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Effect of Rhizobium Inoculation on Seedling Growth of *Albizia niopides* (Spruce Ex Bnth) Burkart

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Abstract: Investigation was carried out on the effect of Rhizobium inoculation on the Seedling Growth and development of *Albizia niopoides* in a glass house at the International Institute of Tropical Agriculture (IITA) Ibadan Nigeria. This involved isolation of Rhizobium from this species microbiologically. There were four treatments in all namely 1 mL Rhizobium inoculation; 5 mL Rhizobium inoculation; 10 mL Rhizobium inoculation and control (without Rhizobium inoculation). They were arranged in a randomized complete block design and replicated three times. The parameters considered went height, collar diameter, leaf number and leaf area. Significant differences were recorded among the treatments with respect to plant height. 10 mL Rhizobium inoculation gave maximum height of 93.6 cm; Diameter increment of 0.77 mm (14 WAP), leaf number 14.7 and leaf area 193.4. The inoculated treatments produced nodule number ranging between 336.7 and 496.0 while uninoculated treatment gave 247.6 nodules dry matter production was directly proportional to the quantity of Rhizobium inoculation applied. 10 mL Rhizobium broth produced the highest nodule dry matter of 24.7 g which was significantly different from the control (13.97 g). No significant difference was recorded among the treatment vis-a-vis leaf and root dry matter production by seedlings of *A. niopoides*. it is recommended that Rhizobia inoculation should be adopted for the establishment of some nitrogen fixing tree where native Rhizobia is not available.

Key words: Rhizobium, seedling, isolation, nitrogen fixation

INTRODUCTION

The population pressure in developing countries has led to increasing agricultural activity. It is estimated that 56% of the total energy used in agriculture in this region is consumed in the production and consumption of nitrogen fertilizer. The more recently developed height yielding varieties of crop require increasing quantities of N fertilizer for optimum production. The amount of commercial N fertilizer available was not sufficient. to meet these requirements. Evidences suggest that biological nitrogen fixation will be viable alternative to sustained availability of nitrogen in the tropics.

Legumes are crucial to the balance of nature for many are able to convert nitrogengas from the air into ammonia, a soluble form of nitrogen, which is readily utilized by plants. Though, other few plant families have species with this ability, legumes produce the greatest mass of biologically fixed nitrogen.

The conversion of atmospheric nitrogen to ammonia is actually accomplished by soil bacteria belonging to the genus Rhizobium. The bacteria infect the legume root and the plant, in reaction, form swellings (nodules) on the root surface. Within these nodules the Rhizobia proliferate and thrive. There they absorb, much of the nitrogenous product and use them to produce protein, vitamins and other nitrogen containing compounds (Sanginga *et al*., 1995).

Stackebrad *et al*. (1998), while considering the occurrences of nitrogen-fixing bacteria phtogenetically, based on 165 RNA analysis is and excluding some green sulphur and non-sulphr bacteria discovered that these bacteria could be found on divergent branches within the alpha-sub/division of purple photosynthetic bacteria. Biological Nitrogen fixation according to Elkan (1992), depends upon a highly conserved enzyme complex common to all bacteria with this property, although organization and genomal location varies. He opined that nitrogen-fixing process is similar indifferent prokaryotic system and depends upon the following factors; nitrogenase enzyme complex; a high energy requirement as ATP; (anaerobic condition for nitrogenase activity) and source of strong redundant (Haaker and Klugkast, 1987).

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They are of the view that biological nitrogen fixation accounts for two-thirds of the N-available to Eukaryotes.

The study was designed to assess the influence of different quantity of Rhizobium strain on the growth and development of *Albizia niopoides*.

MATERIALS AND METHODS

The experiment was conducted in a glass house at the International Institute of Tropical Agriculture, Ibadan.

