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ARBUSCULAR MYCORRHIZAL AND RHIZOBIUM STRAIN TREATMENT ON GROWTH PERFORMANCE AND DEVELOPMENT OF PARKIA *BIGLOBOSA (JACQ.) BENTH*

NGELE, B. A.[1], EFFA, E. A[1], AGBA M. O[1*] AND NKANG, A. E[1].

1: Department of Plant and Ecological Studies, University of Calabar, Calabar, Nigeria

***Corresponding Author: AGBA M. O; E Mail: agbamary03@gmail.com**

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ABSTRACT

Parkia biglobosa (Jacq) Benth (African locust beans) is an important multipurpose tree legume in tropical and subtropical Africa, used for nutritional and medicinal purposes and valued particularly for its seeds. This study investigated the influence on growth performance of *Parkia biglobosa* by a tripartite symbiotic association between the tree legume, a bacterium (R*hizobium sp.)* and an arbuscular mycorrhizal fungus (*Glomus etunicatum).* Seeds of the test plant were subjected to four treatment combinations, namely; control (topsoil with no inoculations), topsoil pre-inoculated with arbuscular mycorrhizal fungus (*Glomus etunicatum*) and *Rhizobium* strain as separate treatments; and a composite treatment (topsoil pre-inoculated with a combination of *G. etunicatum* and *Rhizobium*). Measurements of harvested plants height, girth, dry weight, root: shoot ratio, net assimilation rate and relative growth rate were taken and calculated in triplicates as appropriate, bi-weekly, from the 8th to the 18th week after planting. Single inoculations with *G. etunicatum* and *Rhizobium* compared with the control in their performance at growth improvement. However, dual inoculation with *G. etunicatum* and *Rhizobium* significantly improved ($P \leq 0.05$) plant growth performance.

Keywords: Arbuscular Mycorrhizal Fungus, *Glomus etinicatum*, Rhizobium, *Parkia biglobosa*

INTRODUCTION

Parkia biglobosa is a leguminous, perennial and deciduous tree. They are found on a wide range of natural and semi-natural ecosystems, such as open savannah woodlands. Still, it is most common and abundant in anthropic communities, basically bush fallow and wooded farmland where cultivation is semi-permanent. This plant has found use in many areas, including food, fodder, apiculture, fuel, timber, fibre, gum, tannin, alcohol, poison, medicine, agroforestry, and soil improver.

Some microorganisms colonize plants in their natural environment. Some of these microorganisms, particularly beneficial bacteria and fungi, can improve plant performance under stress environments and consequently enhance yield. Arbuscular mycorrhizal fungi and rhizobia are two of the most important plant symbionts **[1]**. They play a key role in natural ecosystems and influence plant productivity, plant nutrition and plant resistance to stress conditions **[2]**. Arbuscular mycorrhizal fungi (AMF) are associated with the roots of over 80 % of terrestrial plant species **[3-4]**, including halophytes, hydrophytes and xerophytes.

Glomus is an arbuscular mycorrhizal fungus that penetrates its host plant's cell wall **[5]**. It is not known to be pathogenic but contributes to the fungal biomass dominance of soils. Within the cell wall, the fungus forms arbuscules or tree-like structures at the subcellular level. Like all arbuscular mycorrhizal fungi, Glomus is an obligate heterotroph; that is, it requires a host to obtain organic nutrients **[6]**. In return, the host plant acquires inorganic nutrients from the arbuscular mycorrhizal fungus. In this respect, *Glomus* plays an important role in the overall nutrient cycling of ecosystems.

Rhizobium is a **genus of Gram-negative bacteria that fixes nitrogen. They form an endo-symbiotic** relationship with certain plants, such as legumes, by colonizing plant cells within root nodules and converting atmospheric nitrogen into ammonia, which acts as a natural fertilizer. Plants, in turn, provide the bacteria with the organic compounds made by photosynthesis **[7]**.

