Effects Of Rhizobium Inoculants And Growth Stages On Shoot Biomass And Yield Of Soybean (Glycine Max (L.) Merril).

Osei Dominic, Shirley Lamptey, Christian Larbi Ayisi, Andrews Apraku

ABSTRACT: The experiment was conducted during the 2012 farming season at the University for Development Studies, Nyankpala in the Tolon District of the Northern Region of Ghana. The objective of the study was to determine the influence of rhizobium inoculants and growth stages on shoot biomass and yield of soybean. Two levels of inoculation regimes (uninoculation (-In) and inoculation (+In)) were combined with four sampling stages (vegetative stage, flowering stage, pod stage and physiological maturity stage). Parameters measured were canopy spread, leaf area, days to 50% flowering total grain yield as well as biomass. The results indicated that canopy spread was not significantly ($p>0.05$) influenced by the inoculants at 6 WAP and 9 WAP. However, inoculation ($p<0.001$) influenced canopy spread at 3 WAP. Also our results shows that days to 50% flowering was significantly affected by inoculation ($p<0.003$) and varied among treatment. The biomass of the inoculated plot was higher (21.6 Kg) than that of the non- inoculated (15.9Kg) plot in all the sampling stages of the soybean with R2 +In having the highest (22.8 Kg) and R4-In having the least (15.4 g). We therefore document for the first time that high yield of soybean can be obtained under farmer's conditions provided the seed are inoculated with peat base inoculants.

Key words: Rhizobium; Soybean; Inoculants; Growth; Biomass; Spread; Yield.

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1 INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) belongs to the family leguminosae and a subfamily Papilinoideae, tribe Phaseodeae and the genus Glycine (Simmond *et al.,* 1999). It is an oil crop increasing in popularity, especially in Northern Ghana. It is rich in protein and used to fortify various foods, in order to improve their nutritional quality (8). Soybean is also important in the supply of high quality animal feed (14). It has been estimated that 1.6 million metric tons are needed annually to satisfy domestic and industrial needs (15). There is therefore a wide margin between what is needed and what is currently produced. The yield gain of soybean Ghana falls within a range of 0.4-1 t/ha (2). Soybean being a legume crop has the ability to supply their own nitrogen needs provided they have been inoculated and there is efficient nodulation in the plant. Soybean seed inoculants are available in three forms: peat based, liquid, and granular. Peat-based powdered inoculants are the most popular commercial form. Soybean is normally inoculated using laboratory-made peat base inoculants containing bacterial strain. Peat is most commonly used as the carrier for Rhizobium inoculants. The function of peat is to retain the viability of Rhizobium over long periods without loss of effectiveness (1). Peat-based inoculants provide some protection against environmental stresses, particularly lack of moisture and resulting bacterial desiccation (10; 26). Soybean inoculants are inexpensive and becoming more convenient, with new bulk seed applications and bacterial life extenders allowing inoculants to live on stored seed for much longer periods while maintaining effectiveness. Inoculation of soybean seeds can increase yields dramatically by 50% or more (4) and improving soybean root colonization. When soybean roots are infected by the appropriate *Rhizobium,* nodules are generally formed with regard to their potential for Nitrogen fixation (23). According to (17), lack of effective nodulation and unavailability of suitable varieties are among the causes of poor yield. Green manures, often known as cover crop, are plants which are grown to improve soil structure and nutrient content of the soil. Green manures are cheap alternative to artificial fertilizers and can be used to complement animal manures. Green manure means planting a crop (soybean) that is meant to be incorporated into the soil. According to (18), weeds can be a serious problem in soybean cultivation as they may reduce yield in three different ways: which are competition for crop growth factor, harboring pests and interfering with harvest. (9) reported that, 20-100% of yield loss depends on methods of weed control, timing and rate of fertilizer application by farmers. For population, a given stage is reached when more than 50% of the plants sampled have achieved the specified growth stage (3). The R –Stages of the soybean is the various reproductive events that occur during the growth of soybean. The reproductive (R) stages include the vegetative stage (R1), flowering stage (R2), full pod (R3) and harvest maturity (R4). Soybean production has challenges such as low yield due to failure of Crop to establish, planting depth of the crop, unimproved seeds, low soil fertility, and above all lack of effective nodulation (12). The objective of this present work was therefore to evaluate the effects of rhizobium inoculants and growth stages on shoot biomass and yield of soybean (jenguma) focusing on the effect of rhizobium inoculants on soybean yield and determine the effects of different growth stages on shoot biomass of soybean.