Soil preparation: 2.9 kg of soil was collected from IITA, sieved and filled into 7 (17.5×12.5 cm) pots after which they were appropriately labeled according to treatments. Seedlings of *Albizia niopoides* (4 weeks old) raised in the nursery were later planted into the pots and left for another two weeks in the screen house for proper establishment. The seedlings were then inoculated with either 1, 5 or 10 mL Rhizobium broth. The fourth treatment was the control (without Rhizoium inoculation). They were arranged in a randomized complete block design and were watered daily with 20 mL of distilled water the plants were assessed for height, collar diameter, leaf number and leaf area. Nodulation and biomass production were determined after four months.

Isolation of the rhizobium: Several nodules of *Albizia niopoides* seedlings were surface sterilized and Rhizobia in these nodules were isolated according to method used by Vincent. Several nodules were placed in a test tube filled with sterile water and shaking vigorously. In order to drain the water without using the nodules, the tube was covered with a wire mesh and later inverted for proper draining. The above process was repeated several times until the nodules were thoroughly clean and the water used for washing became clear.

95% ethanol was added to the tube to cover the nodules for 10 sec and drained. 3% H_2O_2, a sterilizing agent was introduced into the tube and drained after 4 min. Sterile water was then used to rinse the nodules. This was repeated six times to ensure proper sterilization.

The glass rod meant for crushing the nodules was sterilized by diping in ethanol and passing it through a naked flame. Before crushing, two drops of sterile water were added to the nodules to enhance the crushing and provide enough inoculum. A loopful of the inoculum from the crushed nodule was then streaked on the surface of a Congo Red Agar (CRA) labeled and incubated for 4-6 days at 28°C. The CRA was used to differentiate *A. Niopoides* rhizobium from other rhizobia. After full growth of the mixed culture Rhizoium strain was sub-cultured into another CRA plate to obtain pure culture which was pinkish in colour. The plates were then incubated for about six days. The pure culture was then restreaked on to a new YEM agar plate. After the growth of pure culture on the new plate, a loopful of the culture was used to prepare a smear for gram staining. After the grain staining, the bacteria assumed a red colour which indicated that it was a gram negative strain. From the pure culture a loopful was inoculated into agar slant for preservation. The inoculated slants were incubated and later stored in a refrigerator for future use.

When this bacterium was to be used in the green house for inoculation, a YEM broth was prepared and inoculated with the preserved culture form the slant. The broth was grown in a shaker for 3-6 days.

RESULTS

Height: The height growth of the plants varied in its responses to the quantity of Rhizobium broth introduced. In the second week, the lowest height growth of 13.8 cm with standard error of 0.75 was record for the control experiment while 10 mL of Rhizobium inoculation gave the highest height growth of 20.0 cm with 1.97 standard error.

At 4 Weeks After Planting (WAP), the same trend continued, however, the differences between 1 mL and 10 mL rhizobium inoculation were not significant. They gave heights of 20.12 cm (S.E. 2.04) and 24.62 cm (S.E. 2.54), respectively. The unioculated control experiment still produced the lowest height growth of 17.48 cm with 1.34 S.E.

The advantage of large quantity of Rhizobium inoculation over the rest of the treatment was also visible at 6WAP as 10 mL Rhizobium broth produced seedling height of 31.4 cm (S.E. 2.67) which was significantly different from those given by 5 mL and control which had values of 23.5 (S.E. 2.02) and 22.3 (S.E. 2.44), respectively. At 8WAP, the analysis of variance indicated that no significant differences existed among all the treatments. Similar trend was observed in the 10th week where 10 mL of Rhizobium broth produced *A. niopoidies* seedling height of 50.1 cm (S.E. 4.56). The minimum height of 40.2 cm (S.E.3.4) was recorded for 1 mL treatment. This trend persisted till 20 WAP as analysis of variances indicated that the differences between the treatments were not analysis of variances indicated that the differences between the treatments were not significant. However, on the average 10 mL of Rhizobium inoculation which gave 93.6 cm height (S.E.41) positively influenced height growth of *A. niopoides* seedlings more than the rest of the treatments. The values were significantly different from 1, 5 mL and control treatments. The unioculated trials gave the minimum height growth of 77.2 cm (S.E. 36).

