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Arachis hypogaea Yield Analysis in Rhizobial (*Bradyrhizobium* sp.) Inoculated Post-solarized Soil

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Abstract

In the bid to enhance *A. hypogaea* yield through cheaper, environmentally friendly agronomical techniques, biofertilization through rhizobial (*Bradyrhizobium sp*.) inoculation in post-solarized soil was studied after subjecting soil to the following treatments: solarized inoculated, unsolarized uninoculated (control), solarized uninoculated, and unsolarized inoculated with *Bradyrhizobium sp*. Following these, viable *A. hypogaea* seeds were planted 14.0 days after the treatments and then monitored for 120 days period to ascertain the influence of treatments on germination, root lengths, plant heights, weights (fresh and dry), pod and nodule numbers. Soil temperature fluctuated between 28.0 and 54.0°C within the 16 days period of solarization.

Seed germination was not affected by the various treatments applied. Nevertheless, pod and nodule numbers, weights (fresh and dry), height and root length growth were affected by treatments (P □ 0.05). Weights, pod and nodule numbers were in the order: solarized inoculated □ unsolarized inoculated □ unsolarized uninoculated □ solarized uninoculated. On the other hand, plant height followed the order: solarized inoculated □ unsolarized uninoculated □ unsolarized inoculated □ solarized uninoculated, while root length growth in the order: solarized uninoculated □ solarized inoculated □ unsolarized inoculated □ unsolarized uninoculated. The highest number of pods and nodules which were recorded in solarized inoculated soil were 11.0 and 136.0 per plant respectively, while the lowest pods and nodules which were recorded in solarized uninoculated were 3.0 and 31.0 per plant respectively. Rhizobial inoculation in post-solarized soil enhanced *A. hypogaea* yield.

Key words: *A. hypogaea; Inoculation; Post-solarized; Rhizobia; Soil; Yield*

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Introduction

Arachis hypogaea (Groundnut) is the most significant crop next to soybean among legumes which serve as food for livestock and human, supplying useful dietary protein especially where animal protein is non-existence (Redden., *et al*. 2005 as cited by Grandawa, 2014). Nigeria is one of the major producers of groundnut in Africa with an estimated production level of 172 kilogram per hectare (Grandawa, 2014).

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Although, increase in agricultural productivity through inorganic fertilizers application has helped to a greater extent meet the food demand of the ever soaring human population; this practice is bedevilled with increasing high cost of procurement, environmental pollution accompanying over fertilization and its attendant problems. Cheaper and environmentally friendly agronomical techniques and biofertilization are therefore a more preferable option for plant growth enhancement and crop yield.

While soil solarization (cost-effective and environmentally friendly means of controlling soil-borne plant pathogens) has been shown to enhance plant growth (Pokharel, 2011; Chauhan., *et al*. 1988; Elmore., *et al*. 1997), this technique is detrimental to rhizobial population in soil vital for legume nodulation and yield (Pokharel, 2011; Linke., *et al*. 1991; Chauhan., *et al*. 1988). On the other hand rhizobial inoculation in unsolarized soil faces possible challenges of stiff competition from well adapted soil microflora and microbial grazing phenomenon (Bashan, 1998) which could lead to inoculants failure.

However, the inoculation of rhizobiain a post-solarized soil may help address these challenges highlighted herein. It is on the premise of enhancing the yield of *A. hypogaea* by re-inoculation of rhizobia (*Bradyrhizobium* sp.) in a post-solarized soil that this study was embarked upon.

Materials and Methods

Evaluation of soil baseline physicochemical characteristics

Reference data on the physicochemical characteristics of experimental soil were obtained first before soil treatments and plant propagation. Triplicate soil samples were collected and evaluated for total organic carbon, porosity, nitrogen, phosphorus, pH and textural composition engaging the methods of Black (1965), Ezzati., *et al*. (2012), micro-Kjeldahl procedure (Hesse, 1979), Bray and Kurtz (1971), Black (1965) and the hydrometer technique as stated by Aliyu and Oyeyiola (2011) respectively.