2.0MATERIALS AND METHODS

2.1 Geographical Location of the Experiment

The study was carried out in 2012 at Nyankpala campus of the University for Development Studies farm for the future, Tamale in the Northern Region of Ghana from July to October, 2012. Nyankpala is situated on latitude 90 25"N, longitude 00 58"W and altitude 183m above sea level (21).

2.2 Experimental Design

The experiment was laid in a randomized complete block design (RCBD) with nine treatments and four replications in a 2x4 factorial experiments. The treatments were Inoculated seeds and Non-inoculated seeds (-In, +In) with four different Reproductive growth stages (vegetative, flowering, pod and physiological maturity) and weed plot. Plot size was 2.50m × 2.50m; with inter row and intra row spacing of 1.0m and 1.50m respectively. The total plot size was 14.50m × 30.50m = 442.25m^2. In all nine experimental treatments were used with the inscription T1, T2, T3, T4, T5, T6, T7, T8 and T9. T1, T2, T3 and T4 had R1, R2, R3 and R4 plus inoculated seeds respectively while T5, T6, T7 and T8 had R1, R2, R3 and R4 minus inoculated seeds with T9 being weed plot.

2.3 Agronomic Practices
The soybean variety Jenguma was used. The seeds were obtained from the Soil Microbiology Department of Savanna Agricultural Research Institute (SARI) Nyankpala. For the inoculated plots, 520g of seeds were weighed using an electronic scale and put in a bowl, reasonable amount of water was sprinkle on the seeds and stirred uniformly. 2.6g of rhzobium inoculants was added to the wetted seeds and stirred. The inoculated seeds were allowed to dry under room temperature for 30 minutes and planted on the ridges with a planting distance of 50x10cm on 12th July, 2012. The experiment was done to coincide with the rainfall period, thus the plants were rain fed during the period of the experiment. Weeding was done throughout the growing period after planting. This was done through hoeing at 2, 5 and 9WAP. Fertilizer application was done on 2WAP at 30kg/ha of P_2O_5. The application was done uniformly across the plots because it was not a treatment.

2.4 Data Collection
Data was collected on crop establishment, plant height, leaf area, canopy spread, and shoot biomass at reproductive stages, days to 50% flowering, number of pod per plant, 100 grain weight, number and weight of nodules, and total grain weight as follows.

2.4.1 Plant Biomass sampling
This parameter was taken at different growth stage from the sampled area (the three inner rows) for the soybean biomass determination and nodulation assessment that is the biological nitrogen fixation (BNF) assessment. The various growth stages were vegetative stage (R1), flowering stage (R2), full pod stage (R3) and physiological stage (R4).this was done by Establishing the sampling area for each plot and number of plants counted in the area and the figure recorded; Plants in the sampling area from each plot were harvested; Shoots were separated from the roots; Shoots from each plot were bulked, weighed and recorded; Samples of three were taken from each plot, weighed and recorded; Samples were Air- dried for at least one day and oven dried at 80˚C for 48 hours (to a constant weight) and weight recorded.

2.4.2 Canopy Spread
This parameter was taken at 3, 6, 9 WAP by measuring the diagonals of the canopy of the plants and an average calculated on each of the thirty two (32) plots.

2.4.3 Leaf Area
Leaf area was taken at 3, 6 and 9 WAP. The length and breadth of the leaf from the six tagged plants were recorded and an average calculated on each of the thirty two (32) plots.

2.4.4 Days to 50% flowering
This was taken by counting the number of days from planting to when 50% of the plants in the net plot flowered

2.5 Data Analysis
Data collected were subjected to analyses of variance (ANOVA) using the computer statistical package Genstat (2008 Edition) and treatment means compared using the least significance difference (LSD) at 5% probability level.

