source of materials

The test organism, *Rhizobium leguminosarum* was isolated from legume root rhizospheric soil and from top soil peat in Eastern field (experimental farms) of National Root Crops and Research Institute (NRCRI) Umudike. The test vegetable crops, waterleaf (*Talinum triangulare*) and pumpkin (*Telfairia occidentalis*), were obtained from the school horticulture garden while laboratory and other facilities were sourced from Ceslab Laboratory in association with the research laboratories of National Root Crops Research Institute (NRCRI), Umudike.

Experimentation design

The experimental design was a simple randomized block design involving the use of two controls C_1 , C_2 representing the two unfertilized test vegetable crops, waterleaf and pumpkin respectively as well as three test seedlings groups for each plant three of which were treated with *Rhizobium leguminosarum* at varying doses. The experimental layout is shown in Table I below.

Table I. The Experimental design layout representing the block design

Test crop	Control	R ₁	R ₂	R ₃
<i>Talinum triangulare</i> (T ₁)	T ₁ Co	T ₁ R ₁	T ₁ R ₂	T ₁ R ₃
<i>Telfairia occidentalis</i> (T ₂)	T ₂ Co	T ₂ R ₁	T ₂ R ₂	T ₂ R ₃

Key T1 = Test plant 1 (*Talinum triangulare*) T2, = plant 2 (*Telferia occidentalis*)

Co = Control, R1, R2 and R3 = *Rhizobium leguminosarum* at different doses of 2.5, 5.0 and 7.5 ml of 1x10⁸ cfu/ml

In line with the randomized design shown above, a total of 40 pots (or perforated bags) were used each of which will contain 2 kg of sterile top soil from agricultural farm. The bags were used for each test plant on the test biofertilizer organism (*Rhizobium leguminosarum*), for each test plant for example *Telfairia occidentalis*, the 40 bags were divided into four groups of 5 bags each and designated accordingly for

example T_1C_0 , T_1R_1 , T_1R1_2 and T_1R_3 representing the controls without organisms, replicate 1 with *Rhizobium leguminosarum*, replicate 2 and Replicate 3 respectively. This same scheme was used for the second plant (*Talinium triangulare*).

Isolation of bacterial biofertilizer

The test organism, *Rhizobium leguminosarum* was isolated from legume root rhizospheric soil and from top soil peat in Eastern field (experimental farms) of National Root Crops and Research Institute (NRCRI) Umudike.

Isolation of Rhizobium leguminosarum

The method of Boraste *et al.* (2009), reported by Raj (2007) was employed. Accordingly, root nodules of healthy legume plant were surface disinfected by scrubbing with 70% ethanol-soaked sterile cotton wool. The nodules were crushed in a surface sterilized microbiology laboratory porcelain mortar with pestle and in the presence of drops of sterile distilled water. Loopfuls of the crushed nodule and water mixture were streaked onto sterile Yeast extract mannitol agar (YEMA) containing 1% congo red dye. Incubation was for 72 hours at room temperature. Thereafter, the plates were observed for growth and the presence of distinct colonies from which inoculum were taken for purification.

Purification was by sub-culture in which inoculum (loopful) from the distinct colonies were streaked onto fresh sterile solid medium (YEMA) and incubated as described earlier. The presence of uniform colonies in the sub-cultured plates was considered a condition for acceptance of purity. The resulting pure cultures were used for characterization and subsequent identification.

Preparation of biofertilizer inoculum

The bacterial inoculum used as biofertilizer were prepared to contain 2.5×10^8 cfu/ml, 5.0×10^8 cfu/ml and 7.5×10^8 cfu/ml. The determination of concentration/ cell densities for the different treatments (used as biofertilizer doses) was based on Mcfarland's turbidity standards, Turbidity that contains approximately 1×10^8 cfu/ml (Cockerill, 2012). Suspensions of each of the test bacterial isolates were prepared separately from a 48 hours old broth culture in clear glass test tubes with screw cap covers. The preparation of the bacteria suspension involved the transfer of 2ml of the 48hours old broth culture of the test organism, to 18ml of sterile normal saline solution in a glass test tube with cover. Their respective turbidities were adjusted to match that of the Mcfarland standard in similar glass test tubes when broth was placed against clear white background thus giving an approximate concentration (cell density) of 1×10^8 cfu/ml. The