Table 1: Mean values of *A. niopoides* growth parameters under varying quantities of rhizobium inoculation

	Weeks after planning										
Treatment	2	4	6	8	10	12	14	16	18	20	22
1 mL	15.04b	20.1ab	25.3b	36.0a	40.2a	54.9a	62.4a	67.0a	74.7a	77.3a	85.0a
5 mL	15.8b	18.8b	22.3b	32.7a	41.7a	57.4a	63.4a	69.4a	75.0a	78.2a	82.0a
10 mL	20.0a	24.6a	31.4a	42.7a	50.1a	62.9a	68.6a	75.7a	77.0a	93.59a	102.4b
Control	13.8b	17.5b	23.5b	30.1a	38.7a	51.9a	60.1a	71.0a	74.0a	77.2a	80.41a
DSD at 0.05	3.79	5.32	6.8	9.52	11.8	14.3	13.2	14.9	14.5	14.4	14.4
1 mL	0.14ab	0.26ab	0.43a	0.47a	0.55a	0.62a	0.70a	0.80a	0.89a	0.95a	0.97a
5 mL	0.14ab	0.25ab	0.31a	0.47b	0.51b	0.60ab	0.64a	0.80a	1.01a	1.03a	1.04a
10 mL	0.16a	0.29a	0.45a	0.55a	0.57a	0.68a	0.72a	0.84a	1.06a	1.12a	1.18a
Control	0.13b	0.24b	0.38a	0.48b	0.50b	0.56b	0.60a	0.76b	0.82a	0.91a	0.94a
DSD at 0.05	0.04	0.05	0.08	0.07	0.06	0.06	0.07	0.09	0.22	0.13	0.27
1 mL	6.56a	7.78a	8.9a	10.4b	10.9b	11.9a	12.8b	13.2b	13.78b	14.1b	15.55b
5 mL	6.44a	8.00a	8.9a	11.4b	11.1b	12.7ab	13.3b	13.6b	14.22b	15.0b	15.55b
10 mL	6.67a	8.22a	9.89b	12.4a	13.3a	14.7a	15.1a	16.1a	17.7a	18.3a	20.1a
Control	6.11a	6.67a	8.8a	10.2b	10.8b	11.8b	11.8b	12.3b	13.4b	13.6b	12.22b
DSD at 0.05	2.01	2.30	2.01	2.14	2.70	2.70	2.59	3.19	2.96	3.60	3.51
1 mL	68.0b	121.4a	148.2b	172.2b	174.0a	176.8a	180.4a	188.2a	208.1a	209.4a	214.2a
5 mL	57.0b	100.3a	142.0b	187.9b	192.0a	193.4a	201.0a	206.5a	219.1a	200.2a	224.4a
10 mL	107.5a	143.8a	164.2a	196.8a	206.4a	211.0a	214.1a	218.4a	234.3b	238.8a	241.8a
Control	69.3b	104.9a	144.0b	176.2b	178.2a	184.2a	187.3a	187.5a	190.4b	191.5a	193.4b
DSD at 0.05	43.0	58.7	39.7	58.2	47.2	57.5	43.6	52.7	54.9	43.0	52.6

Means with the same letter are not significantly different

Table 2: Nodule dry matter yield by seedlings of *A. niopoides* with varying levels of rhizobium inoculation

Treatments	Mean
1 mL rhizobium broth	15.08ab
5 mL rhizobium broth	17.43ab
10 mL rhizobium broth	24.76b
Control	13.96b
LSD at 0.05	3.45

Means with the same letter are not significantly different

Diameter increment: The mean diameter increment of *A. niopoides* seedling under various quantities of Rhizobium broth is shown in Table 1.

