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# Impacts of Plant Growth Promoting Rhizobacteria Inoculation Configurations on Vegetative Growth, Nodulation and Yield of Bambara groundnut [*Vigna subterranea* (L.) Verdc.]

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***Abstract—*** *The main objective of this study is determine the responses of Bambara groundnut [Vigna subterranea (L). Verdc.] to application methods of plant growth promoting rhizobacteria inoculant and time of sowing after inoculation in Agbani area of Enugu, South East Nigeria. Field trials were conducted at the Teaching and Research Farm of the Faculty of Agriculture and Natural Resources management, Enugu State University of Science and Technology in 2015 and 2016 planting season. The experiment was a 2 × 3 factorial trial in a randomized complete block design with three replications. The treatments consisted of two different rhizobacteria inoculant application methods (seed applied method and soil applied method) and three different periods of sowing after inoculation (0 min, 15 mins and 30 mins) in all possible combinations (six treatment The obtained results revealed that Bambara groundnut cultivated on soils fertilized with ten grams of rhizobacteria inoculant per planting hole significantly ($p < 0.05$) had highest vegetative growth, number of nodules per plant and yield traits in both planting season. The main effect of time of sowing indicated that plants sown 30 minutes after inoculation significantly ($p < 0.05$) gave the tallest plants and leaf area index per plant in 2015 and 2016 planting season than the other time regime. The interaction effect of rhizobacteria inoculant application methods and time of sowing after inoculation were observed to be significant ($p < 0.05$) in 2015 and 2016 planting season. The results showed that the plants on soil with combined use of soil applied method of inoculation treatment and 30 minutes after inocualtion before planting significantly ($p < 0.05$) had the highest vegetative growth, number of nodules per plant and yield parameters than the other interaction effect. The combined use of soil applied method of inoculation treatment and 30 minutes after inocualtion before planting is recommended for the cultivation of Bambara groundnut in Agbani Area South East Nigeria.*

***Keywords— Bambara groundnut, growth, nodulation, phosphate fertilizer, rhizobacteria inoculant, yield.***

## I. INTRODUCTION

Bambara groundnut [*Vignasubterranea*(L.) Verdc.] is a major source of dietary protein and essential component of the cropping systems in the semi-arid regions of sub-Saharan Africa. Bambara nuts contain 63 % carbohydrates, 19 % protein and 6.5 % oil (Goli, 1997; Ikenganyia *et al*., 2017). The high protein content is useful in combating protein deficiencies in human and animal nutrition (Massawe *et al*., 2002). KariKari *et al*. (1997) characterized its micro nutrient content per 100 mg as calcium (95.5 - 99.0 mg), iron (5.1 - 9.0 mg), potasuim (11.45 – 14.36 mg) and sodium (2.9 – 10.6 mg). Grain yield of Bambara groundnut varies accross countries in African. Nigeria is the highest producer of Bambara groundnut with a production capacity that ranged from 500 -2600 kg (Begemann, 1987). Chad and Cameroon produces 800kg $ha^{-1}$, Benin 450-650kg$ha^{-1}$Congo 350-650 kg $ha^{-1}$ Ghana 714-1100kg $ha^{-1}$ (Karikari and Lavoe, 1977; Linnemann,1987). However, production of Bambara groundnut is challenged by poor crop establishment, inappropriate planting depth and plant spacing, use of unimproved seeds, low soil fertility and lack of effective

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nodulation technology (Ikenganyia *et al*., 2017). *Rhizobium* inoculation is a major agronomic factor that can sustainably influence the yield potential and economic returns of Bambara groundnut. Legume has the capacity to symbiotically fix nitrogen to the soil through the mechanism of biological nitrogen fixation. The greatest success in terms of modified agricultural practices arising from scientific research in biological nitrogen fixation (BNF) has been the development of rhizobial inoculants (Giller and Cadisch, 1995).

Bacterial inoculation to enhance the productivity of different crops is being practiced since the discovery of beneficial effects of plant growth promoting rhizobacteria. The methods used for augmentation of the beneficial plant growth promoting rhizobacteria contributes mainly to the survival efficiency of the bacteria in the soil and on the seeds. Most common methods developed and explored include seed treatment, soil amendment and roots dipping in the bacterial suspensions before transplanting particularly in rice (Mahmood *et al*., 2016; Ikenganyia *et al*., 2017). Other uncommon methods include foliar spray or application of bacteria through drip irrigation (Podile and Kishore, 2006). PGPR are applied to the soil or seeds and/or to the plant parts when there is risk of inhibitors or antagonistic microbes on the plant tissues (Gindrat, 1979). Inoculation methods are yet to be explored as there is scarce information available regarding the delivery and application of bacteria to the soil or the seeds (Mahmood *et al*., 2016). However, it is quite clear that population of the bacteria in soil is mainly dependent on initial stack of inoculums on the seed (Milus and Rothrock, 1993). Hebbar*et al*. (1992) stated that application of more inoculums per seed can increase the efficiency but results are not always steady. Bacteria need to compete with other microbes to colonize the soil rhizosphere so introduced bacteria should be competitive enough to efficiently compete and colonize the roots. The main objective of this study is to determine the effect of application methods of rhizobacteria inoculant and time of sowing after inoculation on the vegetative growth, nodulation and yield of Bambara groundnut [*Vigna subterranea* (L). Verdc.] in Agbani area of Enugu South East

## II. MATERIALS AND METHODS

### Description of the study area

Field trials were conducted at the Teaching and Research Farm of the Faculty of Agriculture and Natural Resources Management, Enugu State University of Science and Technology, Agbani. in 2015 and 2016 planting season. The site is located on latitude $06^0$52' North, longitude $07^0$15' East and altitude 450 meters above sea level (Anikwe *et al*., 2017). The area has an annual rainfall which ranges from 1700 – 2010 mm. The rainfall pattern is bimodal and is between April and October, and the dry season is between November and March. The soil's textural class is sandy loam with an isohyperthermic soil temperature regime (Anikwe *et al*., 2017) and is classified as Typic Paleudults of the order Utisol (Anikwe *et al*., 2016; Ikenganyia *et al*., 2014 ).

### Soil sample collection and analyses

Soil samples were collected from the top soil at a depth of 0 to 15 cm before planting and at four and eight weeks after planting for the three experiments in 2015 and 2016 planting season respectively. Three representative soil samples were randomly collected per plot and bulked to form a composite soil sample for each plot. A total of thirty-six composite soil samples were collected. Samples were air dried, ground and passed through a sieve of 2 mm standard mesh size. The soil pH was determined with a pH meter using 1:2.5 soil to water ratio and 1: 2.5 soil to 0.1 N KCl (potassium chloride) suspension according to Page *et al*., (1982). Organic carbon was determined using the Walkley and Black wet digestion method Bremmer and Mulvane, 1982. Soil organic matter content was obtained by multiplying the value of organic carbon by 1.724 (Van Bemmeler factor). Total nitrogen was determined before the applications of treatments, four and eight weeks after planting by Micro-kjeldahl procedure (Page *et al*., 1982). Available phosphorus was determined before the applications of treatments, four and eight weeks after planting by extraction with Bray II extractant as described by Bray and Kurz 1945 and determined colorimeterically using ascorbic acid method (Murphy and Riley, 1962). Exchangeable potassium was determined before the applications of treatments, four and eight weeks after planting by extraction using 1 ammonium acetate ($NH_4OAC$) solution and determined by the flame emission spectroscopy as outlined by Anderson and Ingram, 1993. Aluminium and hydrogen content (exchangeable acidity) was determined by titrimetric method after extraction with 1.0 N KCl (Mclean, 1982). The cation exchange capacity was determined by $NH_4OAC$ displacement method (Rhoades, 1982). Calcium and magnesium was determined by the complexiometric titration method as described by Chapman (1982). Particle size distribution analysis was done by the hydrometer method (Gee and Bauder, 2002) and the corresponding textual class determined from the United State Department of Agriculture Soil Texture Triangle. Base saturation was determined by the method outlined by Page *et al*., (1982).