aseptic withdrawal of 2.5 ml from the bacteria suspension of 1×10^8 cfu/ml therefore gives an equivalent of 2.5×10^8 cfu/ml. This was then mixed with inert carrier material to form the inoculums at that concentration. Similarly, 5.0ml and 7.5ml of the prepared suspension also gave their respective equivalent concentration of 5.0×10^8 cfu/ml, 7.5×10^8 cfu/ml. The mixing with carrier inert material (activated charcoal) was done without delay following the confirmation of matching turbidity with the Mcfarland Solution to avoid further increase in the bacterial population if left to stand.

Identification of test organisms

Identification of the isolate (*Rhizobium leguminosarum*) was done at both phenotypic and molecular levels. The phenotypic identifications were based on the four-step approach as described by Cheesbrough (2006).

Identification of isolates

Identification of the isolate (*Rhizobium leguminosarum*) was done by cross checking their respective characteristics (as recorded in the characterization test above) against existing taxa in standard manuals for confirmation. Two manuals were used namely *Bergey's Manual of Determinative Bacteriology* (Buchanan *et al.* 1994) and the Manual for Identification of Medical Bacteria (Cowan, 1993). Confirmation of the identity was based on matching characteristics of the isolates vis a vis those contained in the manuals. The isolates were thus identified accordingly.

Molecular identification of isolates

Molecular characterization of the isolates *Rhizobium leguminosarum* was done using DNA sequencing method as described by Cubo *et al.* (1992). The 16s ribosomal DNA genes of the isolates were amplified using a polymerase chain reaction (PCR) machine with a universal bacterial primers Fd_1 . The sequences obtained were compared with deposited available information at a gene bank database. The phylogenetic analysis of the 16s rRNA gene sequence was carried out using MEGA 6.0 programme.

Application of the biofertilizer to the plant

Application of the biofertilizer to the two plants involves the process of embedding the microorganism on solid inert carrier material with large surface area which then applied around the seedling in the normal ring application. In this regard, 1ml of each prepared biofertilizer inoculum was mixed with 5g of activated charcoal as the inert carrier material. The mixing was done in a sterile plastic cup with the aid of a surface sterilized spatula until a homogenous mixture

was obtained. This was done for each chosen biofertilizer dose i.e., 2.5×10^8 fu/g, 5.0×10^8 cfu/g, and 7.5×10^8 cfu/g. This preparation was done separately for each of the microorganisms used. The normal ring application was used as the microorganisms were already embedded on the solid powered carrier. To affect this, a circular ring was drawn around the seedling plant within the rhizospheric soil zone, Groove (depression in the soil) of about 2-3 cm depth was made along the ring and the solid biofertilizer powder was carefully spread evenly in the groove around the seedling. The inoculated grooved (depressions) was covered with soil from its two sides and the fertilized plant was left to grow under close periodic observation.

Test for biofertilizer potential of rhizobium leguminosarum

The method described by Flores-Félix *et al.* (2013) was employed in each case, four groups of the 40 bags that was divided, which includes the control and the test sample soil bags were planted with seeds of the appropriate vegetables (*Talinum triangulare* and *Telfairia occidentalis*) and observed to germinate. The seedlings in each bag were thinned down to contain two plants of about the same size. Thereafter, the seedlings in their respective bags were treated with the different prepared biofertilizer per concentrations (2.5×10^8 cfu/g, 5.0×10^8 cfu/g, and 7.5×10^8 cfu/g) respectively. All the plants in their respective bags were observed to grow under the same conditions of environment for eight weeks. Then they were carefully harvested and measured for the different growth parameters.