The analysis of variance revealed that significant differences existed among the treatments at 2WAP. The lowest diameter increment of 0.13 cm (S.E. 0.02). At 6 weeks after planting the difference between the treatments were not statistically significant. However, the seedling diameter increment was influenced more by 10 mL of Rhizobium inoculation than the rest of the treatments. The values ranged between 0.31cm (S.E. 0.04)and 0.45 cm (S.E. 0.02). In the eight week, greater quantity of rhizobium inoculation appeared to have a considerable influence on diameter growth as depicted by diameter increment of 0.55 cm (S.E. 0.02) which differed significantly form other treatments. The results obtained at 10WAP showed that there were significant differences among the treatments. The control treatment on the average gave the lowest diameter values of -.68 cm (S.E. 0.2) and 0.56 cm (S.E. 0.01), respectively. The responses of diameter increment to Rhizobium inoculation were not significant at 14 weeks for all the treatments but 10 mL Rhizobium inoculation still produced the highest diameter increment of 0.72 (S.E. 0.03. The only significant difference observed at week 16 was between all the inoculated treatments and the control (without inoculation).

As from 18-22 WAP no significant differences were observed. However, 10 mL of Rhizobium inoculation still exhibited the highest diameter increment of 1.18 cm (S.E. 0.03).

Leaf number: Mean leaf number of *A. niopoides* seedlings under different quantities of Rhizobium inoculation is shown in Table 2.

The analysis of variance revealed no statistical differences among the treatments at 2WAP. 10 mL inoculation produced the highest foliage of 6.67 (S.E. 0.62) of *Albizia* seedlings than the rest of the treatments while .11 (S.E. 0.67) minimum number of leaves was recorded under the control. Similar trend was observed at 4WA. However higher quantity of Rhizobium still gave a corresponding increase in the number of leaves available on *A. niopoides* seedlings as much as 8.2 (S.E. 0.82). The result obtained at 6WAP indicated a significant difference between 10 mL Rhizobium broth with a value of 9.9 (S.E. 0.005) and the rest of the treatments. At this period the maximum number of leaves yielded by 10 mL Rhizobium broth exerted the same influence on Albizia seedlings as the foliage number recorded under each of them gave the same value of 8.9 (0.75 S.E.). Also at 8WAP, Significant differences were observed only

between the control and other treatments. The values ranged between 10.4 (S.E. 0.69) and 12.4 (S.E. 1.21), respectively were not significantly different. At 14WAP, leaf senescence was observed, the rate of senescence for *A. niopoides* appeared to be much. For instance, the control (uninoculated) treatment exhibited a decrease in the number of leaves from 11.8 (S.E. 0.62) in the 12th week to 11.6 (S.E. 0.73) in the 14th weeks. However, statistical analysis still indicated an insignificant difference between the control; 1 mL and 5 mL Rhizobium broth which gave 11.6, 12.8 and 13.3 leaf numbers, respectively. On the other hand, application of 10 mL Rhizobium broth still significantly increased the foliage of *A. niopoides* with a value of 15.1 (S.E. 1.5). As from the 16th week after planting to final assessment at 22WAP, the result indicated a similar effect of Rhiobium inoculation on *Albizia* leave when compared to that of week 14. That is, 10 mL Rhizobium inoculation produced more foliage than the other treatments.

Leaf area: The mean leaf area values of *A. niopoides* seedlings under different quantities of Rhizobium inoculation is shown in Table 1.

At the beginning of the assessment (2 WAP), the positive influence of higher quantities of Rhizobium inoculation was again demonstrated as 10 mL Rhizobium broth produced leaf area increment of 107.5 cm^2 (S.E. 20.9) that differed significantly from the rest of the treatments. At week 4, the effect of Rhizobium broth introduced to the seedlings was not significant, on the average however, 10 mL still gave the highest value of 104.9 with 23.4 S.E. The results obtained for 6 weeks after planting revealed that leaf area value for 10 mL Rhizobium broth which gave 164.2cm^2 (S.E. 18.3) was statistically different from others. Similar trend was observed in week 8 when leaf area increment responded significantly to higher quantities of Rhizobium inoculation with values ranging between 176.2 9S.E. 15.2) for control and 196.8 (S.E. 26.8) for 10 mL treatment. As from the 10th to the 16th weeks after planting, analysis of variance showed that the differences in the leaf area values for all the treatments were not statistically significantly. However, on the average, inoculation till contributed greatly to leaf area increment when compared to the uninoculated treatment which gave lowest value of 187.5 (S.E. 12.9) in the 16th week. A significant difference was observed between 10 mL treatment and the rest at 18 weeks after planting. The leaf areas were 208.1, 219.1, 234.3 and 190.4 given by 1 mL, 5 mL, 10 mL and the control treatments, respectively. Similar trend was also repeated at the 20th week after planting (WAP) with the uninoculated plants possessing minimum number of 191.5 (S.E. 11.6) leaf area increment. At the time