### Experimental design

The experiment was a 2 × 3 factorial trial in a randomized

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complete block design with three replications. The treatments consisted of two different rhizobacteria inoculant application methods (seed applied method and soil applied method) and three different periods of sowing after inoculation (0 min, 15 mins and 30 mins) in all possible combinations (six treatment combinations) (Table 1).

**Preparation of inoculant slurry**

**Materials.**

500 ml of bottled water can wooden stirring spoon plastic basin, 3 liter capacity Rhizobia inoculant (Nodumax legume inoculant manufactured by IITA business incubation platform) Bambara groundunt seeds (Cultivar TVSU 1061 from IITA Ibadan Nigeria)white paper

**Seed inoculation methods**

The enclosed gum arabic (sticker) inside the sachet of Nodumax inoculant was dissolved into 300 ml of warm water to form a slurry, Healthy Bambara groundnut seeds (Cultivar TVSU 1061 from IITA Ibadan Nigeria) were selected, washed with 95% ethanol, surface sterilized with 3.5% sodium hypochlorite (commercial bleach) and then rinsed with distilled water before inoculation.15 kg of the sterilized seeds were poured into a basin, the slurry formed was added to the seeds and mixed uniformly, 100 g of nodumax inoculant were added uniformly to the seeds and covered for 10 minutes to avoid direct sunlight. The inoculated Bambara groundnut seeds were planted according to the time specified as the treatment into a moist seed bed.

**Soil inoculation methods**

Planting holes were made to a depth of 10 cm at a spacing of 20 cm × 45 cm (intra × inter row spacing) on each seed bed. Inside the planting hole, ten grams of rhizobacteria inoculant were applied followed by the un-inoculated Bambara groundnut seeds and then covered with the soil.

**Field operations**

A total land area measuring 190 $m^2$ (19 m x 10 m) was used for the experiment. The land was divided into three blocks (columns: north-south direction), and each was sub-divided into six plots (rows: east-west direction) making a total of eighteen plots (Figure 1). Plots (beds) measuring 2 m x 2 m (4 $m^2$) were separated by 1 m x 1 m pathway between and in between plots. Planting was done at the rate of two seeds per hole and thinned to one plant at two weeks after planting. Prophylactic application of 15 ml of Karate (Pyrethroid insecticide) in five liters of water was applied at one, four, six and eight weeks after planting to avert pest incidence. Three plants at the center rows were sampled during data collection. Weeding was done using traditional hoe.

**Data collection**

Plant height was determined at four and eight weeks after planting by measuring the length of the plant from the soil level to the tip of the topmost leaf using a measuring tape. Leaf area per plant was estimated as leaf length (L) x width (W) x 0.85 as described by Blanco and Folegatti (2003). Leaf area index per plant was determined at four and eight weeks after planting as total leaf area per plant divided by the feeding area available for the plant (inter row spacing multiplied by intra row spacing of each plant). Number of nodules per plant and number of leaves per plant were obtained by visual counting of the nodules at the roots of uprooted Bambara groundnut and fully expanded leaves respectively. Number of fresh pods per plant were recorded at harvest obtained by visual counting of fresh pods. Weight per plant of fresh pod per plant was recorded at harvest using electronic weighing balance. In determining the number of nodules and fresh pods, a spade was used to carefully scoop out the soil containing the plant roots. The soil with the roots was then immersed in a basin of water to remove the soil, the roots were recovered and the nodules counted manually. Sampled plants were separated into leaves, stems and roots and put in a paper envelope and oven dried at 80°Celsius to a constant weight for three days for the dry matter determination of leaf, stem and root at harvest.

**Data analyses**

Data collected was subjected to analysis of variance (ANOVA) test for randomized complete block design as outlined by Gomez and Gomez 1984 (Table 2). Significant means were separated using Fisher's least significant difference (F-LSD) at 5% probability level. Statistical analysis was executed using GENSTAT (2007) Statistical Software

**Linear model used for the analysis of variance is shown below (Gomez and Gomez 1984).**

$$Xij_k = \mu + A_i + B_j + R_k + (AB)_{ij} + €ij_k.$$

Where:

$Xij_k$ = Any individual observation on the experiment unit.

$\mu$ = population or general mean

$A_i$ = Effects of inoculant application method

$B_j$ = Effects of time of sowing

$R_k$ = Effects of blocking

$(AB)_{ij}$ = Effects of inoculant application method and time of sowing interaction

€ijk = Experimental error.

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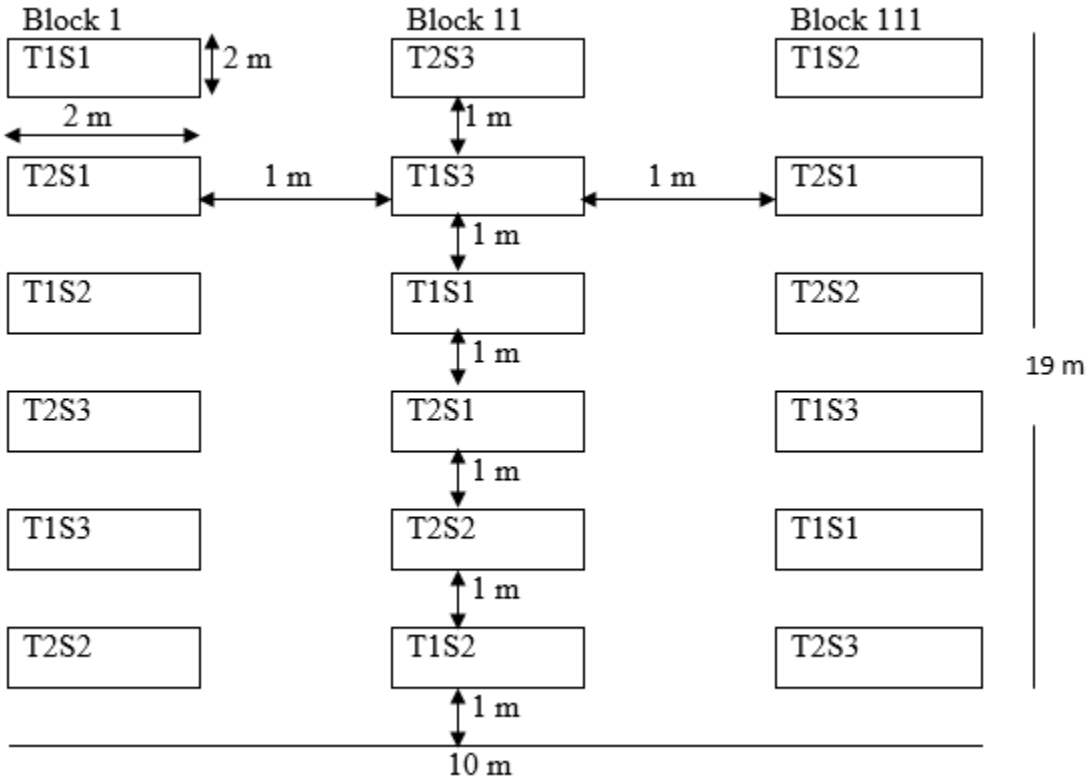
*Table.1: Treatment combinations of two application methods of rhizobacteria inoculant and three levels of time of sowing after inoculation in a randomized complete block design*

