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Growth, Nodulation and Biomass Yield of Soybean (*Glycine max*) as Influenced by Bio-Fertilizers under Simulated Eroded Soil Condition

P.A. Babajide, W.B. Akanbi, L.O. Alamu, E.A. Ewetola and O.O. Olatunji
Department of Agronomy, Ladoke Akintola University of Technology, Ogbomoso, Nigeria

Abstract: Green house experiments were conducted to determine the effect of different rhizobial and mycorrhizal species on growth, nodulation and biomass yield of soybean (*Glycine max*), grown under low fertile eroded soil condition in the South western Nigeria. It was a factorial experiment with 2 levels of soil (sterile and non-sterile), inoculation with *Glomus clarum* (with and without) and inoculation with either R25B or IRJ2180A rhizobial strain, while the control received no inoculation of any of the microbial strains. Each treatment was replicated 3 times and the trial was arranged in a Completely Randomized Design (CRD). Plant growth and biomass yield were significantly enhanced by arbuscular mycorrhiza in both sterile and non-sterile soil conditions, compared to the control. Combined inoculation of mycorrhiza with any of the rhizobial strains significantly improved plant growth and biomass production. The effect of the combined inoculation was particularly more effective with mycorrhiza + R25B rhizobium inoculation ,which had the highest values of plant height (68.8 cm), stem circumference (2.94 cm), number of leaves (39.0), shoot dry weight (16.1 g) and root dry weight (4.6 g), while the control had the least values of 33.2, 0.60 cm, 15.0, 4.4 and 1.6 g, respectively. Nodulation was equally enhanced by mycorrhizal and rhizobial inoculations under sterile and unsterile soil conditions. The percentage mycorrhizal root colonization values ranged from 4.0-42%. Root colonization was highest for mycorrhizal inoculated plants grown on sterile soil. Thus, dual inoculation of mycorrhiza + R25B rhizobium, may be beneficial to soybean production in the tropics, where nutrients particularly available P and total N are very low. Hence, a relatively low input technology, for improved soil fertility/productivity and sustainable arable, green manure and forage crops production.

Key words: Bio-fertilizer, eroded soil, mycorrhiza, rhizobia, biomass yield

INTRODUCTION

Most tropical soils of humid and sub-humid African countries are degraded and therefore, characterized by low levels of essential nutrients (Sanginga *et al*., 1996), low agricultural productivity and high erodibility (Akinbola, 1999; Fagbola *et al*., 2001). These undesirable characteristics are traceable to undesirable environmental conditions and poor management strategies such as; high solar radiation, torrential rainfall, storms, incessant yearly bush burning, mono-cropping, overgrazing, bulldozing, mining activities etc., which may enhance soil erosion/degradation as well as the related consequential effects such as leaching, soil acidity, low C.E.C., poor vegetative cover, poor soil moisture, texture, structure and colour (Barea *et al*., 1997). However, one of the efforts being made towards reversing these hapless tropical soil conditions (as often practiced), is application of inorganic/chemical fertilizers, which are practically unaffordable particularly for the resource-poor local farmers. More so, the reported harmful residual effects of these synthetic fertilizers on soils, plants, water, beneficial soil microbes and even man, cannot be overlooked (Sobulo, 2000).

Soybean, which is one of the important grain legumes commonly grown in the tropics, plays a significant role in supplying N to the succeeding crops (Nwoko and Sanginga, 1999). Generally, the required P for N-fixation, growth and development by grain legumes cannot compensate for the incessant nutrients absorbed by grown crops in marginal tropical soils. Therefore, there is an urgent need for biological and environmentally friendly strategies for reclaiming such badly eroded marginal soils. Such strategies could serve as reliable alternatives to properly replace the incessant application of synthetic fertilizers, which are expensive and mostly associated with residual harmful effects on the lives of crops and other beneficial soil-inhabiting microbes.