3.0 RESULTS AND DISCUSSION

3.1 Canopy spread of soybean plant (cm) and Leaf area (cm^2) of soybean
Fig. 1a and b represents our results for effects of inoculation regime on canopy spread and leaf area of soybean respectively. Canopy spread was not significantly ($p>0.05$) influence by the inoculants at 6 WAP and 9 WAP. However, inoculation ($p<0.001$) influenced canopy spread at 3 WAP. Leaf area was not significantly ($P>0.05$) influenced by inoculation at 3 WAP and 6 WAP at sixty two days after planting. However, the main effects of inoculation ($p<0.001$) significantly influenced leaf area index at 9WAP. Analysis made on canopy spread showed significant difference among treatments. Soybean plants of the inoculated plot at 3 WAP produced significantly higher (25.62 cm) canopy spread than non-inoculated (20.16 cm) plots at 3, 6, 9 WAP. This agrees with the findings of (28) who stated that inoculation promote growth factors such as production of larger leaves. There was significant difference among treatments in leaf area. Soybean plants on inoculated plots recorded the highest (35.5) compared to the non- inoculated plot which recorded the lowest (29.5). Inoculation of the soybean seeds increased nutrients, nitrogen fixation potential and other growth factors for the enhancement of leave production with larger areas as reported by (16) and (7). This is not in line with the study conducted Applied and Environmental Microbiology (1979) which said that inoculants brand or carrier type was not a significant factor for determining leaf area.