Soil solarization treatment

Soil solarization was carried out by adapting the procedure described by Elmore., *et al*. (1997). Soil used for the propagation of plant was collected within the range of 0-15 cm soil horizon. Four kilogram (4.0 kg) of soil collected per container (12 plastic containers in total with a dimension of 22.0 cm width and 20.0 cm depth). Six of these containers together with soil were solarized for the period of 16.0 days, while the other six remained unsolarized.

Soil solarization was embarked upon in mid-November by moisturizing soil and covering it with transparent sheets of polyethylene of 0.025 cm thickness. This was allowed to stay in the open to receive direct solar radiation. Heat escape from soil was prevented by ensuring complete coverage with sheet touching soil surface. Soil daily temperature measurements were taken at 10.00 hours and 16.00 hours using inserted thermometer at 10.0 cm depth.

Rhizobia isolation from *A. hypogaea* nodules

Rhizobial cells was isolated from root nodules of *A. hypogaea* adapting the methods of Deka and Azad (2006) and Ben-Gweirif., *et al*. (2005). Properly formed nodules (matured size and pinkish) were removed from roots and washed properly using tap water, followed by surface sterilization for 2.0 minutes with 70% ethanol. Thereafter, nodules were rinsed in distilled sterile water before engaging sodium hypochlorite (3.5% w/v) for additional surface sterilization of nodule for 2.0 minutes. This was followed by series (thrice) of rinsing using distilled sterile water. Nodules were placed in Mac Cartney bottle and crushed with the addition of few drops of distilled sterilized water.

Wire loop was used to collect loop-full of the crushed nodules. This was streaked on petri dishes containing yeast extract manitol agar (YEMA)incorporated congo red and subsequently incubated at 30.0 ± 2.0°C (room temperature) for 72 hours. Colonies on YEMA plates that were viscous,smooth, musky odour, circular edge, convex and raised, which took up faintly incorporated dye were considered as colonies of rhizobia. These colonies were removed from the plates for further purification and identification before storing in slant of YEMA as stock culture for future use

Identification of *Bradyrhizobium* sp. isolated from *A. hypogaea* nodules

Bradyrhizobium sp. isolated from the nodules of *A. hypogaea* was characterized using Bergey's Manual of Determinative Bacteriology (John., *et al*. 1994) on the basis of morphological, cultural and biochemical features of the isolate as well as its intrinsic resistance to tested array of antibiotics.

***Bradyrhizobium* population scale up and the inoculation of unsolarized and solarized soil**

Three of the unsolarized and solarized plastic containers with soil were inoculated with the previously isolated *bradyrhizobium* sp. From the root nodules of *A. hypogaea* 24 hours after soil solarization activity. Culture of *Bradyrhizobium* sp. that have been purified was scaled up in a four liter jerry can (sterilized with 70.0% ethanol) containing two liter of yeast extract manitol broth (YEMB) after previous transfer from a 500 ml flask containing 200 ml of YEMB culture of Bradyrhizobium. Aliquot of the culture in test tubes were subsequently centrifuged for five minutes at the rate of 4000 rev/minute to harvest cells. Rhizobial cells that were harvested were transferred into a four liter sterile jerry can and the volume made up to three liter mark using physiological saline so as to obtain a concentration of 8.5×10^8 cfu/ml. Each of the solarized and unsolarized soil plastic containers was then inoculated with a 500 ml physiological saline containing rhizobia at the designated cell concentration. The inoculated cells were then plowed into soil with the aid of a sterile hand towel.

Assessing seeds of *A. hypogaea* for viability

Seeds of *A. hypogaea* purchased from a local market (North Bank Market, Benue State, Nigeria) were initially assessed for viability before propagation. Viability test was carried out by adapting the floatation method of Suma and Srimathi (2014). *A. hypogaea* seeds were immersed for 12.00 hours in lukewarm water after which floating seeds which are non-viable were discarded and those that remained immersed (which are viable) were preferentially selected for propagation.

Treatment of soil and propagation of plant

Soil meant for the propagation of *A. hypogaea* was initially subjected to the following treatments: solarized inoculated, unsolarized uninoculated (control), solarized uninoculated and unsolarized inoculated with *Bradyrhizobium* sp. Each treatment was replicated thricewith soil receiving regular watering before seed propagation.