| | | *Application Method (T)* | |
|---|---|---|---|
| | | T1 | T2 |
| ***Time of sowing (S)*** | S1 | T1S1 | T2S1 |
| | S2 | T1S2 | T2S2 |
| | S3 | T1S3 | T2S3 |

*T1 - Soil applied method, T2 - Seed applied method, S1 - 0 min, S2 - 15 mins, S3 - 30 mins*




T1 - Soil applied method, T2 - Seed applied method, S1 - 0 min, S2 - 15 mins, S3 - 30 mins

*Fig.1: Schematic illustration of the field layout design of the experiment*

*Table 2. Outline of analysis of variance of a 2 × 3 factorial trial in a randomized complete block design*

| ***Sources of variation*** | Degree of freedom | Degree of freedom specified |
|---|---|---|
| ***Block (R)*** | (R-1) | 2 |
| ***Application method (T)*** | (T-1) | 1 |
| ***Time of sowing (S)*** | (S-1) | 2 |
| ***Interaction (TS)*** | (T-1)(S-1) | 2 |
| ***Error*** | (TS-1)(R-1) | 10 |
| ***Total*** | (TSR-1) | 17 |

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## III. RESULTS

**Initial soil characteristics of the study area before planting in 2015 and 2016 cropping season**

The pre-planting analyses of soil properties in both years are presented in the Table 3. The result indicated that the textural class of the study site is loamy sand, which contained 46% (2015) and 47% (2016) coarse sand, 41% (2015) and 40% (2016) fine sand, 5% (2015) and 5% (2016) clay and 8% (2015) and 8% (2016) silt. Percentage organic carbon was found to be 0.65% (2015) and 0.72% (2016) and total nitrogen contents were 0.038% (2015) and 0.041% (2016). The low values observed were below the critical levels for the study area. However, these values were higher in 2016 planting season than in 2015 planting season. As expected, this indicated that the soils had higher values for nutrient in 2016 when compared to 2015 planting season because of the influence of the legumes planted in the previous season. The soil pH in KCl ranged from 5.4 to 5.7 and 6.1 to 6.3 in water respectively in both years indicating slight acidity according to the rating by Landon (1991). The pre-planting exchangeable cations content and exchangeable acidity of the soil in 2015 were ($Mg^{2+}$ 0.72 c mol $kg^{-1}$, $K^{+}$ 0.17 c mol $kg^{-1}$, $Na^{+}$ 0.09 c mol $kg^{-1}$, $Ca^{2+}$ 1.2 c mol $kg^{-1}$, $Al^{3+}$ c mol $kg^{-1}$ 3.24 c mol $kg^{-1}$, $H^{+}$ 2.66 c mol $kg^{-1}$ and CEC 8.1 c mol $kg^{-1}$) whereas the values obtained in 2016 were ($Mg^{2+}$ 0.90 c mol $kg^{-1}$, $K^{+}$ 0.10 c mol $kg^{-1}$, $Na^{+}$ 0.17 c mol $kg^{-1}$, $Ca^{2+}$ 1.6 c mol $kg^{-1}$, $Al^{3+}$ 3.06 c mol $kg^{-1}$, $H^{+}$ 2.40 c mol $kg^{-1}$ and CEC 8.90 c mol $kg^{-1}$). The values obtained were low in the two years. Available phosphorus (Bray 11) was found to be 7.91 c mol $kg^{-1}$ (2015) and 8.82 c mol $kg^{-1}$ (2016). Bulk density of soil samples varied from a value of 1.57 mg $m^{-3}$ in 2015 to a lower value of 1.52 mg $m^{-3}$ in 2016. Total porosity of soil indicated that the soil had a total pore space of 41% in 2015 and 43% in 2016 planting season. The saturated hydraulic conductivity of the soil ranged from 21.72 cm $hr^{-1}$in 2015 to 22. 00 cm $hr^{-1}$ in 2016

*Table.3: Initial soil characteristics of the study area before planting in 2015 and 2016 cropping season*

| Parameters | 2015 | 2016 |
|---|---|---|
| **Particle size distribution (%)** | | |
| Coarse sand | 46 | 47 |
| Fine sand | 41 | 40 |
| Clay | 5 | 5 |
| Silt | 8 | 8 |
| Textural class | Loamy sand | Loamy sand |
| Bulk Density (mg $m^{-3}$) | 1.57 | 1.52 |
| Total Porosity (%) | 41 | 43 |
| Saturated Hydraulic Conductivity (cm $hr^{-1}$) | 21.72 | 22.00 |
| pH (water) | 6.1 | 6.3 |
| pH (KCl) | 5.4 | 5.7 |
| Organic carbon (%) | 0.65 | 0.72 |
| Total nitrogen (%) | 0.038 | 0.041 |
| Available phosphorus (c mol $kg^{-1}$) | 7.91 | 8.82 |
| **Exchangeable bases (c mol $kg^{-1}$)** | | |
| Calcium | 1.2 | 1.6 |
| Magnesium | 0.72 | 0.90 |
| Potassium | 0.17 | 0.10 |
| Sodium | 0.90 | 0.17 |
| **Exchangeable acidity (c mol $kg^{-1}$)** | | |
| Hydrogen | 2.66 | 2.40 |
| Aluminum | 3.24 | 3.06 |
| Cation exchangeable capacity (c mol $kg^{-1}$) | 8.10 | 8.90 |

**Effect of rhizobacteria inoculant application methods and time of sowing after inoculation on plant height and number of leaves per plant of Bambara groundnut [*Vignasubterranea*(L.) Verdc.] at four and eight weeks after planting in 2015 and 2016 planting season**

The results presented in Table 4 showed that the main effect of rhizobacteria inoculant application methods on

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plant height of Bambara groundnut were significant ($p < 0.05$) at four and eight weeks after planting in 2015 and 2016 planting season. Bambara groundnut cultivated on soils fertilized with ten grams of rhizobacteria inoculant per planting hole significantly ($p < 0.05$) produced the tallest plants at four weeks after planting (19.02 cm in 2015 and 22.96 cm in 2016) and eight weeks after planting (24.29 cm in 2015 and 26.29 cm in 2016) than seed applied method. The main effect of time of sowing after inoculation on plant height of Bambara groundnut were also significant ($p < 0.05$). The results showed that Bambara groundnut planted 30 minutes after inoculation significantly ($p < 0.05$) gave the tallest plants which varied from 17.70 cm (2015) to 21.56 cm (2016) at four weeks after planting and 27.30 cm (2015) to 23.60 cm (2016) at eight weeks after planting than the other time of sowing after of inoculation.Consequently, the interaction effect of rhizobacteria inoculant application methods and time of sowing after inoculation on plant height of Bambara groundnut were observed to be non- significant ($p > 0.05$) at four and eight weeks after planting in both planting season.