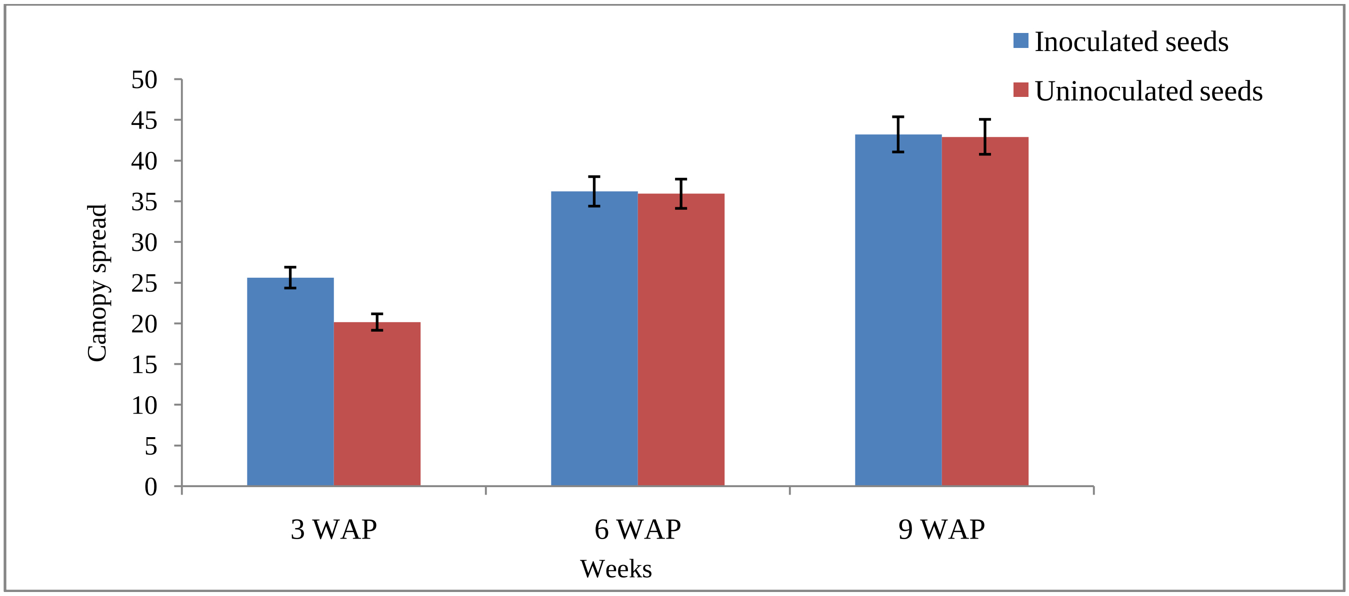

Fig. 1a

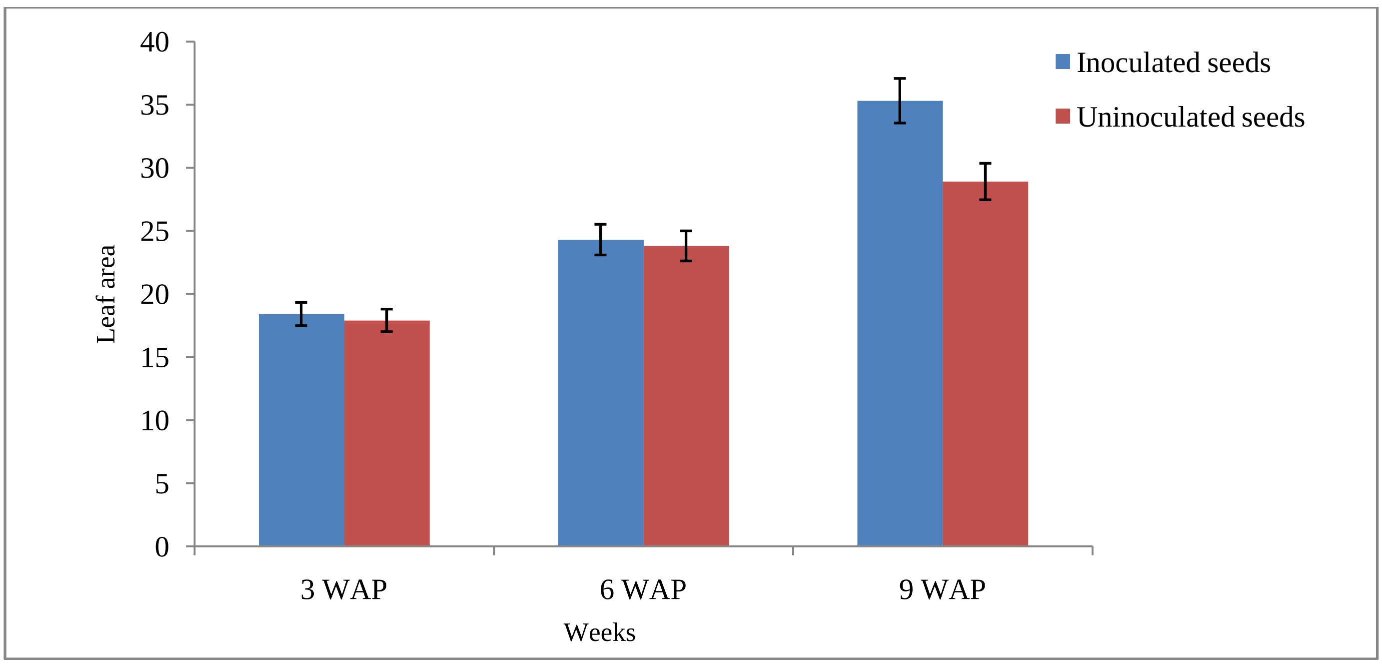

Fig.1b

Fig.1 Effect of inoculation regime on (a) canopy spread of soybean; (b) soybean leaf area. Bars represent SEM.

3.2 Days to 50% flowering of soybean

In this present study, our results shows that days to 50% flowering was significantly affected by inoculation ($p<0.003$) and varied among treatment. The uninoculated seeds treatment produced highest number of days to flower. (Fig.2)