Seed propagation was done two weeks after rhizobial inoculation into soil. Viable seeds were propagated five per container of soil ensuring consistency in depth (2.0 cm deep). Thinning of germinated seeds was done leaving the best growth, two stands per container of soil. Propagated plants were monitored for four months ensuring regular watering (avoiding flooding condition).

Evaluating treatment effect on *A. hypogaea* germination

The effect of the various treatments on *A. hypogaea* germination was assessed by observing the earliest time of shoot appearance in each of the treated soils, while percentage germination was evaluated using the formula:

$$\text{Percentage germination (\%)} = \frac{\text{Number of germinated seeds}}{\text{Number of propagated seeds}} \times 100$$

Evaluating treatment effect on pod and nodule formation in *A. hypogaea*

Nodule and pod numbers per plant under the influence of the various treatments were evaluated after 4 months of plant propagation. Running tap water at slow speed was used to wash uprooted roots making sure there was no root damage. The number of nodules and pods per plant were then evaluated by enumeration after roots have been air-dried for 30 minutes.

Evaluating treatment effects on the weights (fresh and dry), root length and height growth of A. hypogaea

The effect of treatments on the weights (fresh and dry), root length and height growth were determined after 4 months of planting. For the evaluation of weights, plant was uprooted with roots washed in slow running tap water to free all adhering soil particles. Fresh weight was then taken after air-drying for 30 minutes, while dry weight taken after heating to constant weight in hot-air oven at 60°C with the aid of electronic weighing balance.

On the other hand, treatment effect on plant height growth was evaluated by measuring plant from it base to the tip of the tallest leaf, and root length measured from base to the tip of the longest root with the aid of a calibrated meter rule.

Analysis of data

Numerical data obtained from the study were evaluated employing statistical tools such dispersion, measure of central tendency, analysis of variance and Student's t-test (P □ 0.05).

Results

Baseline physicochemical characteristics of soil

Baseline data from this study showed that the soil employed for the propagation of *A. hypogaea* has a neutral pH with fair amount of nitrogen content and porosity sufficient for *A. hypogae* cultivation. Based on its textural constituents the soil may describe as loamy sand (Table 1).

Solarized soil temperature

Temperature of solarized soil varied between 28 and 32°C in the morning hours (10.00 hours), and 39 and 54°C in the evening hours (16.00 hours) within the solarization period. Comparatively, soil temperatures were consistently higher in the evening than morning hours. (Figure 1)

Treatments effects on *A. hypogaea* germination

In this study, not minding the fact that solarized inoculated treatment recorded the fastest germination time of 3.7 days, while unsolarized uninoculated recorded the most delayed germination (6.0 days); the differences in the germination time among the array of treatments were not statistically significant (P □ 0.05). Similarly, all the various soil treatments witnessed a 100% germination of propagated *A. hypogae* (Table 2).

Treatments effects on nodule and pod formation in *A. hypogae*

The number of nodules and pods per *A. hypogaea* plant were significantly affected by the applied treatments (P □ 0.05) (Table 3). While the lowest number of nodules and pods (31.0 and 3.0 per plant respectively) were recorded in solarized uninoculated soil, the highest number of nodules and pods (136.0 and 11.0 per plant respectively) were recorded in solarized inoculated soil.

Treatments effects on the weights (fresh and dry), height and root length growth in *A. hypogaea*

Fresh and dry weights of the plant were significantly affected by the various soil treatments (P □ 0.05) (Table 4). The highest fresh and dry weights were recorded in Solarized inoculated (20.3 and 7.6 g respectively), while the lowest was in solarized uninoculated treatment (10.0 and 3.0 g respectively).

Similarly, plant height and root length growth also varied with soil treatments (P □ 0.05) (Figure 2). While the highest height growth occurred in solarized inoculated (48.5 cm), the lowest occurred in solarized uninoculated soil (22.1 cm). In contrast to the height growth; the longest root length occurred in solarized uninoculated (43.7 cm) and the shortest in usolarized uninoculated soil (24.3 cm). However, while the root length growth in solarized inoculated did not differ significantly from solarized uninoculated, that of unsolarized uninoculated was also statistically the same with unsolarized inoculated (P □ 0.05).