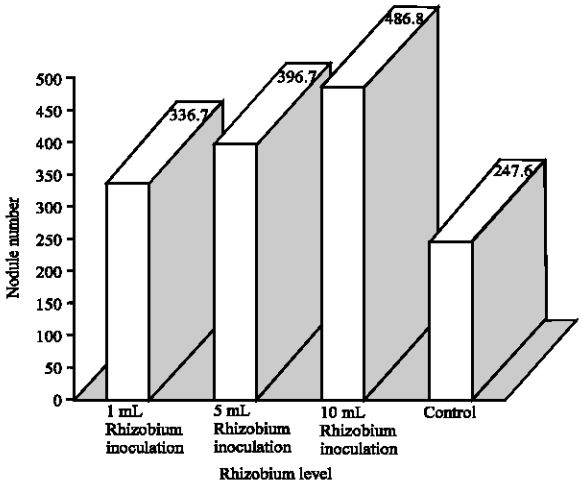

Fig. 1: Nodulation by albizia niopoides under rhizobium inculation

of final assessment there existed no significant difference among the inoculated treatments, however, they differed statistically from the control which give 193.4 (S.E. 15.6).

Nodule number: Inoculation had a profound effect on nodulation. uninoculated seedlings produced an average of 247.6 nodules (S.E. 40.6) as shown in Fig. 1, while the inoculated treatment gave nodule numbers ranging between 336.7 (S.E. 70.2) and 486.0 (S.E. 59.9). Quantities of rhizobium broth introduced also determined to a great extent the rate of nodulation. For instance, 10 mL of rhizobium inoculation gave 486.8 with 59.9 S.E. that differed significantly from other treatments. However, 1 mL and 5 mL produced more nodules than the control. They were 336.7 (S.E. 70.2) for control and 396.7 (S.E. 40.6) for 5 mL.

Dry weight: Dry matter yield by seedlings of *A. niopoides* with varying levels of Rhizobium inoculation is shown in Table 3 mL of Rhizobium inoculation gave 61.1 g (S.E. 6.3) dry matter that was more than the rest of the treatments. The uninoculated seedlings produced the lowest dry matter accumulation of 29.03 g (S.E. 3.13) with respect to stem.

The influence of Rhizombium inoculation on leaf and root dry matter production by *A. niopoides* seedlings followed the same pattern to that of stem. There were no significant differences among all the treatments except 10 mL Rhizobium inoculation which gave 18.83 g (S.E. 1.05) and 78.80 g (4.65 S.E.) of dry matter for leaf and stem, respectively.

Dry matter yield of nodules under different quantities of Rhizobium inoculation is shown in Table 3.

Table 3: Dry matter yield by seedlings of *A. niopoides* with varying levels of rhizobium inoculation. (Means with the same letter are not significantly different)

Treatments (X)	Stem (g)	Leaf (g)	Root (g)
1 mL rhizobium broth	30.70b	12.89b	53.96b
5 mL rhizobium broth	30.78b	13.53b	54.71b
10 mL rhizobium broth	61.07a	18.83a	78.90a
Control	29.03b	11.80b	53.18b
LSD at 0.05	9.09	2.22	9.94

Means with the same letter are not significantly different

The results indicated that dry matter production was directly proportional to the quantity of Rhizobium inoculation applied. As expected, 10 mL of rhizobium broth produced the highest nodule dry matter accumulation of 24.76 g. (S.E .4.89) while the uninoculated control treatment gave 13.97 (S.E. 2.64), which was the lowest. The analysis of variance table revealed that the differences were statistically significant at 5% alpha level of significance.