Determination of plant height

Determination of plant height was done by direct linear measurement of the shoot using a flexible calibrated rule (Tape). Measurement of each plant covered the distance from the base of attachment to the soil, to the tip of the plant. Where different plant shoots arose from the base, each branch was measured and a mean value was taken (as in the waterleaf). However, for the pumpkin, the tip of the longest vine of the main plant was taken (not the branches that arose after the base). All measurement was done on each treatment plant covering the triplicated treatment bags, (Khalid and Mahmoud 2015)

Determination of plant biomass

The plant biological mass was determined as the mass (weight) of the entire freshly harvested plant in their fresh form. The gravimetric method involved a process in which the freshly harvested plant (devoid of any extraneous materials) was carefully stuffed into a previously weighed and labelled large paper envelope. Care was taken to ensure non-loss of leaves or tender shoot parts in the process. The

envelope containing the fresh plant was placed on a top loading sensitive electronic weighing balance and its weight was registered and recorded in grams.

The formula below was used to calculate the biomass

$$\text{Biomass (g)} = W_2 - W_1$$

where W_1 = weight of empty paper envelope

W_2 = weight of the envelope + fresh plants

Determination of plant leaf area

This was determined using the weight area relationship method. In this process, the area of the entire leaf of each plant was determined using the relationship between the area of a part of the leaf and its weight. All the leaves in each test plant were first plucked out carefully with caution to avoid loss of any single leaf or leaves. The plucked leaves from each test plant were quantitatively put in a weighed paper envelope and the weight was measured as described earlier. Having determined the total weight of the entire leaves of the test plant (W_1), ten leaves were selected and placed on top of one another with the aid of a cork borer of known diameter; ten circles of the leaves were cut out of the stacked leaves. The weight of the ten circles of the leaves was obtained by direct weighing on the weighing balance (W_2).

The area of the ten circles of leaves cut out of the plant was determined using the formula below:

Leaf area (circles) = $\pi r^2 = A_1$ whereby r is the radius of the cork borer.

To calculate the Area (A_2) of the entire leaves, the weight to area relation was used wherein

$$\frac{A_2}{W_1} = \frac{A_1}{W_2}$$

W_1 = weight of all the leaves of the test plant

W_2 = weight of 10 circle of leaf

A_1 = area of the 10 circles of leaf

A_2 = area of all the leaves of the test plant

Assessment of the effects of biofertilizer on test plants

The effect of the applied biofertilizer (*Rhizobium leguminosarum*) on the growth indices of the test vegetables was done by an assessment of the impact on the tested parameters including plant height, biomass, and leaf area.

Effect of biofertilizer application on the plant biomass

The effect on biomass was monitored by calculating the percentage different between the weight of the vegetable from the control and that of the vegetable from biofertilized treatment. The effect was expressed as a percentage ratio of the difference between the biomass of biofertilized plant and that of the control biomass of plant to the height of the control the (unfertilized) plant where the result is in the positive, the effect is on increase and where the result is in the negative, the effect is a reduction

The formula below was used:

$$\% \text{ Effect} = \frac{B_t - B_c}{B_c} \times \frac{100}{1}$$

B_c = biomass of control plant

B_t = biomass of biofertilizer treated plant

Effect of biofertilizer on the plant height

This was also calculated in a way similar to the biomass effect whereby consideration was given to the ratio of the difference between the height of the biofertilized plant and the height of the control, to the control.

The formula below was used:

$$\% \text{ Effect} = \frac{H_t - H_c}{H_c} \times \frac{100}{1}$$

H_c = height of control plant

H_t = height of biofertilizer treated plant

$$\frac{\text{Trt} - \text{Control}}{\text{Control}} \times \frac{100}{1} = \% \text{ Effect}$$

Effect of biofertilizer on the leaf area

To determine the effect of biofertilizer on the leaf area of the test plants, was subtracted from the leaf area of the biofertilizer treated plant and expressed as a percentage ratio of the leaf area of the control plant. The formula below was used:

$$\% \text{ Effect} = \frac{A_t - A_c}{A_c} \times \frac{100}{1}$$

A_c = leaf area of the control plant

A_t = leaf area of biofertilized plant

Statistical analysis

Obtained data was subjected to statistical analysis of various (ANOVA) using the statistical package for social sciences (SPSS) version 20.

Results and discussion

Figure 1 depicts the molecular characterization of *Rhizobium leguminosarum* through a visual representation in the form of a phylogenetic tree.