Among the promising strategies for sustainable crop production in the tropics are artificial inoculations of soil

Corresponding Author: P.A. Babajide, Department of Agronomy, Ladoke Akintola University of Technology, Ogbomoso, Nigeria

with beneficial soil-inhabiting microbes/biofertilizers. Availability of such beneficial microbes in the rhizosphere improves soil fertility level. Mycorrhiza is a good example, which improves water and nutrients uptake in many crops found in both tropical and temperate regions of the world (Fagbola *et al*., 2001). Also, rhizobium, azotobacter, azospirillum etc. could also be used as supplements to chemical fertilizers (Indu and Savithri, 2003) and organic fertilizers/manures (Jeyabal *et al*., 1997). However, it has been scientifically established that organic resources, which are often proposed as alternatives to commercial mineral fertilizers cannot solely and effectively reverse the degraded soil fertility condition (Ames *et al*., 1991), particularly in the tropics, because of the fact that, they are; mostly lowin nutrients insufficient in quantityto meet up the requirements on most farms labour-demanding, in terms of preparation/processing and application and mostly having competitive uses e.g., mulching, fuel, fodder, demarcation of boundaries, fencing, staking, building, medicine, erosion control etc. (Palm and Rowland, 1997; Subramanian and Charest, 1999). More so, since chemical fertilizers are generally highly priced, scarce and grossly associated with residual/harmful effects, a careful integration of plant residues with bio-fertilizers, may be beneficial to improved soil fertility and crop yields, in the tropics (particularly south-western Nigeria, where cultivation of many arable and tree crops are favourably influenced by bi-modal rainfall distribution. Therefore, this research is aimed at assessing the response of soybean to sole or combined inoculation with bio-fertilizers such as *Glomus clarum* (mycorrhizal strain) and or either of the 2 rhizobial strains (R25B or IRJ2180A), in a low fertile soil. Such leguminomicrobial symbiotic associations (e.g. soybean-mycorrhiza and soybean-rhizobium) could be easily exploited and incorporated into arable and forage crop production as well as green manure making, so as to enhance plant growth, nodulation and biomass accumulation. This approach may be equally beneficial to local farmers by alleviating/eliminating their total dependence on the scarce and highly priced nitrogen and phosphorus fertilizers, through improved P and N nutrition of the soil.

MATERIALS AND METHODS

This experiment was carried out in the year 2005 (between mid-June to mid-August), in the greenhouse of Department of Agronomy, University of Ibadan, Nigeria. The soil sample (alfisol) used was collected from a degraded field located at Parry Road, University of Ibadan, Nigeria. The soil was sampled and analyzed for its physicochemical properties (IITA, 1982), which is as follows; sand (74.8%), silt (15.2%) and clay (10.0%) i.e. sandy-loam. Also, total N (0.08%), extractable P Bray 1 (1.40 mg kg^{-1}), pH (H_2O) (6.4). Values of the exchangeable bases (in Cmol kg^{-1}) were K^+ (0.2), Ca^+ (1.70), Mg^{2+} (0.40) and Na^+ (0.2). One half of the total amount of soil required was sterilized by using autoclave, while the remaining half was left unsterilized. A total number of 36 pots filled with 5.0 kg soil each were used. Plant residues found at the nearby plots were used as basal application of organic manure for all the soil samples used. Soybean seeds (i.e., variety TGX 1485-2E) obtained from the International Institute of Tropical Agriculture (IITA, 1982), were used for the experiment. The seeds were surface sterilized by using 95% ethanol for 10 sec and were later rinsed 6 times with sterile water after shaking for 3-5 min in 3% hydrogen peroxide (H_2O_2). Three seeds per pot were sown at 2 cm depth and were later thinned to one at one week after emergence. Manual uprooting of weeds was done regularly throughout the growing period. Chopped root fragments of maize plant containing *Glomus clarum* mycorrhizal strain were used as an inoculum. Each inoculum weighing 20 g was first placed at 3 cm soil depth per required pot, at about 1 h before seed sowing. One mL each of inoculum containing approximately 10^8 cells per mL of either R25B or IRJ 2180A rhizobial strain was applied to every required soil at 1 week after sowing, by using a sterile pipette. The experiment was a factorial combination of 2 levels of soil (S^+ = sterile and S^- = non-sterile), 2 levels of mycorrhizal inoculation (M^+ = inoculated and M^- = without inoculation) and 3 levels of rhizobial inoculation (A = without inoculation, B = inoculated with R25B strain and C = inoculated with IRJ 2180A strain). Each treatment was replicated 3 times and the trial was arranged in Completely Randomized Design (CRD). Data collection started at 2 Weeks After Sowing (WAS) and was repeated at 2 weeks interval. The growth parameters determined were plant height (by using measuring tape), stem circumference (by using calipers which first gave the value of the diameter, which was later converted to circumference using a formula of πD (i.e. 3.142 multiplied by the obtained diameter (D) value) and number of leaves (determined by direct counting of fully opened leaves per plant). Biomass yield parameters such as root and shoot dry weights were determined by weighing (after oven drying of plant samples at 80°C for 48 h). Number andeight of nodules were determined by first placing roots in a sieve and carefully washing under a slow running tap water. Later the nodules were then carefully