Furthermore, Table 5 revealed that there was a non-significant ($p > 0.05$) effect of rhizobacteria inoculant application methods at four weeks after planting in 2015 but at eight weeks after planting, plants sown on plots treated with ten grams of rhizobacteria inoculant per planting hole significantly ($p < 0.05$) had more number of leaves per plant which ranged from 44 to 45 in 2015 and 2016 planting season respectively than the non-inoculated plots (seed applied method). Similarly, the main effect of time of sowing after inoculation and the interaction effect of rhizobacteria inoculant application methods and time of sowing after inoculation on number of leaves per plant of Bambara groundnut were observed to be non- significant ($p > 0.05$) at four and eight weeks after planting in 2015 and 2016 planting season respectively.

*Table.4: Effect of rhizobacteria inoculant application methods and time of sowing after inoculation on plant height (cm) of Bambara groundnut [Vigna subterranea* (L.) Verdc.] *at four and eight weeks after planting in 2015 and 2016 planting season*

| | **2015** | | | | | |
|---|---|---|---|---|---|---|
| | **Rhizobacteria inoculant application methods (T)** | | | | | |
| **Time of sowing (Minutes) (S)** | **4WAP** | | | **8WAP** | | |
| | **Soil applied method** | **Seed applied method** | **Mean (S)** | **Soil applied method** | **Seed applied method** | **Mean (S)** |
| 0 | 17.23 | 10.50 | 13.87 | 22.63 | 24.63 | 23.63 |
| 15 | 18.97 | 12.67 | 15.82 | 24.43 | 26.43 | 25.43 |
| 30 | 20.87 | 14.53 | 17.70 | 25.80 | 28.80 | 27.30 |
| Mean (T) | 19.02 | 12.57 | 15.80 | 24.29 | 26.62 | 25.46 |
| | **4WAP** | | | **8WAP** | | |
| $F\text{-}LSD_{(0.05)}$ for any 2 application methods | 1.94 | | | 0.31 | | |
| $F\text{-}LSD_{(0.05)}$ for any 2 time of sowing | 1.82 | | | 1.00 | | |
| $F\text{-}LSD_{(0.05)}$ for any 2 T X S | NS | | | NS | | |
| | **2016** | | | | | |
| | **Rhizobacteria inoculant application methods (T)** | | | | | |
| **Time of sowing (Minutes) (S)** | **4WAP** | | | **8WAP** | | |
| | **Soil applied method** | **Seed applied method** | **Mean (S)** | **Soil applied method** | **Seed applied method** | **Mean (S)** |
| 0 | 20.32 | 11.05 | 15.68 | 24.36 | 15.36 | 19.86 |
| 15 | 22.79 | 15.76 | 19.27 | 26.43 | 16.00 | 21.21 |
| 30 | 25.78 | 17.35 | 21.56 | 28.08 | 19.12 | 23.60 |
| Mean (T) | 22.96 | 14.72 | 18.84 | 26.29 | 16.83 | 21.56 |
| | **4WAP** | | | **8WAP** | | |
| $F\text{-}LSD_{(0.05)}$ for any 2 application methods | 2.33 | | | 1.45 | | |
| $F\text{-}LSD_{(0.05)}$ for any 2 time of sowing | 0.71 | | | 0.32 | | |
| $F\text{-}LSD_{(0.05)}$ for any 2 T X S | NS | | | NS | | |

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WAP – weeks after planting, F-LSD$_{(0.05)}$ – Fisher's least significant difference at 0.05 probability level, NS - non-significant at 0.05 probability level

*Table.5: Effect of rhizobacteria inoculant application methods and time of sowing after inoculation on number of leaves per plant of Bambara groundnut [Vigna subterranea* (L.) Verdc.] *at four and eight weeks after planting in 2015 and 2016 planting season*

| Time of sowing (Minutes) (S) | 2015 | | | | | |
|---|---|---|---|---|---|---|
| | Rhizobacteria inoculant application methods (T) | | | | | |
| | 4WAP | | | 8WAP | | |
| | Soil applied method | Seed applied method | Mean (S) | Soil applied method | Seed applied method | Mean (S) |
| 0 | 18 | 18 | 18 | 39 | 33 | 36 |
| 15 | 22 | 20 | 21 | 43 | 41 | 42 |
| 30 | 27 | 27 | 27 | 50 | 46 | 48 |
| Mean (T) | 22 | 22 | 22 | 44 | 40 | 42 |
| | 4WAP | | | 8WAP | | |
| F-LSD$_{(0.05)}$ for any 2 application methods | NS | | | 0.6 | | |
| F-LSD$_{(0.05)}$ for any 2 time of sowing | NS | | | NS | | |
| F-LSD$_{(0.05)}$ for any 2 T X S | NS | | | NS | | |
| **Time of sowing (Minutes) (S)** | **2016** | | | | | |
| | Rhizobacteria inoculant application methods (T) | | | | | |
| | 4WAP | | | 8WAP | | |
| | Soil applied method | Seed applied method | Mean (S) | Soil applied method | Seed applied method | Mean (S) |
| 0 | 21 | 20 | 21 | 40 | 38 | 39 |
| 15 | 22 | 22 | 22 | 40 | 44 | 42 |
| 30 | 29 | 27 | 28 | 54 | 50 | 52 |
| Mean (T) | 24 | 23 | 24 | 45 | 44 | 44 |
| | 4WAP | | | 8WAP | | |
| F-LSD$_{(0.05)}$ for any 2 application methods | 0.4 | | | 0.1 | | |
| F-LSD$_{(0.05)}$ for any 2 time of sowing | NS | | | NS | | |
| F-LSD$_{(0.05)}$ for any 2 T X S | NS | | | NS | | |

WAP – weeks after planting, F-LSD$_{(0.05)}$ – Fisher's least significant difference at 0.05 probability level, NS - non-significant at 0.05 probability level

**Effect of rhizobacteria inoculant application methods and time of sowing after inoculation on leave area index per plant of Bambara groundnut [*Vignasubterranea*(L.) Verdc.] at four and eight weeks after planting in 2015 and 2016 planting season**