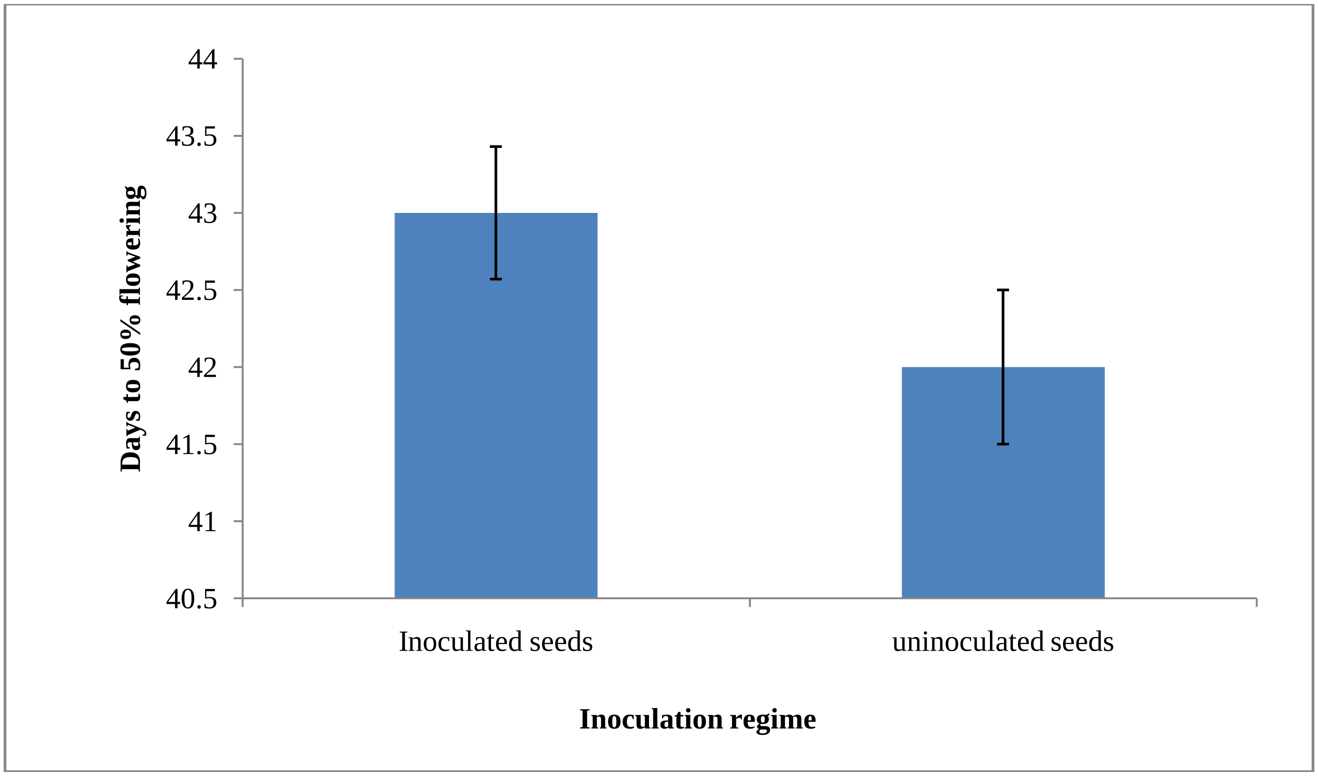

Fig.2. Effect of inoculation regime on days to 50% flowering of soybean. Bars represent SEM

Data analyzed revealed that soybean plants on (weed free) inoculated plots recorded the lowest number of days to 50% flowering while non inoculated plots had the highest. The application of inoculants resulted in prolonged number of days to flowering. (23) reported that application of inoculants hastened plant growth so that flowering and fruit do not start whilst the plants are too small as it could results in stunted growth which could adversely affect fruit and quality. Notwithstanding, plots with no inoculants application shortened (43 days) number of days to flowering.

3.3 Biomass Sampling

The results reported for biomass sampling is shown in figure 3. The oven dry weight of the sample shoot were not significantly ($p>0.05$) affected by the interaction between growth stage and inoculation regimes. However, the biomass of sample shoots was significantly ($p<0.002$) influenced by inoculation regimes and growth stages. The biomass of the inoculated plot was higher (21.6 Kg) than that of the non- inoculated (15.9Kg) plot in all the sampling stages of the soybean with R2 +In having the highest (22.8 Kg) and R4-In having the least (15.4 g). This was due to the effective nodulation of the rhizombium. This confirms the study conducted by (22) which revealed that Plant biomass of the inoculated treatments were significantly greater than that of the control. With respect to the growth stages, flowering stage had the highest biomass (22.8 Kg), followed by vegetative stage and pod stage and maturity stage having the least (8.2 Kg). This confirms the study conducted by (27) which states that maximum N2 fixation occurs between the flowering and early pod stages of soybean development. However the generally observed reduction in N2 fixation activity between the full pod and maturity stages could lead to a shortage of N during seed-filling in high-yielding environments. Also confirms by (19) which states that the demand for nitrogen is extremely high during seed formation immediately after flowering. Nitrogen concentration in vegetative biomass (leaves plus stems) at flowering stage was high. Hence, partitioning of N between vegetative biomass and seeds varied widely too, since a great amount of N is mobilized to the grain in relation to that remaining in the residues (13). Also, a study conducted by (20) which state that the higher number of nodules per plant, the higher the biomass. Therefore, flowering stage is the most appropriate stage to be used for green manuring.