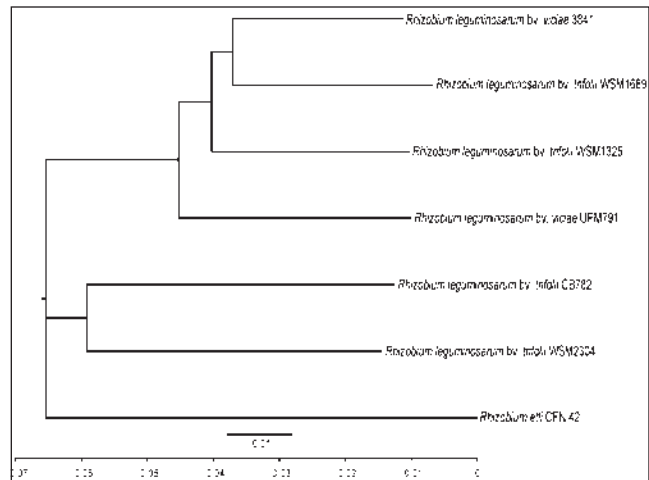


Fig. 1. Phylogenetic tree of *Rhizobium leguminosarum*

The biomass of the vegetables grown with microbial biofertilizer and the controls

From the results there was a variation of significant differences between the biomass of vegetables grown with different doses of the biofertilizers, those grown with chemical fertilizer and the control grown in unfertilized soil. With regards to *Telfairia occidentalis* (Ugu), the biomass increased from an average of 20.28g in the control to 92.53g in the *Rhizobium leguminosarum* fertilized crops whereas the NPK fertilized crops recorded an average 96.56g as biomass. From the result also, the corresponding increase in the *Rhizobium leguminosarum* fertilized *Talinum* crops recorded a highest mean biomass of 74.4g and the increase in the chemically fertilized crops was a maximum of 62.38g.

Similarly, the corresponding increase in the *Rhizobium leguminosarum* fertilized *Telfairia occidentalis* crops at the same doses of inoculants from 20.28g (control) to 54.05g, 68.14g and 92.53g. Similar trends were recorded for the *Talinum triangulare* crops with increase from 20.33g (control) to 40.79g, 46.47g and 49.26g for the three doses of inoculants respectively. On the whole there was no significant difference in the biomasses of the two biofertilizers and the chemical fertilizer (NPK) at the highest dose of application

However, the biofertilizer had higher biomass values than the chemical fertilizer crops (Ugu) at lower application dosages. The impact of the biofertilizers was higher in *Telferia occidentalis* than in the *Talinum triangulare* crop. While the Ugu biofertilized crops performed better than the NPK fertilized crops, the chemical (NPK) fertilized water leaf plants, performed better than the bio-fertilizer treated *Talinum triangulare* crops (Table II).

Table II. Mean biomass of vegetables grown with bio-fertilizer

Treatment	<i>Telfairia occidentalis</i>	<i>Talinum triangulare</i> (cm)
Control	20.28± 4.07 ^c	20.33± 4.01 ^f
<i>Rhizobium leguminosarum</i>		
2.5ml	54.05± 2.38 ^d	42.26± 6.64 ^e
5.0ml	68.14± 5.71 ^{bc}	55.65± 0.74 ^e
7.5ml	92.53± 7.35 ^a	74.40± 1.85 ^a
NPK		
2.5ml {T ₁ }	30.84± 2.34 ^e	22.94± 5.12 ^f
5.0ml {T ₂ }	57.24 ± 3.5 ^{cd}	53.15± 1.75 ^c
7.5ml {T ₃ }	96.56 ± 3.49 ^a	62.38 ± 4.05 ^b

Values shown means of triplicate measurement ± standard deviation. Figures with different superscripts in the columns are significantly different (P<0.05)
Vegetative growth (plant height) of vegetables grown with biofertilizer and the controls.