DISCUSSION

Atayese *et al.* (1993) studied that influence of drought and inoculation with mycorrhiza on dry matter production, nutrient uptake and water relations of some species. They found out in general, that Vesicular Arbuscula Mycorrhiza (VAM) plants survived better and had more dry matter and nutrients and a larger leaf area, than uninoculated plants. The results obtained here for *A. niopoides* seedlings are in agreement with the above findings. The study by Mulongoy and Owoaje (1992) also lend more credence to this. This significant increase in height observed at 4WAP between 10 mL Rhizobium broth and the control is still indicative of the amount of nitrogen fixed by this species, which in this case could be higher. Adeola and Sumaila (1999), opined that the amount of nitrogen fixed by any nitrogen-fixing tree is related to its nitrogen fixing potential, i.e its ability to fix nitrogen in the absence of any limiting factor.

The significant response in diameter increment obtained between the inoculated treatments and the control could be ascribed to the quantity of nodules advanced by Mac Dicksen (1994), which would lead to an increase in plant growth, including seed production and an increase in N or protein level of the seed at 4 WAP. The significant increase in diameter as influenced by the 10m broth reported in this study could be due partly to nitrogen addition through its nodules. Similar result was obtained for Paraserianthes falcataria in philipine (Smith 1992).

Nitrogen deficiency is a common cause of chlorosis which can be corrected through fertilization or Rhizobium inoculation. The results obtained here attested to this. Leaf number increased considerably in the control experiment until week 14 where thee was a reduction which probably was due to lower rate of nodulation. Kramer and Kozlowoki (1994), however, noted that a relatively large area is necessary for the photosynthesis needed to support good growth. This in turn will depend on the total number of leaves available on the leguminous species. The insignificant differences obtained in the first few weeks among the treatments could be due to a slow initial phase of nitrogen fixation; and the seedling growth as reflected in the rate of leaf production, which was probably initiated by the residual nitrogen in the soil.

The importance of inoculation in seedling establishment was clearly demonstrated at 22WAP as well inoculated seedlings differed significantly from the uninoculated one in their leaf area values. Chaboot and Hicks (1982), considered this as part other strategy of a species to maximize productivity under different environmental conditions. Moreover, extended leaf longevity is required to pay back the investment in leaves displayed in less productive condition (Khosla *et al.*, 1992).

Application of mineral nitrogen is known to delay senescence of leaves and prolong photosynthetic activity. The higher leaf dry weight obtained here in all the uninoculated treatments as compared to the control was indicative of the amount of nitrogen present, which in this case was greater probably due to higher nodulation rate. Similar results have been reported by Ezenwa and Atta-Krah (1992).

As regards nodulation and nodule dry weight, significant ($p<0.05$) differences were actually observed between 10 mL Rhizobium inoculation and the control which could have been due to increased nodulation from the latter. Similar result was reported for inoculated *Leucaena leucocephala* seedlings, but occurred only among those with P fertilization Osonubi *et al.* (1995). Okon, (1996), also reported increases in nodule number as a result of inoculation. Mulongoy and Owoaje (1992) also observed tremendous increase in nodulation of *G. sepium* after 6 months of establishment.

CONCLUSION

This study has demonstrated unequivocally the ability of Rhizobium inoculation to enhance establishment of *A. niopides* species where native rhizobia is

probably not available. This is extremely relevant to the farming system of lowland humid tropics where the soils are lacking adequate nutrient for crop growth and development. If Rhizobium inoculation is incorporated into our highly fragile, degraded and impoverished soil it will go a long way in improving soil fertility and eventually enhance crop productivity; thereby reduce heavy reliance on industrial fertilizer which is always beyond the reach of our local farmers because of its prohibitive cost.

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