The main effect of rhizobacteria inoculant application methods on leaf area index per plant of Bambara groundnut at four and eight weeks after planting in 2015 and 2016 planting season were significant. (Table 6). Leaf area index of Bambara groundnut cultivated on soil applied method of rhizobacteria inoculation plots were significantly ($p < 0.05$) the highest ( 1.71 in 2015 and 1.74 in 2016 at four weeks after planting and (9.11 in 2015 and 9.37 in 2016 at eight weeks after planting) than the plants on seed applied method of rhizobacteria inoculation. A significant ($p < 0.05$) effect was recorded on the main effect of time of sowing after inoculation on the leaf area index of Bambara groundnut at four and eight weeks after planting in 2015 and 2016 planting season. Plants sown after 30 minutes of rhizobacteria inoculant application significantly ($p < 0.05$) had the highest leaf area index per plant (1.60 in 2015 and 1.62 in 2016 at four weeks after planting and 8.12 in 2015 and 8.46 in 2016 at eight weeks after planting) followed by plants grown after 15 minutes of inoculant application (1.20

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in 2015 and 1.25 in 2016 at four weeks after planting and 6.95 in 2015 and 7.01 in 2016 at eight weeks after planting) and the lest was recorded on plants grown immediately after inoculation (0 minutes after inoculation). The values ranged from 0.88 (2015) to 0.93 (2016) at four weeks after planting and 5.83 (2015) to 5.90 (2016) at eight weeks after planting.

Furthermore, the combined effect of rhizobacteria inoculant application methods and time of sowing after inoculation on leaf area index per plant of Bambara groundnut were observed to be non- significant ($p > 0.05$) at four and eight weeks after planting in 2015 and 2016 planting season respectively. Consequently, the data in Table 6 indicated that leaf area index per plant of Bambara groundnut were higher in 2016 than in 2015 planting season. Observation made at eight weeks after planting were greater than what was observed at four weeks after planting.

*Table.6: Effect of rhizobacteria inoculant application methods and time of sowing after inoculation on leave area index per plant of Bambara groundnut [Vigna subterranea* (L.) Verdc.] *at four and eight weeks after planting in 2015 and 2016 planting season*

| **Time of sowing (Minutes) (S)** | **2015** | | | | | |
|---|---|---|---|---|---|---|
| | **Rhizobacteria inoculant application methods (T)** | | | | | |
| | **4WAP** | | | **8WAP** | | |
| | **Soil applied method** | **Seed applied method** | **Mean (S)** | **Soil applied method** | **Seed applied method** | **Mean (S)** |
| 0 | 1.22 | 0.55 | 0.88 | 7.79 | 3.87 | 5.83 |
| 15 | 1.68 | 0.73 | 1.20 | 9.16 | 4.74 | 6.95 |
| 30 | 2.24 | 0.96 | 1.60 | 10.37 | 5.86 | 8.12 |
| Mean (T) | 1.71 | 0.75 | 1.23 | 9.11 | 4.82 | 6.96 |
| | **4WAP** | | | **8WAP** | | |
| F-LSD$_{(0.05)}$ for any 2 application methods | 0.02 | | | 2.11 | | |
| F-LSD$_{(0.05)}$ for any 2 time of sowing | 0.01 | | | 1.31 | | |
| F-LSD$_{(0.05)}$ for any 2 T X S | NS | | | NS | | |
| **Time of sowing (Minutes) (S)** | **2016** | | | | | |
| | **Rhizobacteria inoculant application methods (T)** | | | | | |
| | **4WAP** | | | **8WAP** | | |
| | **Soil applied method** | **Seed applied method** | **Mean (S)** | **Soil applied method** | **Seed applied method** | **Mean (S)** |
| 0 | 1.28 | 0.57 | 0.93 | 7.90 | 3.90 | 5.90 |
| 15 | 1.70 | 0.81 | 1.25 | 9.21 | 4.80 | 7.01 |
| 30 | 2.25 | 1.00 | 1.62 | 11.00 | 5.91 | 8.46 |
| Mean (T) | 1.74 | 0.79 | 1.27 | 9.37 | 4.87 | 7.12 |
| | **4WAP** | | | **8WAP** | | |
| F-LSD$_{(0.05)}$ for any 2 application methods | 0.02 | | | 3.49 | | |
| F-LSD$_{(0.05)}$ for any 2 time of sowing | 1.00 | | | 0.22 | | |
| F-LSD$_{(0.05)}$ for any 2 T X S | NS | | | NS | | |

WAP – weeks after planting, F-LSD$_{(0.05)}$ – Fisher's least significant difference at 0.05 probability level, NS - non-significant at 0.05 probability level

**Effect of rhizobacteria inoculant application methods and time of sowing after inoculation on number of nodules per plant of Bambara groundnut [*Vignasubterranea*(L.) Verdc.] at four and eight weeks after planting in 2015 and 2016 planting season**

The results presented in Table 7 indicated that the main effect of inoculant application methods on number of nodules produced per plant of Bambara groundnut were significant ($p < 0.05$). Plants grown on soil applied method of inoculation had significantly ($p < 0.05$) more number of nodules per plant which varied from 27 (2015) to 29 (2016) at four weeks after planting and 41 (2015) to 43 (2016) at eight weeks after planting than the seed applied method of inoculation (14 in 2015 and 15 in 2016 at four weeks after

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planting and 26 in 2015 and 28 in 2016 at eight weeks after planting). The main effect of time of sowing after inoculation were non- significant ($p > 0.05$) at four and eight weeks after planting in 2015 and 2016 planting season respectively. The interaction effect of inoculant application methods and time of sowing after inoculation were significant ($p < 0.05$).

Furthermore, plants cultivated on soils which received ten grams of rhizobacteria inoculant at 30 minutes after inoculation produced more number of nodules per plant at four weeks after the plant the value was 30 in 2015 and 33 in 2016 and at eight weeks after planting the value was 42 in 2015 and 45 in 2016. The combined effect of seed applied methods and 0 minute after inoculation produced the lowest number of nodules per plant (10 in 2015 and 11 in 2016 at four weeks after planting and 18 in 2015 and 20 in 2016 at eight weeks after planting). The results in Table 7 showed that number of nodules per plant were higher in 2016 than in 2015 planting season. Observation made at eight weeks after planting were greater than recorded at four weeks after planting.