It is well documented that arbuscular mycorrhizal fungi colonization and activity in leguminous plants is enhanced by rhizobium, resulting in better plant performance **[8-11]**. Arbuscular mycorrhizal fungi and *Rhizobium spp*. form an intimate association with leguminous plants, which is termed the "tripartite symbiosis". Plants benefit from this association in many ways, including enhanced plant growth, yield and

nutrient uptake, especially nitrogen and phosphorus **[12-13]**. Research reports have shown that plant benefits derived from this tripartite symbiosis are greater than that of plants inoculated with either arbuscular mycorrhizal fungi or Rhizobium alone **[8-11]**. Ngele **[14]** also reported that dual inoculation with *Glomus etunicatum* and *Rhizobium* enhanced tolerance to environmental stress in seedling of *Parkia biglobosa*. Therefore, this study aims to investigate the effect of arbuscular mycorrhizal fungi (*Glomus etunicatum*), *Rhizobium* strain and composite inoculation with arbuscular mycorrhizal fungi and Rhizobium on the growth and development of *the Parkia biglobosa* plant.

MATERIALS AND METHOD

Soil Preparation

Topsoil samples, sieved through a 0.5 x 0.5 cm wire mesh to remove large objects and root fragments, was used as the growth medium.

Seed Collection

Dried viable seeds of *P. biglobosa* were obtained from the Cross River State Forestry Commission Calabar, Cross River State, Nigeria.

Source of Fungus:

The arbuscular mycorrhizal fungus *G. etunicatum*was obtained from the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria.

Isolation and Culturing of Rhizobium

The culture media, Yeast Mannitol Agar, was used for the culturing of Rhizobium and was prepared as follows: 15 g of the agar power, 3 g of yeast extract, 5 g of peptone, and 5 g of mannitol was dissolved in 1 litre of distilled water. The mixture was first brought to the solution by boiling and stirring until all the suspension particles disappeared. The media was dispensed into 250 ml conical flasks and sterilized by autoclaving at 121^{o}C for 25 minutes.

Ten freshly collected nodules with roots from an established *Cajanuscajan* plant were washed thoroughly. The nodules were severed from the root by cutting the root about 0.5 cm on each side of the nodules using a pair of sterile scissors; 0.5 cm root appendages were left to enhance easy manipulation of the nodules with forceps. Nodules were immersed for 5-10 seconds in 95 % ethanol to break the surface tension and remove air bubbles from the tissue. They were then transferred to a 3 % solution of sodium hypochlorite and soaked for 4-6 minutes, after which they were removed and rinsed in five changes of distilled water, using sterile forceps for transferring. The nodules were finally transferred into a sterile

test tube and crushed in a drop of sterile water using a sterile glass rod. One loopful of the nodule suspension was streaked on each yeast mannitol agar plate. The plates were incubated at 26 ^{o} C in the dark under sterile conditions.

Daily observation was made for a week at the end of which isolated colonies were sub-cultured on yeast mannitol agar plates to ensure they were pure *Rhizobium* strains. After seven days of growth, a loopful of pure rhizobium culture was transferred with a sterile inoculating loop into 250 ml sterile yeast mannitol broth in each conical flask and incubated for 14 days in a rotary shaker (New Brunswick Scientific) in a controlled environment at 27 ^{o}C and 120 rpm. The rhizobium culture was stored in the refrigerator until used.

Seed Planting and Experimental Layout

This experiment was conducted at the Botanic Garden of the University of Calabar, Cross River State, Nigeria. Seventy-two polythene nursery bags, each measuring 39 X 49 cm, were filled with 6 kg of soil each and grouped into four experimental units, each containing 18 bags. These four experimental groups were designated thus; arbuscular mycorrhizal fungus inoculated (AMF), Rhizobium inoculated (Rhz), dual inoculation with arbuscular mycorrhizal fungus and Rhizobium (AMFRhz) and control (topsoil without inoculum). The soil in each bag was watered to field capacity and left to drain overnight before seeds were planted. The experiment was laid out in a complete randomized design (CRD) and replicated thrice.

Each nursery bag was sown with four seeds of *P. biglobosa,* and treatments were applied accordingly. AMF inoculation was carried out by placing the seeds on 50 g crude inocula of *G. etunicatum* consisting of spores, infected maize root fragments in the planting holes, and covered with soil. Inoculation with Rhizobium was done by applying 20 ml of *Rhizobium spp* suspension in yeast mannitol broth containing approximately 109 bacterial cells per ml, close to the sown seeds. The composite treatment was administered by inoculating a combination of AMF and Rhz. The seedlings were thinned to two per bag three weeks after germination. The subsequent growth period is referred to as "weeks after seedling establishment" (WAE).