Soil Properties	Values
Texture	
Sand (%)	73.0 ± 1.0
Silt (%)	18.0 ± 2.0
Clay (%)	9.0 ± 1.0
pH	7.2 ± 0.2
Water Holding Capacity (%)	71.0 ± 1.7
Nitrogen (%)	0.08 ± 0.01
Phosphorus (mg/kg)	0.7 ± 0.17
Organic Carbon (%)	1.5 ± 0.1
Organic Matter (%)	1.6 ± 0.3

Table 1: *Baseline physicochemical properties of experimental soil.*

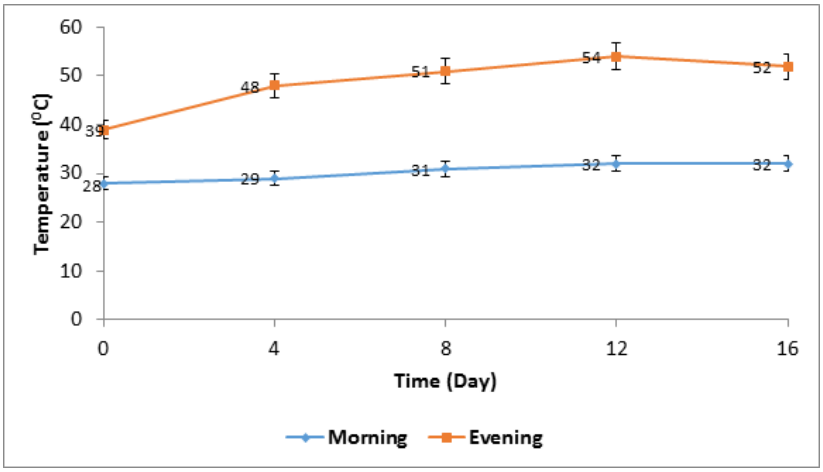

Figure 1: *Temperature fluctuation within the period of soil solarisation.*

Treatment	Germination Time (Days)	% Germination
Unsolarized uninoculated	6.0 ± 1.0[a]	100.0
Unsolarized inoculated	4.3 ± 0.6[a]	100.0
Solarized uninoculated	4.2 ± 0.8[a]	100.0
Solarized inoculated	3.7 ± 0.6[a]	100.0

Table 2: *Influence of different treatments on germination in A. hypogaea.*

Key: *Values with the same superscript alphabet in the same column did not differ significantly (P □ 0.05).

Treatment	Nodules/plant	Pods/plant
Unsolarized uninoculated	36.0 ± 7.8[a]	5.3 ± 0.6[a]
Unsolarized inoculated	70.3 ± 1.5[b]	8.3 ± 1.5[b]
Solarized uninoculated	31.0 ± 6.1[c]	3.0 ± 0.0[c]
Solarized inoculated	136.0 ± 17.8[d]	11.0 ± 1.0[d]

Table 3: *Influence of different treatments on nodule and pod formation in A. hypogaea.*

Key: *Values with the same superscript alphabet in the same column did not differ significantly (P □ 0.05).

Treatment	Fresh weight (g)	Dry weight (g)
Unsolarized uninoculated	12.7 ± 2.1[a]	3.8 ± 0.6[a]
Unsolarized inoculated	15.7 ± 1.5[b]	5.2 ± 0.4[b]
Solarized uninoculated	10.0 ± 2.0[a]	3.0 ± 0.6[a]
Solarized inoculated	20.3 ± 2.1[c]	7.6 ± 1.2[c]

Table 4: *Influence of treatments on fresh and dry weights of A. hypogaea.*

Key: *Values with the same superscript alphabet in the same column did not differ significantly (P □ 0.05).