*Table.7: Effect of rhizobacteria inoculant application methods and time of sowing after inoculation on number of nodules per plant of Bambara groundnut [Vigna subterranea* (L.) Verdc.] *at four and eight weeks after planting in 2015 and 2016 planting season*

| **Time of sowing (Minutes) (S)** | **2015** | | | | | |
|---|---|---|---|---|---|---|
| | **Rhizobacteria inoculant application methods (T)** | | | | | |
| | **4WAP** | | | **8WAP** | | |
| | **Soil applied method** | **Seed applied method** | **Mean (S)** | **Soil applied method** | **Seed applied method** | **Mean (S)** |
| 0 | 22 | 10 | 16 | 39 | 18 | 28 |
| 15 | 28 | 14 | 21 | 41 | 26 | 34 |
| 30 | 30 | 18 | 24 | 42 | 33 | 38 |
| Mean (T) | 27 | 14 | 41 | 41 | 26 | 34 |
| | **4WAP** | | | **8WAP** | | |
| F-LSD$_{(0.05)}$ for any 2 application methods | 3.0 | | | 5.3 | | |
| F-LSD$_{(0.05)}$ for any 2 time of sowing | NS | | | NS | | |
| F-LSD$_{(0.05)}$ for any 2 T X S | 2.1 | | | 8.4 | | |
| **Time of sowing (Minutes) (S)** | **2016** | | | | | |
| | **Rhizobacteria inoculant application methods (T)** | | | | | |
| | **4WAP** | | | **8WAP** | | |
| | **Soil applied method** | **Seed applied method** | **Mean (S)** | **Soil applied method** | **Seed applied method** | **Mean (S)** |
| 0 | 24 | 11 | 17 | 41 | 20 | 30 |
| 15 | 30 | 14 | 22 | 44 | 29 | 36 |
| 30 | 33 | 20 | 26 | 45 | 35 | 40 |
| Mean (T) | 29 | 15 | 22 | 43 | 28 | 35 |
| | **4WAP** | | | **8WAP** | | |
| F-LSD$_{(0.05)}$ for any 2 application methods | 7.7 | | | 9.8 | | |
| F-LSD$_{(0.05)}$ for any 2 time of sowing | NS | | | NS | | |
| F-LSD$_{(0.05)}$ for any 2 T X S | 5.1 | | | 10.8 | | |

WAP – weeks after planting, F-LSD$_{(0.05)}$ – Fisher's least significant difference at 0.05 probability level, NS - non-significant at 0.05 probability level

**Effects of rhizobacteria inoculant application methods and time of sowing after inoculation on number of fresh pods, weight of fresh pods, leaf, stem and root dry weight per plant of Bambara groundnut [*Vignasubterranea*(L.) Verdc.] in 2015 and 2016 planting season**

The data presented in Table 8 revealed that the main effect of rhizobacteria inoculant application methods on

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number of fresh pods per plant and weight of fresh pods per plant at harvest in 2015 and 2016 planting season were significant ($p < 0.05$). Consequently, the main effect of time of sowing after inoculation and the interaction effect of inoculant application methods and time of sowing after inoculation gave a non- significant ($p > 0.05$) effect on the number of fresh pod at harvest in 2015 and 2016 planting season. Bambara groundnut planted on plots which received ten grams of rhizobacteria inoculant per planting hole had significantly ( $p < 0.05$) more number of pods per plant at harvest (8 in 2015 and 9 in 2016) than the seed applied method of inoculation..More so, Table 8 showed that the main effect of time of sowing after inoculation and the interaction effect of inoculant application methods and time of sowing after inoculation gave a non-significant ($p > 0.05$) effect on the weight of fresh pods produced at harvest in 2015 and 2016 planting season. The weight of pods of plants grown on soil applied method of inoculation significantly ($p < 0.05$) was the highest in comparison with the seed applied method of inoculation. At four weeks after planting, the weight ranged from 143.90 g plant$^{-1}$ to 149.33 g plant$^{-1}$ in 2015 and 2016 planting season.

Furthermore, the results in Table 9 indicated that the main effect of inoculant application methods on leaf dry weight per plant and stem dry weight per plant of Bambara groundnut were significant ($p < 0.05$). Soil applied method of inoculation gave the highest dry weight of leaf (27.66 g plant$^{-1}$ in 2015 and 30.04 g plant$^{-1}$ in 2016) and stem (19.05 g plant$^{-1}$ in 2015 and 23.04 g plant$^{-1}$ in 2016) at eight weeks after planting in 2015 and 2016 planting season than the seed applied method of inoculation. The results also showed that the main effect of time of sowing after inoculation and the interaction effect of inoculant application methods and time of sowing after inoculation gave a non-significant ($p > 0.05$) effect on the leaf dry weight per plant and stem dry weight per plant of Bambara groundnut at eight weeks after planting in 2015 and 2016 planting season

Similarly , Table 10 showed that the main effect of inoculant application methods on root dry weight were significant ($p < 0.05$). Soil applied method of inoculation produced the highest (3.53 g plant$^{-1}$ in 2015 and 3.64 g plant$^{-1}$ in 2016) root dry weight than the seed applied method. The main effect of time of sowing after inoculation and the interaction effect of inoculant application methods and time of sowing after inoculation gave a non-significant ($p > 0.05$) effect on the root dry weight per plant of Bambara groundnut at eight weeks after planting in 2015 and 2016 planting season ( Table 10).

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*Table.8: Effects of rhizobacteria inoculant application methods and time of sowing after inoculation on number of fresh pods per plant and weight of fresh pods per plant of Bambara groundnut [Vigna subterranea* (L.) Verdc.] *at harvest in 2015 and 2016 planting season*

| **Time of sowing (Minutes) (S)** | **2015** | | | | | |
|---|---|---|---|---|---|---|
| | **Rhizobacteria inoculant application methods (T)** | | | | | |
| | **Number of fresh pods per plant** | | | **Weight of fresh pods per plant (g plant$^{-1}$)** | | |
| | **Soil applied method** | **Seed applied method** | **Mean (S)** | **Soil applied method** | **Seed applied method** | **Mean (S)** |
| 0 | 7 | 3 | 5 | 135.60 | 39.60 | 87.60 |
| 15 | 9 | 4 | 6 | 146.10 | 57.70 | 101.90 |
| 30 | 9 | 6 | 7 | 150.00 | 98.60 | 124.30 |
| Mean (T) | 8 | 4 | 6 | 143.90 | 65.30 | 104.60 |
| F-LSD$_{(0.05)}$ for any 2 application methods | | 1.4 | | | 20.40 | |
| F-LSD$_{(0.05)}$ for any 2 time of sowing | | NS | | | NS | |
| F-LSD$_{(0.05)}$ for any 2 T X S | | NS | | | NS | |
| **Time of sowing (Minutes) (S)** | **2016** | | | | | |
| | **Rhizobacteria inoculant application methods (T)** | | | | | |
| | **Number of fresh pods per plant** | | | **Weight of fresh pods per plant (g plant$^{-1}$)** | | |
| | **Soil applied method** | **Seed applied method** | **Mean (S)** | **Soil applied method** | **Seed applied method** | **Mean (S)** |
| 0 | 8 | 3 | 5 | 140.00 | 42.51 | 91.25 |

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| | | | | | | |
|---|---|---|---|---|---|---|
| 15 | 10 | 5 | 7 | 151.00 | 62.00 | 106.50 |
| 30 | 11 | 7 | 9 | 157.00 | 100.00 | 128.50 |
| Mean (T) | 9 | 5 | 7 | 149.33 | 68.17 | 108.75 |
| F-LSD$_{(0.05)}$ for any 2 application methods | | 0.9 | | | 23.54 | |
| F-LSD$_{(0.05)}$ for any 2 time of sowing | | NS | | | NS | |
| F-LSD$_{(0.05)}$ for any 2 T X S | | NS | | | NS | |

F-LSD$_{(0.05)}$ – Fisher's least significant difference at 0.05 probability level, NS - non-significant at 0.05 probability level