Growth Measurements

Eight weeks after germination, three plants from each treatment group were randomly selected, thoroughly watered to loosen the soil and then harvested for growth measurements and analysis. Subsequent

harvests were done bi-weekly. Growth parameters used in the evaluation of the effect of treatments applied in this experiment are plant height, plant girth, dry weights root: shoot ratio, relative growth rate and net assimilation rate**.**

Relative growth rate was calculated after Hunt **[15]** as:

$$RGR = \frac{\log W_2 - \log W_1}{T_2 - T_1} \ gg^{-1}wk^{-1} \quad \textbf{Equation 1}$$

Where: W_1 = total dry weight at first harvest

W_2 = total plant dry weight at second harvest

$T_2 - T_1$ = the time interval between first and second harvest in weeks

Log_e = Natural Logarithm

Net assimilation rate (NAR): the Net Assimilation Rate was calculated as the change in total plant biomass per leaf weight and time **[16]**:

$$NAR = \frac{2(W_2 - W_1)}{(T_2 - T_1)(LDW_2 + LDW_1)} \ gg^{-1}wk^{-1} \quad \textbf{Equation 2}$$

Where LDW_1 = leaf dry weight at first harvest

LDW_2 = leaf dry weight at second harvest

RESULTS

The growth performance of *P. biglobosa*, evaluated by some growth parameters, was better in plants with composite inoculation with AMF and RHz and was particularly so in the 18th week after seedling establishment. The following results were obtained from this research:

Plant height

The influence of *G. etunicatum* and rhizobium inoculation on the height of *P. biglobosa* is presented in **Table 1**.

Inoculation with *G. etunicatum* alone (AMF) gave shorter plants that were comparable with the control plants at 8WAE. However, at 10, 12, 14, 16 and 18 WAE height was significantly lower ($P \leq 0.05$) than the control plants. Plants grown in Rhizobium treated soils (Rhz) were significantly shorter than those in the control ($P \leq 0.05$), particularly at the 8, 10 and 18 WAE. Treatment with a combination of *G. etunicatum* and Rhizobium (AMFRhz) produced plants with height significantly higher than the control 8, 10 and 12 WAE, comparable with the control plants at the 14 and 16 WAE, but significantly lower ($P \leq 0.05$) at the 18 WAE.

Plant Girth

The influence of *G. etunicatum* and rhizobium inoculation on the girth of *P. biglobosa* is presented in **Table 2**.

P. biglobosa plants' girth in AMF inoculated soil was significantly larger ($P \leq 0.05$) than the control plants at the 8WAE, but was significantly lower ($P \leq 0.05$) in subsequent

weeks. Plants from the Rhz treatment had significantly larger girths ($P \leq 0.05$) than the control at 8 WAE, significantly lower ($P \leq 0.05$) at the 10, 12 and 18 WAE, while at the 14 and 16 WAE girth was comparable with the control plants. Plants grown in AMFRhz inoculated soil had a significantly larger girth ($P \leq 0.05$) than the control at 8 WAE, significantly lower at the 10 and 12 WAE, comparable at the 14 and 16 WAE, but significantly larger than the control at the 18th week after planting.

Dry weight

The influence of arbuscular mycorrhizal fungi and rhizobium inoculation on the dry weight of *Parkia biglobosa*is presented in **Table 3**.

The dry weight of plants grown in AMF inoculated soil was significantly lower ($P \leq 0.05$) than that of the control plants from the 8th to the 18th WAE. Rhizobium treatment produced plants with total dry weight significantly lower ($P \leq 0.05$) than those in the control at the 10th, 12th and 14th WAE, but not significantly different at the 16th and 18th WAE. Plants grown in AMFRHz inoculated soil had dry weight comparable with the control at the 8th, 10th, 12th, 14th and 16th WAE, but was significantly higher ($P \leq 0.05$) at the 18th WAE.

Root: Shoot Ratio

The influence *of G. etunicatum* and rhizobium inoculation on the root: shoot ratio of *P. biglobosa* is presented in **Table 4**.