The results show that both test vegetables *Telfairia occidentalis* and *Talinum triangulare* responded well to the bio plant foods but to varying extents. The mean *Telfairia occidentalis* plant heights in the *Rhizobium leguminosarum* fertilized plants were 33.23cm, 37.37cm and 39.93cm. For the waterleaf plants, the increases were from 17.33cm to 26.87cm in the *Rhizobium leguminosarum* fertilized plants at the same dosage. Interestingly, the Ugu plants performed better than the waterleaf plants for test organism. Also, *Rhizobium leguminosarum* fertilized waterleaf had higher height than the chemically (NPK) fertilized plants whereas the contrast was recorded with the *Telfairia occidentalis* plants where the NPK fertilized plant had higher height (36.07-78.60cm) than the bio-fertilized plant 33.23cm-39.93cm for *Rhizobium leguminosarum*. There were significant differences between the plant heights of the fertilized plants and the control in all cases (Table III, Fig. 2 and 3).

The increase in the *Rhizobium leguminosarum* fertilized “*Telfairia occidentalis*” plants was from 461.76cm² to 569.32cm² (2.50 x 10⁸cells/kg) and to a maximum leaf area of 1220.3cm² in the 7.5x10⁸ cell/kg fertilized plants. There was significant difference between the leaf areas of the bio-fertilizer grown *Telfairia occidentalis* and those grown with NPK which recorded 686.77cm² to 1327.90cm² as leaf areas at the lowest to the highest doses applied.

Table III. Plant height of vegetables grown with biofertilizer (cm)

Treatment	<i>Telfairia occidentalis</i>	<i>Talinum triangulare</i>
Control	29.27±5.80 ^e	17.33±1.26 ^{cd}
<i>Rhizobium leguminosarum</i>		
2.5ml	33.23± 0.49 ^{de}	23.63 ± 0.65 ^b
50ml	37.37± 1.48 ^{cd}	25.40± 1.10 ^a
75ml	39.93 ±1.50 ^c	26.87± 0.12 ^a
NPK		
2.5ml {T ₁ }	36.07 ± 1.50 ^{cd}	15.70± 0.36 ^c
5.0ml {T ₂ }	48.47 ±1.92 ^b	17.04 ±0.63 ^{de}
7.5ml {T ₃ }	78.60± 2.65 ^a	18.73± 0.21 ^c

Values show means of triplicate measurements± standard deviation. Figures with different superscripts in the column are significantly different (P <0.05)



Fig. 2. *Telfairia occidentalis* (pumpkin) before application of biofertilizer and NPK

In the *Talinum triangulare*, there were increases in the leaf area also in a dose dependent manner, from 93.69cm² in the unfertilized plant (control) to maximum values of 287.64cm² and 301.83cm² in the plants with high doses of *Rhizobium leguminosarum* and NPK respectively. The results showed



Fig. 3. *Telfairia occidentalis* and *Talinum triangulare* after application of biofertilizer

that, at the high dose of application of the bio-fertilizers, the leaf areas did not show statistically significance difference between the bio-fertilizer on one hand and with the chemical fertilizer on the other but leaf areas of the control plants (unfertilized) were much lower than those of the bio-fertilized ones and the differences between then was statistically significant. The general trend however is that in each case, the increase in leaf area was dependent on application dose of the fertilizers notwithstanding the type.

Leaf area measurement of the vegetables grown with biofertilizers and their controls

The result show significant variations in the leaf areas of the vegetables (*Telfairia occidentalis* and *Talinum triangulare*)

Table IV. Leaf area of vegetable grown with bio-fertilizers and control (cm²)

Treatment	<i>Telfairia occidentalis</i>	<i>Talinum triangulare</i>
Control	461.76± 182.63 ^g	93.69± 20.45 ^f
<i>Rhizobium leguminosarum</i>		
2.5	569.32±102.23 ^{fg}	136.02 ±24.24 ^c
5.0	1006.00±12.69 ^{cd}	255.20± 1.00 ^b
7.5	1220.3± 19.26 ^{bc}	287.64± 5.70 ^a
NPK		
T ₁	686.77± 46.13 ^f	102.03± 1.96 ^f
T ₂	1022. 0± 11.62 ^d	108.55± 1.74 ^f
T ₃	1327.90± 25.49 ^a	301.83±3.75 ^a

Values show means of triplicate analysis ± standard deviation. Figures with different superscripts in the same column are significantly different (P<0.05)

grown with biofertilizer (*Rhizobium leguminosarum*) as well as those grown with NPK fertilizer and the control (unfertilized vegetables) (Table IV).