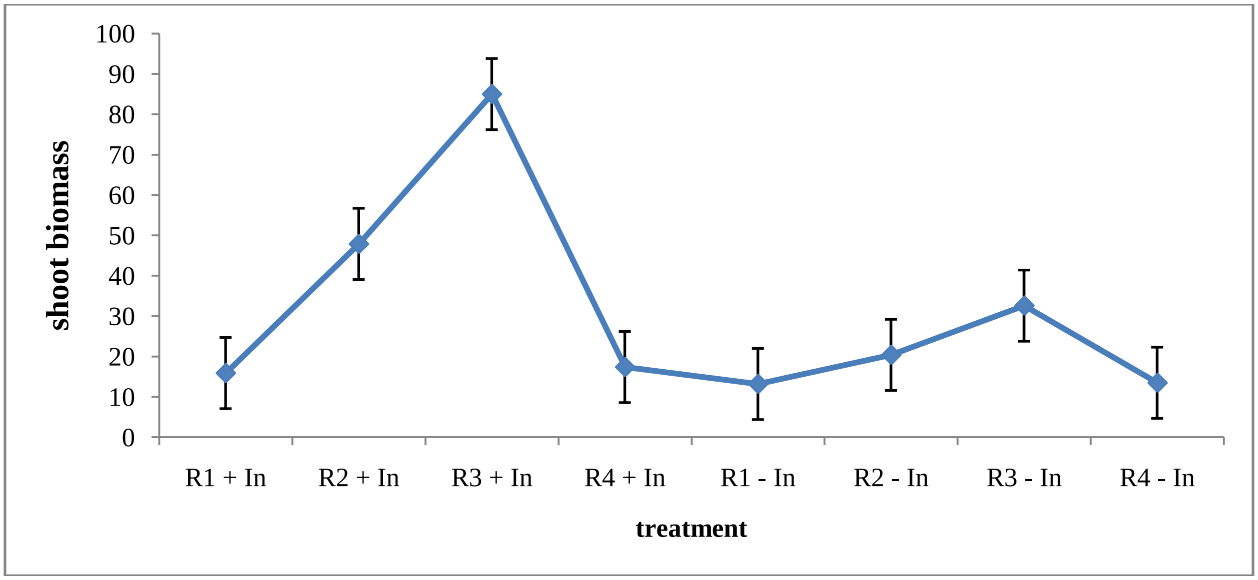

Fig.3. Effect inoculation regime on weight of shoots of soybean. Bars represent SEM

3.4 Total grain yield (kg/ha) of soybean

Fig. 4 shows that total grain yield of the soybean was significantly ($p<0.003$) influenced by the inoculation regimes. T_3 (R3+In) recorded the highest total grain yield followed by T_1 (R1+In) whiles T_6 (R2-In) produced the least total grain yield. Soybean plants of the plots inoculated produced significantly higher (3.24kg) grain yield while the non inculcated plots produced lowest (2.44kg). this is in line with the study conducted in Saginaw by (5) which states that grain yield increased to 100 kg ha−1 in 2003 and also in Hillsdale also recorded yield increases in both 2004 (280 kg ha−1) and 2005 (110 kg ha−1). T1 (R1+In) produced the highest (1.42kg) grain weight followed by T3 (R3+I) (1.39kg/ha) and T5 (R1-In) (0.94S kg/ha) produced the least yield. Inoculation of soybean is a significant agency for the manipulation of rhizombium for improving crop productivity and soil fertility (11). Also, the study confirms that of (5) which states that the yield of the inoculated plot is higher than that of the uninoculated plots.