detached from the roots and counted together with those that were accidentally detached which could be easily seen and picked up from the sieve). The nodules were immediately air-dried for 15 min before weighing and recording of the values observed. Mycorrhizal root colonization was quantified after cleaning of root samples for 15 min at 121°C in 10% KOH and staining in chlorazol black E (Brundrett *et al*., 1984) by the gridline intersect method (Giovannetti and Mosse, 1980). All data collected were analyzed following the procedures of Analysis of Variance (ANOVA). Where differences were observed, Duncan's Multiple Range Test (DMRT) was used to compare differences between the treatment means.

RESULTS AND DISCUSSION

As earlier indicated in the materials and methods, the results obtained from the physicochemical properties of the soil sample used showed that the soil was mildly acidic (i.e. pH 6.4). Also, the nutrient levels were very low e.g., available P (1.40 mg kg^{-1}) and total N (0.08%). Arbuscular mycorrhiza contributed significantly to plant height under both sterile and non-sterile soil conditions (Table 1). More so, insignificant differences were observed among the treatment means of all growth parameters (plant height, number of leaves and stem circumference) measured as at 2 weeks after sowing (WAS), as shown on Table 1-3. A combined inoculation of *Glomus clarum* and R25B rhizobium received by soybean grown on sterile soil (M^+S^+B) produced the highest number of nodules and best biomass yield (Table 4). The best growth parameters were also observed in treatment combination M^+S^+B as from 4-8 WAS (Table 1-4). In contrast, the highest mycorrhizal root colonization was observed in soybean grown on mychorrhizal inoculated sterile soil which received no rhizobial inoculation i.e., M^+S^+A (Table 4). This shows that soybean responded better to mycorrhizal inoculation under sterile soil condition than non-sterile condition,

Table 1: Effect of inoculated biofertilizers on plant height (cm) of soybean at different ages

	Weeks After Sowing (WAS)			
Treatment	2	4	6	8
Control (M^-S^-A)	12.1	22.5ab	23.2de	32.2e
M^-S^-B	12.9	20.5b	26.5d	36.6e
M^-S^-C	12.5	20.5b	25.1d	33.1e
M^-S^+A	12.6	18.8b	24.1de	31.4ef
M^-S^+B	12.6	22.3ab	28.9c	38.7d
M^-S^+C	12.2	21.3b	26.1d	36.2e
M^+S^+A	13.4	27.5a	34.9b	46.1d
M^+S^+B	13.1	29.7a	45.5a	68.8a
M^+S^+C	13.2	28.9a	35.4b	51.6c
M^+S^-A	13.1	26.5a	32.0c	45.5d
M^+S^-B	13.0	26.3a	37.8b	55.7b
M^+S^-C	13.0	26.8a	33.3c	50.4c
	Ns			

Means followed by same letters are not significantly different at p = 0.05 using DMRT; M^+ = mycorrhizal inoculated; M^- = non-inoculated with mycorrhiza; S^+ = sterilized soil; S^- = unsterilized soil; A = noninoculated with rhizobium; B = inoculated with R25B rhizobium; C = inoculated with IRJ2180A rhizobium; Ns = Not significant

Table 2: Effect of inoculated biofertilizers on stem circumference (cm^{-1}) of soybean at different ages