The root: shoot ratio of plants grown in AMF inoculated soil was comparable with the control plants but was only significantly lower ($P \leq 0.05$) at the 10th WAE. Plants grown in Rhz inoculated soil had a root: shoot ratio comparable with the ones in the control at the 8th, 10th, 14th and 16th WAE, but was significantly lower ($P \leq 0.05$) at 10th WAE, and significantly higher at the 18th WAE. The root: shoot ratio of plants from AMFRhz treated soil was slightly higher than the control plants at the 8th and 10th WAE. However, these values were comparable with the control at the 12th, 14th and 16th WAE, but significantly higher at the 18th WAE.

Net Assimilation Rate

The influence of *G. etunicatum* and rhizobium inoculation on the net assimilation rate of *P. biglobosa* is presented in **Table 5**.

Plants grown in AMF inoculated soil had a significantly higher ($P \leq 0.05$) net assimilation rate than the control at the 16th WAE, significantly lower ($P \leq 0.05$) at the 12th WAE, but comparable at other weeks. The net assimilation rate of plants from the Rhz treated soil was significantly higher than the control at the 8th week but was comparable with the control in subsequent

weeks. AMFRhz inoculation produced plants with net assimilation rates comparable with the control plants but only significantly higher ($P \leq 0.05$) at the 16th WAE.

Relative Growth Rate

The influence of *G. etunicatum* and rhizobium inoculation on the relative growth rate of *P. biglobosa* under salt stress is presented in **Table 6**.

Plants grown in AMF inoculated soil had a relative growth rate similar to that recorded for the control plants. The relative growth rate of plants grown in Rhz inoculated soil was also comparable with the control plants but was only significantly higher ($P \leq 0.05$) at 16 WAE. The relative growth rate of plants grown in AMFRhz inoculated soil was similar to the control plants but was only significantly higher ($P \leq 0.05$) at the 18th WAE.

Table 1: Influence of *Glomus etunicatum* and *Rhizobium* inoculation on height (cm) of *Parkia biglobosa*):

TREATMENT	8 WAE	10 WAE	12 WAE	14 WAE	16WAE	18WAE
CONTROL	*13.40^{b}±0.10	25.83^{b}±4.0	26.50^{b} ±1.50	30.33^{a}±0.76	33.58^{ab}±3.92	46.00^{a}±1.00
AMF	12.50^{b} ±2.50	16.43^{d} ±2.22	20.10^{c}±2.53	21.50^{b}±1.32	29.66^{b}±1.52	32.33^{c}±3.05
RHz	9.66^{c} ±1.52	20.40^{c} ±0.90	24.25^{bc}±1.95	27.66^{a}±1.52	29.91^{b}±2.32	39.16^{b}±4.93
AMFRHz	17.40^{a} ±0.40	29.08^{a}±3.57	30.08^{a}±1.46	32.50^{a} ±2.50	38.33^{a}±2.92	40.00^{b}±1.00

*** Means of three replicates ± SEM. According to Duncan Multiple Range Test, means within each column followed by different letters are significantly different at P ≤ 0.05. WAE: weeks after establishment; AMF: arbuscular mycorrhizal fungi; RHz: Rhizobium; AMFRHz: arbuscular mycorrhizal fungi + Rhizobium.**

Table 2: Influence of *Glomus etunicatum* and *Rhizobium* inoculation on Girth (cm) of *Parkia biglobosa*

TREATMENT	8 WAE	10 WAE	12 WAE	14 WAE	16WAE	18WAE
CONTROL	*2.34^{c}±0.13	4.73^{a}±0.21	5.94^{a} ±0.37	5.60^{a}±1.13	5.81^{a}±0.16	9.12^{b}±0.22
AMF	2.54^{b} ±0.08	3.31^{d} ±0.27	3.09^{c}±0.04	3.45^{b}±0.62	4.56^{b}±0.32	5.71^{d}±0.87
RHz	2.26^{b} ±0.04	3.81^{c} ±0.18	3.82^{c}±0.39	5.00^{a}±0.23	6.45^{a}±1.03	7.15^{c}±0.19
AMFRHz	4.66^{a} ±0.04	4.36^{b}±0.04	4.96^{b}±0.65	5.49^{a} ±0.34	6.20^{a}±0.49	10.18^{a}±0.25

*** Means of three replicates ± SEM. According to Duncan Multiple Range Test, means within each column followed by different letters are significantly different at P ≤ 0.05. WAE: weeks after establishment; AMF: arbuscular mycorrhizal fungi; RHz: Rhizobium; AMFRHz: arbuscular mycorrhizal fungi + Rhizobium**