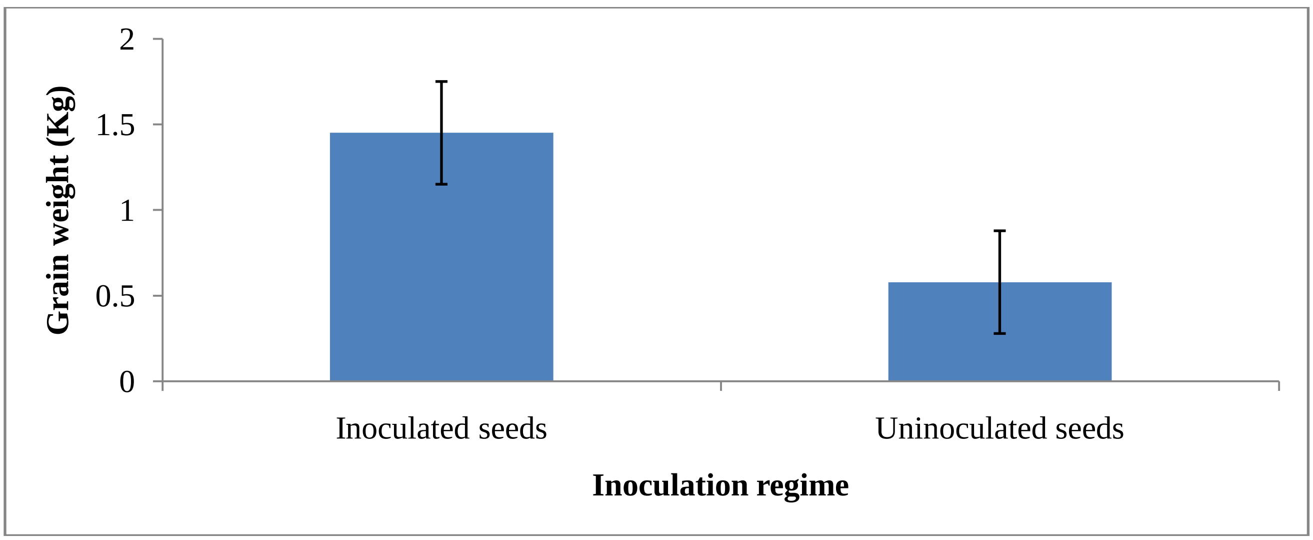

Fig. 4 Effect inoculation regime on total grain weight of soybean. Bars represent SEM

4 CONCLUSION

The results indicated that canopy spread was not significantly ($p>0.05$) influenced by the inoculants at 6 WAP and 9 WAP. However, inoculation ($p<0.001$) influenced canopy spread at 3 WAP. Also our results shows that days to 50% flowering was significantly affected by inoculation ($p<0.003$) and varied among treatment. The biomass of the inoculated plot was higher (21.6 Kg) than that of the non-inoculated (15.9Kg) plot in all the sampling stages of the soybean with R2 +In having the highest (22.8 Kg) and R4-In having the least (15.4 g). We therefore document for the first time that high yield of soybean can be obtained under farmer's conditions provided the seed are inoculated with peat base inoculants.