*Table.9: Effects of rhizobacteria inoculant application methods and time of sowing after inoculation on leaf dry weight per plant (g plant$^{-1}$) and stem dry weight per palnt (g plant$^{-1}$) of Bambara groundnut* [*Vigna subterranea* (L.) Verdc.] *at eight weeks after planting in 2015 and 2016 planting season*

| **Time of sowing (Minutes) (S)** | **2015** | | | | | |
|---|---|---|---|---|---|---|
| | **Rhizobacteria inoculant application methods (T)** | | | | | |
| | **Leaf dry weight per plant** | | | **Stem dry weight per plant** | | |
| | **Soil applied method** | **Seed applied method** | **Mean (S)** | **Soil applied method** | **Seed applied method** | **Mean (S)** |
| 0 | 20.11 | 19.67 | 19.89 | 16.37 | 16.00 | 16.19 |
| 15 | 25.89 | 23.34 | 24.62 | 19.77 | 19.34 | 19.56 |
| 30 | 36.99 | 30.76 | 33.88 | 21.00 | 20.21 | 20.61 |
| Mean (T) | 27.66 | 24.59 | 26.13 | 19.05 | 18.52 | 18.79 |
| F-LSD$_{(0.05)}$ for any 2 application methods | | 1.11 | | | 0.01 | |
| F-LSD$_{(0.05)}$ for any 2 time of sowing | | NS | | | NS | |
| F-LSD$_{(0.05)}$ for any 2 T X S | | NS | | | NS | |
| **Time of sowing (Minutes) (S)** | **2016** | | | | | |
| | **Rhizobacteria inoculant application methods (T)** | | | | | |
| | **Leaf dry weight per plant** | | | **Stem dry weight per plant** | | |
| | **Soil applied method** | **Seed applied method** | **Mean (S)** | **Soil applied method** | **Seed applied method** | **Mean (S)** |
| 0 | 22.11 | 20.00 | 21.06 | 18.00 | 16.56 | 17.28 |
| 15 | 29.00 | 26.31 | 27.66 | 25.00 | 20.91 | 22.96 |
| 30 | 39.00 | 37.12 | 38.06 | 26.11 | 21.00 | 23.56 |
| Mean (T) | 30.04 | 27.81 | 28.93 | 23.04 | 19.49 | 21.27 |
| F-LSD$_{(0.05)}$ for any 2 application methods | | 2.10 | | | 1.72 | |
| F-LSD$_{(0.05)}$ for any 2 time of sowing | | NS | | | NS | |
| F-LSD$_{(0.05)}$ for any 2 T X S | | NS | | | NS | |

F-LSD$_{(0.05)}$ – Fisher's least significant difference at 0.05 probability level, NS - non-significant at 0.05 probability level

*Table.10: Effect of rhizobacteria inoculant application methods and time of sowing after inoculation on root dry weight per palnt of Bambara groundnut [Vignasubterranea* (L.) Verdc.] *at eight weeks after planting in 2015 and 2016 planting season*

| **Time of sowing (Minutes) (S)** | **2015** | | |
|---|---|---|---|
| | **Rhizobacteria inoculant application methods (T)** | | |
| | **Soil applied method** | **Seed applied method** | **Mean (S)** |
| 0 | 2.90 | 2.61 | 2.76 |
| 15 | 3.71 | 3.00 | 3.36 |
| 30 | 3.99 | 3.73 | 3.86 |
| Mean (T) | 3.53 | 3.11 | 3.32 |

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| | |
|---|---|
| F-LSD$_{(0.05)}$ for any 2 application methods | 0.10 |
| F-LSD$_{(0.05)}$ for any 2 time of sowing | NS |
| F-LSD$_{(0.05)}$ for any 2 T X S | NS |

| **Time of sowing (Minutes) (S)** | **2016** | | |
|---|---|---|---|
| | **Rhizobacteria inoculant application methods (T)** | | |
| | **Soil applied method** | **Seed applied method** | **Mean (S)** |
| 0 | 3.21 | 2.87 | 3.04 |
| 15 | 3.70 | 3.22 | 3.46 |
| 30 | 4.01 | 3.70 | 3.86 |
| Mean (T) | 3.64 | 3.26 | 3.45 |
| F-LSD$_{(0.05)}$ for any 2 application methods | | 0.03 | |
| F-LSD$_{(0.05)}$ for any 2 time of sowing | | NS | |
| F-LSD$_{(0.05)}$ for any 2 T X S | | NS | |

F-LSD$_{(0.05)}$ – Fisher's least significant difference at 0.05 probability level, NS - non-significant at 0.05 probability level

## IV. DISCUSSION

**Effects of rhizobacteria inoculant application methods and time of sowing after inoculation on vegetative growth of Bambara groundnut [*Vigna subterranea* (L.) Verdc.]**

Despite the poor edaphic conditions of soils in Agbani area of Enugu state which is characterized by low fertility and high acidity caused by over exploitation, nutrient volatilization, erosion, leaching and runoff (Anikwe , 2006), the use of rhizobacteria inoculant was effective in Bambara groundnut vegetative growth promotion. The main effect of rhizobacteria inoculant application methods on plant height, number of leaves per plant at eight weeks after planting in 2015, four weeks after planting and eight week after planting in 2016 and leaf area index per plant at four and eight weeks after planting in 2015 and 2016 planting season were significant ($p < 0.05$). Soil applied method treatment gave taller plants with more number of leaves per plant and highest leaf area index per plant than the seed applied method of inoculation. This is due to the fact that the externally soil applied bacteria like rhizobium were effective in their ability to promote vegetative growth of Bambara groundnut. Adesemoye *et al*., (2008b); Shen *et al.,* (2012) noted that when these organisnms are introduced into the rhizospere, they have the potential to alter microbial populations in the rhizosphere and influence nutrient transformation, availablity and uptake by plants. This findings also suggest that the bacteria inhabiting in the rhizosphere of Bambara groundnut were able to tolerant the abiotic stress conditions in the soils of Agbani area. This findings is in accordance with the investigations of Gururani *et al.,* (2012) who reported an increase in plant height and number of leaves per plant of potato when inoculated with *Bacillus pumilus* under saline, drought and heavy metal stress conditions. Similar results was obtained on Mungbean and chickpea by Chakraborty *et al* (2011), who noted that inoculation with *Bacillus cereus* caused an increase in seedling height, number and length of leaves per plant under saline stress condition. More so, the rhizobim bacteria out competed other soil microbes in colonizing the roots of Bambara groundnut, thereby enhancing the nitrogen fixation that contributed to the increase in the plant height, number of leaves per plant and leaf area index per plant in Bambara groundnut. Also, This finding suggest that the exudate secreted by the root of Bambara groundnut were not antimicrobial to the applied rhizobium. Cai *et al*., 2009, 2012; Carvlhais *et al*., 2013 reported that plant roots respond to environmental conditions through the secretion of a wide range of compounds, according to nutritional status and soil conditions. This action interferes with the plant- bacteria interaction and is an important factor contributing to efficiency of the inoculant. According to Bais *et al*., (2006) root exudation includes the secretion of ions, free oxygen and water, enzymes, mucilage and a diverse array of carbon containing primary and secondary metabolites. Also, Guttman *et al*., (2014) noted that roots of plants excrete 10 - 44% of photosynthetically fixed carbon which serve as an energy source, signaling molecules or antimicrobial for soil microorganisms. The inferiority of the seed coated method of rhizobacteria inoculant application methods over the soil applied method of inoculation could be attributed to the big seed size and texture of Bambara groundnut. The seed might not has imbibe the optimum quantity of liquid bacterial suspension that is capable of effectively promoting vegetative growth. Milus and Rothrock (1993) noted that the population of bacteria in the soil is mainly dependent on the initial stack of inoculums on the seeds. Hebber*et al.,* (1992) stated that application of more inoculums per seed can