Table 3: Influence of *Glomus etunicatum* and *Rhizobium* inoculation on the total dry weight of *Parkia biglobosa* (g)

TREATMENT	8 WAE	10 WAE	12 WAE	14 WAE	16 WAE	18 WAE
CONTROL	*0.72^{ab}±0.18	3.25^{a}±0.65	6.22^{a}±1.41	7.35^{a}±1.14	11.41^{a}±0.40	18.03^{b}±0.96
AMF	0.21^{b}±0.06	0.86^{b}±0.27	1.09^{c}±0.25	1.41^{c}±0.25	2.43^{b}±0.50	4.43^{c}±0.82
RHz	0.17^{b}±0.04	1.15^{b}±0.26	1.90^{bc}±0.64	4.31^{b}±0.39	11.67^{a}±1.84	15.88^{b}±2.86
AMFRHz	1.25^{a}±0.36	2.92^{a}±0.74	4.47^{ab}±0.58	6.86^{a}±0.81	11.97^{a}±1.34	25.91^{a}±2.99

*** Means of three replicates ± SEM. According to Duncan Multiple Range Test, means within each column followed by different letters are significantly different at P ≤ 0.05. WAE: weeks after establishment; AMF: arbuscular mycorrhizal fungi; RHz: Rhizobium; AMFRHz: arbuscular mycorrhizal fungi + Rhizobium**

Table 4: Influence of *Glomus etunicatum* and *Rhizobium* inoculation on the root: shoot ratio of *Parkia biglobosa*:

TREATMENT	8 WAP	10 WAP	12 WAP	14 WAP	16 WAP	18 WAP
CONTROL	*0.23^{b}±0.02	0.22^{c}±0.05	0.8^{a}±0.32	0.63^{a}±0.05	1.00^{a}±0.14	0.73^{b}±0.08
AMF	0.24^{ab}±0.03	0.61^{a}±0.14	0.44^{ab}±0.08	0.71^{a}±0.29	1.22^{a}±0.26	0.80^{b}±0.06
RHz	0.27^{ab}±0.02	0.37^{bc}±0.11	0.37^{c}±0.09	0.43^{a}±0.08	1.16^{a}±0.17	1.09^{a}±0.12
AMFRHz	0.39^{a}±0.15	0.45^{ab}±0.12	0.47^{ab}±0.07	0.65^{a}±0.04	0.97^{a}±0.04	1.23^{a}±0.12

*** Means of three replicates ± SEM. According to Duncan Multiple Range Test, means within each column followed by different letters are significantly different at P ≤ 0.05. WAE: weeks after establishment; AMF: arbuscular mycorrhizal fungi; RHz: Rhizobium; AMFRHz: arbuscular mycorrhizal fungi + Rhizobium**

Table 5: Influence of *Glomus etunicatum* and *Rhizobium* inoculation on the net assimilation rate of *Parkia biglobosa* (gg^{-1} wk^{-1}):

TREATMENT	10 WAE	12 WAE	14 WAE	16 WAE	18 WAE
CONTROL	1.01^{b}±0.20	0.69^{a}±0.06	0.33^{a}±0.48	0.33^{d}±0.24	1.17^{a}±0.59
AMF	1.20^{b}±0.06	0.30^{b}±0.20	0.39^{a}±0.21	1.04^{bc}±0.31	0.92^{a}±0.25
RHz	1.60^{a}±0.32	0.39^{ab}±0.23	0.48^{a}±0.27	1.27^{a}±0.12	0.68^{a}±0.11
AMFRHz	0.85^{b}±0.01	0.46^{ab}±0.22	0.50^{a}±0.15	0.81^{c}±0.06	1.40^{a}±0.33

*** Means of three replicates ± SEM. According to Duncan Multiple Range Test, means within each column followed by different letters are significantly different at P ≤ 0.05. WAE: weeks after establishment; AMF: arbuscular mycorrhizal fungi; RHz: Rhizobium; AMFRHz: arbuscular mycorrhizal fungi + Rhizobium**

Table 6: Influence of *Glomus etunicatum* and *Rhizobium* inoculation on the relative growth rate of *Parkia biglobosa* (gg^{-1} wk^{-1}):