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REFERENCES
1. Y.R. Dommergues, G.D. Hoang and C. Divies, Applied and Environmental Microbiology, Polyacrylamide-Entrapped Rhizobium as an Inoculants for Legumes, Vol. 37, No. 4. Pg 779-781, 1979.
2. K. A. Awuku, Agriculture and Environmental Studies. Rebl. Evans Brothers Ltd. London, 344 pp, 1991.
3. K. J. Boote, S. S. Grimm, J. W .Jones, and J. D. Hesketh, "Parameter estimation for predicting flowering dates of soybean cultivars". Crop Sci. 33:137-144, 1993.
4. T.P. Duong, C.N. Diep, N.H. Khiem, N.T. Hiep, N.V. Toi, and T.K.L. Nhan, "Rhizobium inoculant for soybean [Glycine max (L.) Merrill] in Mekong Delta". Plant soil. Pg 235-770, 1984.
5. S. Conley and E. Christmas, "Utilizing inoculants in a corn–soybean rotation". Purdue Ext. Bull. SPS-100-W. Purdue Univ., West Lafayette, IN, 2006
6. C.R. Glover and W.C. Lindemann, "Nitrogen fixation by legumes". New Mexico State Univ. Coop. Ext. Serv. Guide A-129. New Mexico State Univ., Las Cruces, 2003.
7. D. B. Harder, C. L. Sprague and K. A. Renner, "Effect of soybean row width and population on weed, crop yield, and economic return". Weed Technology 21:744–752, 2007.
8. IITA, Annual Report of the International Institute of Tropical Agriculture, Ibadan Nigeria, 45-47, 1990.
9. R. R. Johnson and L. M. Wax, "Soybean stand establishment and yield as affected by herbicides and cultural practices". Agron J. 71: 880–884, 1996
10. S. Kyei-Boahen, A.E. Slinkard and F.L. Walley, "Evaluation of rhizobial inoculation methods for chickpea", 2002.
11. H. H. Keyser and F. Li, "Potential for increasing biological nitrogen fixation in soybean", Plant Soil 141 (1992), pp. 119–135, 1992.
12. I. Y. D. Lawson and A. Quainoo, "Response of soybean to organic matter application and inoculation with Bradyrhizobium japonicum in the Guinea savanna of Ghana". J. Ghana Sci. Ass. 4(1): 53–57, 2008.
13. R.J. Lawn, Lawn, Agronomic and physiological constraints to the productivity of tropical grain legumes and prospects for improvement, Exp. Agric. 25 (1989), pp. 509–528, 1989.
14. D.R. Miller, N.J. Rosenberg and W.T. Bagly, "Soybean water use in the shelter of a saltfence windbreak". Agric. Meteorol, 11: 405-418, 1997.
15. I. Mamman, Soybean in Nigeria Agriculture. Paper delivered by the Honourable Minister of Agriculture and Natural Resources 3rd Annual conference of Nigeria Soybean Association, Badeggi (March,1990), 26-29, 1990.
16. D. A. McWilliams, D. R. Berglund, and G. J. Endres, Soybean Growth and Development Quick Guide. North Dakota State University; University of Minnesota. Reviewed and Reprinted August 2004, pp 1-5, 2004.
17. G. U. Okereke, C. Onochie, A. Onunkwo, and E. Onyeagba, "Effectiveness of foreign bradyrhizobia strains in enhancing nodulation, dry matter and seed yield of soybean (Glycine max L.) cultivars in Nigeria". Biol. Fertil. Soils, 33: 3-9, 2004.
18. I. C. Onwueme, and T. D. Sinha, Soybean: In: Field Crop Production in Tropical Africa. Tropical Centre for Agricultural and Rural Corporation (C.T.A), Ede, the Netherlands, 337-343, 1991.
19. P. Pedersen, Managing soybean for high yield. Iowa State University, DepartmentofAgronomy,2009.(http://www.extension.agron.iastate.edu/soybean/documents/HighYield.pdf) Accessed July 12, 2011.
20. C. Revellin, G. Meunier, J.J. Giraud, G. Sommer, P. Wadoux, and G. Catroux, Changes in the physiological and agricultural characteristics of peat-based Bradyrhizobium japonicum inoculants after long-term storage, Appl. Microbiol. Biotechnol. 54, pp. 206–211, 2000.
21. SARI, Annual report for the year 2000. Savannah Agricultural Research Institute, P. O. Box 52, Tamale, Ghana, 1997.
22. G. Seneviratne, L.H.J. Van Holm, and E.M.H.G.S Ekanayake, Agronomic benefits of rhizobial inoculant use over nitrogen fertilizer application in tropical soybean. field crops Res. 199-203, 2000.
23. M. Shu-Jie, Q. Yun-Fa, H. Xiao-Zeng, and M. An, Nodule formation and development in soybeans (Glycine mux L.) in response to phosphorus supply in solution culture. Pedosphere, 17: 36-43, 2007.
24. S. Smith, R.M. Osborn, and J.W. Kosanke, Methods and compositions providing agronomically beneficial effects in legumes and non-legumes. U.S. Patent Application 20050187107, 2004.

25. H.H. Tien, T.M. Hien, M.T. Son and D. Herridge, Rhizobial inoculation and N2 fixation of soybean and mungbean in the eastern region of South Vietnam. In D. Herridge (ed.) Inoculants and nitrogen fixation of legumes in Vietnam. ACIAR Proceedings 109e. ACIAR, Canberra, Australia, 2002.

26. Yoav Bashan Department of Microbiology, Division of Experimental Biology,the Center for Biological Research of the Northwest(CIB), La Paz, A.P.128, B.C.S., 23000, Mexico, 2001.

27. F. Zapata, S.K.A. Danso, G. Hardarson and M. Fried, Time course of nitrogen fixation in field-grown soybean using nitrogen-15 methodology, Agron. J. 79, pp. 172–176,1987.

28. K.A Malik, R. Bilal, S. Mehnaz, G. Rasul, M.S Mirza, and S. Ali "Association of nitrogen-fixing, plant promoting rhizobacteria(PGPR) with kallar grass and rice" Plant Soil 194:37–44, 1997.