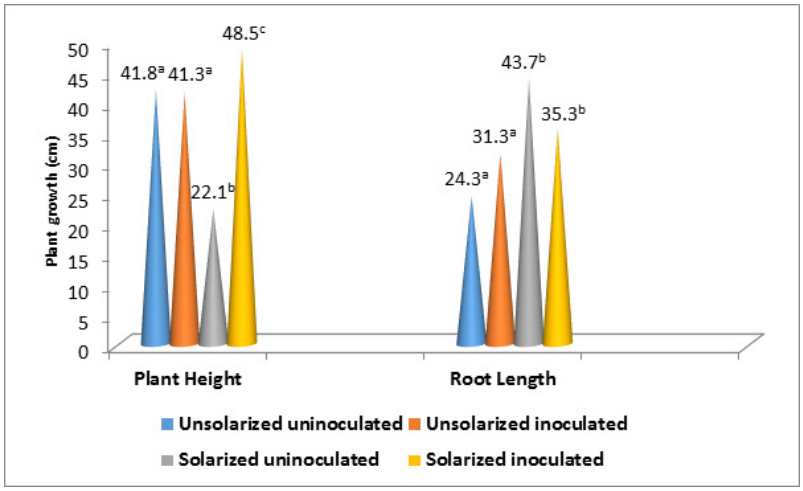

Figure 2: *Influence of treatments on the height and root length growth in A. hypogaea.*

*Key: Values with the same superscript alphabet for the same growth parameter did not differ significantly (P □ 0.05)

Discussion

Soil with a pH of 5.3 to 7.3, moderate organic matter, light coloured, sandy loam, loamy sand texture, with moderate water holding capacity and very low level of clay are deemed best for the growth of *A. hypogaea* (Nyambok., *et al*. 2011; Ajeigbe., *et al*. 2014; Okello., *et al*. 2014). Though the degree of tolerance to acidic pH depend on species; legume and rhizobial cells are generally sensitive to pH less than 5.0 as a result of magnesium and aluminum induced toxicity, which result in impaired nodulation and symbiotic nitrogen fixation (Lowendorf., *et al*. 1981; Munns., *et al*. 1981; Grahamp., *et al*. 1994; Mohammadi., *et al*. 2012), the overall consequent of these is a reduction in plant biomass and yield (Rice., *et al*. 2000). The soil engaged in this study typified texturally a loamy sand soil, having a moderate water holding capacity with a neutral pH (7.2). In line with the aforementioned reports, the baseline properties suggest that the soil is considerably adequate for the propagation of *A. hypogaea*.

Soil temperature under the influence of solarization fluctuated in both morning and evening hours within the 16.0 days of treatment with higher temperatures in the evening than morning. Previous report by Chauhan., *et al*. (1988), corroborate this result. The highest temperature attained at the depth of 10 cm in both morning and evening was 39 and 54°C respectively. These temperatures are sufficient to achieve effective solarization within the period of exposure. Elmore., *et al*. (1997) reported that solarization of soil at temperature of 53°C at depth of 10 cm in greenhouse treatment was sufficient to decimate the population of soil microorganisms within 16 days. Similarly, Sonku and Nomura (1987) maintained that temperature sustenance at 40°C on a daily basis for 10 successive days is what is required to kill soil-borne microorganisms including pathogens.

Notwithstanding the fact that the time and percentage germination in *A. hypogaea* were not statistically different among the various treatments applied to soil, it is pertinent to point out that Solarized inoculated recorded the fastest germination time followed by Solarized uninoculated soil. This seemingly infinitesimal speed in germination observed may be attributed to solarization effect and not the consequence of rhizobial inoculation. Accelerated germination has been reported to occur in plant due to the influence of solarization (Stapletonand Devay, 1986; Jat., *et al*. 2014) but not as a result of the inoculation of rhizobia (Argaw, 2012; Ndlovu, 2015), since it is irrelevant to germination (Ndlovu, 2015) as the fixation of nitrogen starts around 14-35 after infection by rhizobial cells (Dupont., *et al*. 2012).