TREATMENT	10 WAE	12 WAE	14 WAE	16 WAE	18 WAE
CONTROL	0.33^{ab}±0.03	0.13^{a}±0.01	0.06^{a}±0.09	0.10^{b}±0.05	0.10^{b}±0.01
AMF	0.29^{b}±0.03	0.06^{a}±0.03	0.06^{a}±0.02	0.14^{b}±0.03	0.13^{b}±0.03
RHz	0.41^{a}±0.07	0.09^{a}±0.05	0.20^{a}±0.11	0.21^{a}±0.02	0.09^{b}±0.02
AMFRHz	0.18^{c}±0.01	0.10^{a}±0.05	0.09^{a}±0.03	0.12^{b}±0.01	0.16^{a}±0.03

*** Means of three replicates ± SEM. According to Duncan Multiple Range Test, means within each column followed by different letters are significantly different at P ≤ 0.05. WAE: weeks after establishment; AMF: arbuscular mycorrhizal fungi; RHz: Rhizobium; AMFRHz: arbuscular mycorrhizal fungi + Rhizobium**

DISCUSSION

There was enhanced growth in *Parkia biglobosa* plants grown in soil inoculated with *G. etunicatum* and Rhizobium. The significantly shorter plants grown in either *G. etunicatum* inoculated soil or rhizobium inoculated soil are possibly legumes that form a tripartite symbiotic association with arbuscular mycorrhizal fungi and Rhizobium; thus, *P. biglobosa* could not thrive well without both symbionts. Tanja and Marcel **[17]** reported that many legumes form tripartite symbiotic associations with rhizobia and arbuscular mycorrhizal fungi and may not thrive very well in the absence of any of the symbionts. The test plants' heights were significantly improved in soil with both symbionts (AMFRhz), particularly at 8, 10 and 12 weeks after growth. This improved plant height suggests that the enhanced growth vigourwas promoted by both symbionts' synergistic action in transferring nutrients to the test plant. Van der Heijden*et al*. **[18]** also reported that the dual symbiosis with arbuscular mycorrhizal fungi and rhizobia is necessary for legume growth

within plant communities. However, the higher length observed in the control plants at 18 WAP over the AMFRhz plants could be due to branching and vegetative growth in the AMFRhz plants, evidenced by the higher dry matter accumulation recorded for these plants.

Plants grown in soil with dual inoculation with *G. etunicatum* and Rhizobium had the highest girth. The enlargement in girth could also be due to increased plant productivity by the synergistic interactions among the tripartite symbiotic association components. The girth was significantly lower in Plants inoculated separately with AMF and Rhz. The decline in performance may be attributed to the absence of synergy, as only a single symbiont was present **[17]**.

Xavier and Germida **[19]** reported that co-inoculation with both symbionts (arbuscular mycorrhizal fungi and Rhizobium) resulted in enhanced plant in pot and field experiments biomass and better nutrient acquisition. The tripartite association is beneficial to plants in various ways, including improved plant growth, yield and nutrient content **[13, 12]**. In the present study, the root, stem, leaf and total dry weight of plants grown in soils treated to *G. etunicatum* and Rhizobium had higher values when compared with values recorded for plants from other treatments, including the control plants.

Paul and Kucey **[20]** reported that sixty per cent of photosynthetic carbon flux is partitioned into the below-ground root nodule- mycorrhizal association. The root: shoot ratios of the test plants with both symbionts (AMFRHZ) were higher than that obtained from the control plants and plants from other treatments. The higher net assimilation rate recorded in plants grown in AMFRhz inoculated soils explains the plants' increased growth and biomass. The significantly higher relative growth rate in the AMFRhz inoculated plants in this study indicates improved nutrient acquisition brought about by the synergistic action and the expansive soil coverage by the symbionts' symbionts (*G. etunicatum* and *Rhizobium*).

P. biglobosa plants grown in topsoil pre-inoculated with both symbionts (*Glomus etunicatum* and Rhizobium) had better growth, biomass and performed better in all the parameters measured.

CONCLUSION

Composite inoculation with *G. etunicatum* and rhizobium, as evidenced in the finding from this research, enhanced growth in *Parkia biglobosa*. However, more studies of an extended duration are required to further assess the enhancement in growth under field

conditions. This study has practical implications in the development of techniques to improve growth in legumes and other plants.

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