Effect of biofertilizer *rhizobium leguminosarum*, on the biomass of the two test vegetables *telfairia occidentalis* and *talinum triangulare*.

From the result the addition of the fertilizer resulted in increases in the biomass of the vegetables but to varying extents. At three doses applied (2.5 x 10⁸ cells/kg, 5.0 x 10⁸ cells/kg and 7.5 x 10⁸ cells/kg) respectively, *Rhizobium leguminosarum* fertilized *Telfairia occidentalis* plants recorded 166.5%, 235.9% and 356.3% whereas the *Talinum triangulare* counterparts recorded 107.9%, 173.7% and 265.9%. This result also show that the positive impact of the bio-fertilizer was higher in *Telfairia occidentalis* plant than in the *Talinum triangulare* plant. Furthermore, the bio-fertilizer had much more affect than the chemical fertilizer (NPK) except at high dosage (Table V, Fig. 4 and 5).

Table V. Effect of biofertilizers on biomass of vegetables *Telfairia occidentalis* and *Talinum triangulare* (%)

Treatment	<i>Telfaira occidentalis</i>	<i>Talinum triangulare</i>
Control	-	-
<i>Rhizobium leguminosarum</i>		
2.5	166.5%	107.9%
5.0	235.9%	173.7%
7.5	356.3%	265.9%
NPK		
1	52.0%	12.8%
2	182.2%	161.4%
3	376.1%	206.8%

This table shows that the positive impact of the bio-fertilizers was higher in *Telfairia occidentalis* plant than in the *Talinum triangulare* plant.



Fig. 4. *Telfairia occidentalis* after harvest, Treatment A and Treatment B (*Rhizobium*) and Treatment C (NPK)



Fig. 5. *Talinum triangulare* after harvest

Effect of the biofertilizer on the plant height of the test vegetables telfaira occidentalis and talinum triangulare.

In the *Rhizobium leguminosarum* fertilized vegetables increases of 13.53% and 36.35% were needed at low dose (2.5×10^8 cells/kg) in *Telfairia occidentalis* and *Talinum triangulare* respectively while increase of 27.67% and 46.57% and 36.64% and 55.05% were recorded for the respective plant at 5.0×10^8 cells/kg and 7.5×10^8 cells/kg respectively, the NPK fertilized *Telfairia occidentalis* increases by 22.23%, 65.60% and 168.53% at the three levels of application of the fertilizer these figures are lower than their biofertilized *Telfairia occidentalis* control parts except of the high close of application. Generally, therefore the result shows that there were variations in the levels of effect

Table VII. Effect of biofertilizer on the leaf area of the grown *Telfairia occidentalis* and *Talinum triangulare*

Treatment	<i>Telfairia occidentalis</i>	<i>Talinum triangulare</i>
Control	-	-
<i>Rhizobium leguminosarum</i>		
2.5	13.53%	36.35%
5.0	27.67%	46.57%
7.50	36.64%	55.05%
NPK		
2.50	22.23%	- 9.41
5.0	65.60%	- 1.67
7.50	168.53%	8.09%

The table shows that there were variations in the levels of effect of the biofertilizer on the apical growth of the vegetables.

of the biofertilizer on the apical growth of the vegetables. Not with standing, there were increases in lengths of the vegetables in all cases of fertilizers application (Table VI).

Effect of Biofertilizer on the Leaf Area of the Vegetables (Telfairia occidentalis and Talinum triangulare).

Increases were recorded for both plants and biofertilizer organism. At the three doses of application (2.5×10^8 cells/kg, 5.0×10^8 cells/kg and 7.5×10^8 cells/kg) the percentage increases were recorded with *Rhizobium leguminosarum* fertilized vegetables to the times of 23.29%, 117.17% and 164.27% for *Telfairia occidentalis* and 45.52%, 172.17%, and 207.01% for *Talinum triangulare*. This tables show that the biofertilizer had more impact on the leaf areas of *Talinum triangulare* than that of *Telfairia occidentalis*. The levels increase in the leaf area of the test vegetables compared quite favorably with those of NPK fertilized vegetables which recorded 48.73%, 121.33% and 187.57%, for *Telfairia occidentalis* and 8.90%, 15.86% and 222.16% for *Talinum triangulare* (Table VII, Fig. 6, 7 and 8).