	Weeks After Sowing (WAS)			
Treatment	2	4	6	8
Control (M-S-A)	0.40	0.48d	0.56d	0.60d
M^-S^-B	0.43	0.54cd	0.72cd	0.84cd
$M\text{-}S^-C$	0.43	0.46d	0.51d	0.55d
M^-S^+A	0.40	0.42d	0.50d	0.52d
M^-S^+B	0.45	0.60c	0.84cd	1.02cd
M^-S^+C	0.45	0.50d	0.60d	0.75d
M^+S^+A	0.47	0.70c	1.06c	1.47c
M^+S^+B	0.53	1.37a	2.31a	2.94a
M^+S^+C	0.50	0.88b	1.22b	2.49b
M^+S^-A	0.46	0.65c	1.00c	1.16c
M^+S^-B	0.45	0.92b	1.82b	2.21b
M^+S^-C	0.47	0.80b	1.11c	1.56c
	Ns			

Means followed by same letters are not significant different at p = 0.05 using DMRT

Table 3: Effect of inoculated biofertilizers on number of leaves of soybean at different ages

	Weeks After Sowing (WAS)			
Treatment	2	4	6	8
Control (M^-S^-A)	6	12	14d	15d
M^-S^-B	6	11	16d	20c
M^-S^-C	6	10	12d	13d
M^-S^+A	6	11	11d	10e
M^-S^+B	5	11	18d	21c
M^-S^+C	5	11	15d	17d
M^+S^+A	6	13	21c	24c
M^+S^+B	6	18	35a	43a
M^+S^+C	5	15	24c	31bc
M^+S^-A	6	12	20c	23c
M^+S^-B	5	15	29b	35b
M^+S^-C	6	13	22c	27c
	Ns	Ns		

Means followed by same letters are not significant different at p = 0.05 using DMRT

Table 4: Effect of rhizobia and arbuscular mycorrhizal fungus on mycorrhizal root colonization/infection and nodules parameters

Treatment	Mycorrhizal root infection	Number of Nodules	Nodules weight per plant (g)
Control (M^-S^-A)	16.0c	23e	0.50d
M^-S^-B	12.0c	50d	2.50b
M^-S^-C	10.0d	40d	1.80c
M^-S^+A	4.0e	10f	0.30e
M^-S^+B	5.0e	50d	2.93b
M^-S^+C	4.0e	46d	1.81c
M^+S^+A	42.0a	12f	0.31e
M^+S^+B	38.0a	82a	4.80a
M^+S^+C	28.0b	61c	3.00b
M^+S^-A	26.0b	26e	0.70d
M^+S^-B	30.0b	72b	3.80a
M^+S^-C	26.0b	60c	2.70b

Means followed by same letters are not significant different at p = 0.05 using DMRT

which may be due to absence of competitions/interactions with other indigenous soil microbes which may alter root infection and efficiency of the inoculation. General poor performance of the control regarding the growth and yield parameters measured, indicates the effect of the inoculated microbes (mycorrhiza and rhizobia) on soybean performance on such low fertile soil, which is consistent with earlier research reports that mycorrhizal inoculation was efficient in low P soil and high P depresses mycorrhizal root infection (Osonubi *et al*., 1995; Suhardi *et al*., 1993). It is generally assumed that legumes will benefit mostly from mycorrhizal association in nutrients-poor soil conditions, especially where P is limiting. This research results were therefore, contrary to a part of the results of Nwoko and Sanginga (1999), where Arbuscular Mycorrhizal Fungi (AMF) inoculation did not affect the growth of some soybean lines, whereas, soybean line 1039 and mucuna required mycorrhizal inoculation, in order to grow well in the absence of p-application.

CONCLUSION

Mycorrhizal root colonization/infectivity and effectiveness had been reported to be improved in low P soil (Awotoye, 1994; Fagbola *et al*., 2001). Therefore, since most tropical soils are degraded or low in most nutrients, particularly N and P and that the plant breeders are not yet successful in breeding a soybean variety which can fix excessive nitrogen to compensate for the nitrogen removed in the form of grains, farmers are advised to adopt a low input biological strategy of reclaiming or replenishing their depleted soil nutrients (as established in this research results) and incorporate it into arable, forage and green manure crop production). This technology will encourage sustainable crop and animal production in the tropics, where most soils are badly eroded and relatively infertile. More so, the observed preferential compatibility of mycorrhizal strain with different rhizobial strains by soybean as observed in the research results, makes it necessary in the future research to screen other genotypes of soybean concurrently with other mycorrhizal and rhizobial strains for compatibility, in order to effect potential enhancement of this promising technology.

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