This study indicate there were significant differences in the number of nodules and pods formed in *A. hpogaea* as a result of the various treatments applied to soil. All the soils inoculated with *Bradyrhizobium* sp. Recorded higher yield of nodules and pods than the uninoculated, with Solarized inoculated producing the highest yield while, solarized uninoculated producing the least. These findings are in consonant with the reports of previous workers. Although, Ahmed., *et al*. (2006), in their own studies maintained that while nodulation was enhanced in *Vigna radiata* (Mungbean) in soil inoculated with *Rhizobuim* over uninoculated, their yield did not differ significantly. However, in a similar study, Megueni., *et al*. (2006), observed enhanced nodule and seed yield in soybean planted in solarized inoculated soil with *Rhizobium* over solarized uninoculated, unsolarized uninoculated and unsolarized inoculated soils with *Rhizobium*. In the same vein, Habete and Buraka (2016); Nambiar (1985), reported a higher level of nodulation and pod formation in two varieties of *P. vulgaris*; and *A. hypogaea* respectively in soil inoculated with *Rhizobium* over the uninoculated. Furthermore, Yusif., *et al*. (2016), also recorded higher number of effective nodules in *A. hypogaea* inoculated with *Rhizobium* in contrast to uninoculated.

Increasing compactable rhizobia inoculum size in soil has been shown to increase yield in leguminous plants (Rice, 1975). Nevertheless, microbial soil inoculants are faced with several difficulties such as ability to effectively out-compete indigenous soil microflora that are well adapted to the environment and repel protozoan grazing (Bashan, 1998).

Soil solarisation may help in overcoming these challenges as resident microflora in solarised soil are either killed or weakened resulting in their diminished competitive ability. The increased nodulation and pod formation recorded in *A. hypogaea* in the solarized inoculated soil over the rest treatments in this study may therefore be attributed to the favourable environment that allowed the survival of a critical number of *Bradyrhizobium* sp. and their proliferation upon their re-inoculation in the post-solarized soil.

A. hypogaea weights (fresh and dry) measurement revealed that these growth parameters varied with the applied treatments. Solarized inoculated soil produced a higher plant biomass than the rest treatments. This improved plant biomass in the solarized inoculated soil over the rest treatments may once again be ascribed to increased availability and utilization of fixed nitrogen consequent upon the proliferation of inoculated *Bradyrhizobium* sp. and increased nodulation efficiency in a less competitive solarized soil. *Rhizobium* is a plant growth promoting rhizobacteria (Ahmad and Khan, 2010) and has been shown to enhance plant dry matter yield (Thakur and Panwar, 1995). While investigating A. hypogaea response to exogenous inoculation of some stains of *Bradyrhizobium*, Baba- Moussa., *et al*. (2014), reported that strain STM 5945 produced a higher aerial and root dry biomass than the uninoculated control. Similarly, Yusif., *et al*. (2016), also observed a higher root and shoot dry weights in *A. hypogaea* inoculated with Rhizobium than uninoculated control.

The various treatments resulted in differential height and root growth in *A. hypogaea*. While the highest height was registered in solarized inoculated soil, solarized uninoculated which recorded the lowest height, also had the longest root growth which was however statistically the same with solarized inoculated soil. The enhanced height growth in *A. hypogaea* in the solarized inoculated soil could be attributed at least in part to *Bradyrhizobium sp*. inoculation. Enormous body of unsubtle evidence supporting this submission are well documented. Shaheen and Rahmatullah (1994), Basu., *et al*. (2006), Baba- Moussa., *et al*. (2014) and Yusif., *et al*. (2016), have all reported previously that increased height growth in *A. hypogaea* occurred following rhizobia inoculations as compared to uninoculated controls. The increased root length growth in the solarized inoculated and solarized uninoculated over the rest treatments may be credited to solarization effect, a common denominator separating the two other treatments in this case. Soil solarization has been reported to stimulate plant root growth. Root growth stimulation have been attributed to increased dissolution and availability of nutrients such

as Mg_2^+, NH_4^+, K^+, NO_3^+, Ca_2^+ and fulvic acid during thermal breakdown of soil organic matter (Elmore., *et al*. 1997; Pokharel, 2011; Chauhan., *et al*. 1988).

Conclusion

It is evidently clear from this study that the yield of *A. hypogaea* an important dietary legume to man and animal can be improved through the inoculation of *Bradyrhizobium* sp. (a symbiotic nitrogen fixing bacteria) in a post-solarized soil.

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