Table VII. Effect of biofertilizer on the leaf area of the grown *Telfairia occidentalis* and *Talinum triangulare*

Treatment	<i>Telfairia occidentalis</i>	<i>Talinum triangulare</i>
Control	-	-
<i>Rhizobium leguminosarum</i>		
2.5	13.53%	36.35%
5.0	27.67%	46.57%
7.50	36.64%	55.05%
NPK		
2.50	22.23%	- 9.41
5.0	65.60%	- 1.67
7.50	168.53%	8.09%



Fig. 6. Field Experiment on *Talinum triangulare* growth without Biofertilizer and NPK (July, 2020)

The examination of test vegetables revealed the remarkable effectiveness of *Rhizobium leguminosarum* as a bio-fertilizer in enhancing their growth. These inoculated plants displayed significantly higher plant weight (biomass) compared to their



Fig. 7. Field experiment on *Talinium triangulare* growth with biofertilizer (July, 2020)



Fig. 8. Field experiment on *Talinium triangulare* growth with NPK (July, 2020)

unfertilized counterparts. Biomass is a crucial factor influencing crop growth and market value. This finding aligns with prior research by Joiner and Coyne and Goyal *et al.* (2021), highlighting the pivotal role of Rhizobia as crucial microorganisms for enhancing plant growth, particularly in legumes. Additionally, reported substantial increases in plant weight in broad beans in Egypt following *Rhizobium leguminosarum* inoculation.

Furthermore, the performance of the bio-fertilizer was notably competitive with chemical fertilizers. In all cases, a dose-dependent efficacy was observed, suggesting that further optimization of bio-fertilizer doses could potentially boost plant yields. The relatively superior performance of chemical fertilizers in terms of plant height may be attributed to their quick solubility and immediate availability, granting plants an early advantage in terms of fertilizer effects. This might explain why some researchers have suggested that, while organic fertilizers are valuable, they may not entirely replace the indispensable role of chemical fertilizers at present (Subba-Rao *et al.* 1999).

Leaves are a vital part of most vegetable crops, representing the primary edible portions (Sagar *et al.* 2018). *Telfairia occidentalis* and *Talinum triangulare* fall into the category of vegetables where leaves are the primary consumable parts (Ebabhi and Adebayo, 2022). The bio-fertilizers significantly impacted the leaf area of these vegetables, with dose-dependent effects observed in both test plants compared to the unfertilized controls. Although slightly less effective than chemical fertilizer in the case of *Talinum triangulare*, *Rhizobium leguminosarum*'s relative performance reached 93.18%. For *Telfairia occidentalis*, *Rhizobium leguminosarum* exhibited a notable 87.58% increase in leaf area. This finding, supports the work of El-Shimi (2022) on the effect of Biochar, Compost and bio-fertilizer on Pea Yield Then, Study its Residual Effect on the Subsequent Pepper Crop.

In light of the positive impacts recorded for *Rhizobium leguminosarum* as a bio-fertilizer, which encompass increased plant biomass, enhanced apical growth (plant length), and improved overall vegetation (leaf area), it is reasonable to categorize the two test organisms as part of the group known as plant growth-promoting bacteria (PGPB) (Bertrand *et al.*, 2000). This study underscores the potential of bio-fertilizers, such as *Rhizobium leguminosarum*, in contributing to sustainable and effective agricultural practices.

Conclusion

Based on the findings of this study, *Rhizobium leguminosarum* bio-fertilizer significantly improved plant growth, outperforming chemical fertilizers and offering a sustainable alternative for enhancing crop yield.

Recommendation

Based on the conclusive findings of this study, we strongly recommend the adoption of *Rhizobium leguminosarum* bio-fertilizer as a highly effective and sustainable option for promoting plant growth. This bio-fertilizer has demonstrated remarkable performance, surpassed chemical fertilizers, and stood as a promising solution to enhance crop yields while also contributing to environmentally-friendly agriculture.

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