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increase efficiency but results are not always steady. Also, the presence of microniches in the soil enabled the bacteria to survive after their application to soil otherwise reduction in bacterial number will occur (Van Elsas *et al*., 1986; Heijnen *et al*., 1988; Van Elsas and Heijnen 1990). More so, the sticking agent (Gum arabic) used in the preparation of the inoculant slurry for seed biopriming may not have adhere well to the seed coat of Bambara groundnut, this in turn will affect imbibitions of the inoculant solution in to the seed. Seed coating also hinders the gaseous exchange to the leguminous seeds which causes reduction in nitrogen fixation (Duarte *et al.,* 2004)

Furthermore, the main effect of the time of sowing after inoculation was observed to be significant ($p < 0.05$) on plant height and leaf area index per plant and non-significant ($p > 0.05$) on the number of leaves produced per plant at four and eight weeks after in 2015 and 2015. Imbibition time is an important factor in seed coating method of inoculant application method. The better influence of 30 minutes time of sowing after inoculation is due to that seed have ample time to imbibe some quantity of bacterial suspension that can initiate physiological processes in the seed of Bambara groundnut. On the contrary, the interaction effect of rhizobacteria inoculant application methods and time of sowing after inoculation on all the vegetative growth parameters were observed to be non-significant ($p > 0.05$) at four and eight weeks after planting in 2015 and 2016 planting season.

**Effects of rhizobacteria inoculant application methods and time of sowing after inoculation on nodulation of Bambara groundnut [*Vigna subterranea* (L.)Verdc.]**

Nodulation is pivotal in nitrogen fixation by leguminous plants. Bambara groundnut benefited significantly from the main effect of rhizobacteria inoculant application methods on the number of nodules produced per plant at four and eight weeks after planting in 2015 and 2016 planting season. The highest number of nodules per plant were observed on Bambara groundnut cultivated on soils treated with ten grams or rhizobacteria inoculant per planting hole than the seed applied method treatment. This findings is attributed to the role of rhizbium in the production of plant hormones such as auxin, giberellin and cytokinin which are known to enhance plant physiological processes such as nodualtion. Spaepen *et al*., (2007) reported that the influence of bacteria in the rhizosphere of plants is largely due to the production of auxinphytohormones. Several bacterial species can produce indolic compounds such as auxinphytohormone indole-3-acetic acid (IAA), which present great physiological relevance for bacteria-plant interaction, varying from pathogenesis to phytostimulation. Indole acetic acid (IAA) is the most common natural auxin found in plants and its positive effect on root growth (Miransari and Smith 2014). Up to 80% of rhizobacteria can synthesize indole acetic acid (IAA) colonized the seed or root surfaces is proposed to act in conjunction with endogenous IAA in plant to stimulate cell proliferation and enhance the host's uptake of minerals and nutrients from the soil (Vessey, 2003). Indole acetic acid affects plant cell division, extension, and differentiation; stimulates seed and tuber germination; increases the rate of xylem and root development; controls processes of vegetative growth; initiates lateral and adventitious root formation; mediates responses to light, gravity and florescence; affects photosynthesis, pigment formation, biosynthesis of various metabolites, and resistance to stressful conditions. On the other hand, the main effect of time of sowing after inoculation was observed to be non-significant ($p > 0.05$) on the number of nodules produced per plant at four and eight weeks after planting in 2015 and 2016 planting season respectively.

The interaction effect of rhizobacteria inoculant application methods and time of sowing after inoculation on number of nodules per plant was significant ($p < 0.05$). The combined effect of soil applied method of inoculation and 30 minutes time of sowing after inoculation produced more number of nodules per plant than the other interaction effect. This result shows that nodulation is increased when adequate time is given for bacteria colonization of the rhizosphere.

**Effects of rhizobacteria inoculant application methods and time of sowing after inoculation on yield of Bambara groundnut [*Vigna subterranea* (L.)Verdc.]**

The main effect of rhizobacteria inoculant application methods on number of fresh pods per plant, weight of pods per plant, dry matter accumulation of leaf, stem and root per plant in 2015 and 2016 planting season were significant ($p < 0.05$). Plants grown on soil applied method plots had the highest yield traits than the seed applied method plots. This is due to effective nodulation of the rhizobium inoculant. Revellin *et al*., (2000) observed that higher number of nodules per plant resulted in higher plant biomass. The significant effect on the number of nodules produced per plant from this study is sufficient to maintain a high number of pod and weight of fresh pod at hervest. This findings agreed with the investigations of Onduru *et al*., (2008) and Nyoki and Ndakidemi (2013) who reported that inoculation increased nodulation, shoot dry weight, grain yield and other growth variables. Ethylene is a key phytohormone has a wide range of biological activities can

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affect plant growth and development in a large number of different ways including promoting root initiation, inhibiting root elongation, promoting fruit ripening, promoting lower wilting, stimulating seed germination, promoting leaf abscission, activating the synthesis of other plant hormones (Glick *et al*., 2007). The high concentration of ethylene induces defoliation and other cellular processes that may lead to reduced crop performance (Bhattacharyya and Jha, 2012). The enzyme 1-aminocyclopropane-1 carboxylic acid (ACC) is a pre-requisite for ethylene production, catalyzed by ACC oxidase. Iqbal *et al*., (2012) reported improved nodule number, nodule dry weight, fresh biomass, grain yield, straw yield, and nitrogen content in grains of lentil as a result of lowering of the ethylene production via inoculation with plant growth promoting bacteria.

Most of the *Rhizobium* species have been found to produce indole acetic acid (Ahemad and Khan, 2012), which is essential for process of nodule formation through cell division and differentiation along with vascular tissue formation (Ahemad and Kibret, 2014). Thus, higher auxin levels in legume plants are responsible for nodule formation (Spaepen *et al*., 2007; Glick, 2012) and symbiotic relationships Cong *et al*., (2009) observed that inoculation significantly increased grain and straw strain of rice. On the other hand the main effect of time of sowing after inoculation and the interaction effect of rhizobacteria inoculant application methods and time of sowing after inoculation was observed to be non-significant ($p > 0.05$) in both planting season.

## V. CONCLUSION

Bambara groundnut responded well to the influence of rhizobacteria inoculation and time of sowing after inoculation. Significant variations were observed in all the growth and yield traits determined in this study. The combined use soil applied method of inoculation and 30 minutes of after inoculation before planting is recommended for the cultivation of Bambara groundnut in Agbani Area South East Nigeria

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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