GLOBAL JOURNAL OF BIOLOGY, AGRICULTURE & HEALTH SCIENCES
(**Published By**: *Global Institute for Research & Education)*
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INTERACTIVE EFFECT OF NITROGEN FERTILIZATION AND RHIZOBIUM INOCULATION ON NODULATION AND YIELD OF SOYBEAN (Glycine max L. Merrill)

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Abstract

A field experiment was conducted for two consecutive seasons (2009/10 and 2010/11) in the Demonstration Farm of the Faculty of Agriculture at Shambat-Sudan, to study the interactive effect of nitrogen fertilization and rhizobium inoculation on nodulation and yield of soybean (*Glycine max* [L.] Merrill) plants. The experiment was laid out in a randomized complete block design with four replications. The treatments consist of increasing doses of nitrogen (0, 40 and 80 kg ha^{-1}urea) and one strain of rhizobium. The seeds of cultivar Giza 22 were either uninoculated or inoculated with *Rhizobium japonicum* strain TAL 110 before sowing. The results showed that nodules were only formed on inoculated plants and their number varied with the level of nitrogen fertilizer. The number of nodules and their dry weight per plant declined as the rate of nitrogen application increased. Inoculated or fertilized plants significantly increased seed yield relative to uninoculated non-fertilized control plants by 83% and 89%, respectively. However; the high dose of nitrogen fertilizer (80 Kg ha^{-1}) depressed the positive effect of inoculum on seed yield. The high seed yield was associated with significantly higher number of pods per plant and number of seeds per pod. *Rhizobium* inoculated seeds exhibited increased protein content than that of control (63.3 vs 59.9) but with no effect on seed oil content. Application of small N-fertilizer (40 kg ha^{-1}) in combination with rhizobial inoculation seemed to be an appropriate cultivation practice for soybean production under Sudan condition.

Key words: Soybean; nitrogen fertilizer; rhizobium inoculum; seed yield; seed chemical composition.

Introduction

Legumes can meet most of their N needs and contribute to soil N through symbiotic nitrogen fixation. Estimations indicate that legumes can fix up to 200 kg N ha^{-1} $year^{-1}$ under optimal field conditions (Giller, 2000; Graham and Vanace, 2003). Full legume N-benefits can only be achieved in the presence of efficient rhizobial strain which can be native to the soil or introduced in a form of commercial inoculants (Stephens and Rask, 2000; Deaker *et al.,* 2004). Seed inoculation immediately prior to sowing is the most popular method used and it can leads to the establishment of large rhizobial population in the rhizosphere and therefore improved nodulation and nitrogen fixation (Hungria *et al.,* 2000).

Soybean {*Glycine max* (L.) Merrill}, being a worldwide important legume crop, was tried in several parts of the Sudan since 1920s. However, yields were characteristically low and crop production faced many constraints most notably among which are the scarcities or complete absence of soybean-nodulation bacteria especially in the new areas (Khidir, 1997). Therefore, for better yields, the crop should be inoculated with the appropriate rhizobium strain as reported by many researchers (Hardarson and Atkins, 2003; Agha et *al.,* 2004). In this regard, Mahdi (1993) reported that inoculation of soybean with root nodule bacteria can be considered as a useful alterative to nitrogen fertilization. Similarly, Kala *et al.,* (2011) indicated that rhizobium is a promising fertilizer because it is cheap and improves plant growth and seed quality.

Although, additions of small nitrogen fertilizer to the soil enhance nodulation in legumes, it is well known that the development of nodules and nitrogen fixation activity of root nodules is depressed when the plants are exposed to high concentrations of nitrogen (Gibson and Harper 1985; Imsande 1986; Streeter 1988). Previous research on the interactive effect of nitrogen fertilization and inoculation on leguminous crops is scant and contradicting. Therefore, the objective of the present investigation is to study the interactive effect of nitrogen fertilization and rhizobium inoculation on nodulation and yield of soybean.

Materials and Methods

A field experiment was conducted for two consecutive seasons (2009/10 and 2010/11) in the Demonstration Farm of the Faculty of Agriculture at Shambat (latitude 15˚40′ N, longitude 32˚32′ E and altitude 230 m *asl*). The soil of the experimental site is clayey (Fine montomorilonitic, Isohyperthermic Entic Chromustert) with alkaline pH. The climate of the locality is semi-arid with mean annual rainfall of 100 to 200 mm and maximum temperature of about 42˚C in summer and 21˚C in winter. The experiment was designed to study the interactive effect of nitrogen fertilization and rhizobium inoculation on nodulation and yield of soybean.

The experimental treatments and layout

The treatments consist of increasing doses of nitrogen (0, 40 and 80 kg of N ha^{-1}) designated in this study as 0N, 1N and 2N, respectively. Nitrogen fertilizer was applied in a form of urea (46% N). The seeds of cultivar Giza 22 were either uninoculated (R0) or inoculated (R1) by a charcoal-based inoculum obtained from National Research Center in

Khartoum. The inoculum was identified as *Rhizobium japonicum* strain TAL 110. A small amount of gum arabic solution was used as an adhesive material to insure seed coating with the inoculum. In both seasons, seeds were inoculated just before planting, sown and irrigated immediately on the same day. The treatments were arranged in a randomized complete block design with four replications.

The land was prepared by disc ploughing, harrowing and ridging 70 cm apart. The size of the individual plot was 4x5 m consisting of five ridges four-meters in length. The two outer ridges were left as guard ridges and the inner three ridges were used for nodules and yield determinations. A basal dose (50 kg ha^{-1}) of triple super phosphate fertilizer (48% P_2O_5) was used in the two seasons. Sowing was done manually on the shoulder of the ridges on the third week of July in the two seasons. Seeds were sown at a rate of 2-3 seeds per hole and later thinned to one plant per hole. Spacing between plants was 15 cm in both seasons.

Characters studied

One month after planting, three plants per plot, from the inner three ridges, were dug out carefully to avoid root injury. The roots were then separated, washed in a water stream to remove mud and soil particles and examined for the presence of nodules. Nodule dry weight was determined by drying nodule samples after being detached from roots in an oven set at 80 ºC for 48 hrs. At harvest, ten plants in each plot were randomly selected to determine number of pods per plant, number of seeds per pod, 100-seed weight (g). The pods of the harvested area (0.7 m^2) were dried, threshed and cleaned to determine seed yield per unit area. A sample from the harvested seeds was used to determine oil percentage by the Soxhlet extraction method (AOAC 1990). The nitrogen content of each sample was determined by micro-kjeldahl method and the value obtained was multiplied by a factor (6.25) to determine the percentage of total crude protein in the seeds.

Statistical analysis

Analysis of variance (ANOVA) appropriate for randomized complete block design was used according to Gomez and Gomez (1984). Means separation was carried out using the least significant difference (LSD) for the different characters.

Results and Discussion

Number and dry weight of nodules

Nodules were only formed on inoculated plants and their number increased progressively until 60 days after sowing and then decreased with the advancement of growth (Fig. 1). Differences due to nitrogen application on mean number of nodules per plant were significant and increasing nitrogen level decreased the mean number of nodules per plant at all sampling occasions and in both seasons (Fig. 1A). The mean nodules dry weight per plant followed similar pattern to the mean number of nodules per plant (Fig. 1B)**.** The lack of nodules in non-inoculated plants indicates the absence of Rhizobium japonicum in the soil. Therefore, in any future cultivation of soybean the seeds should be appropriately inoculated. Supporting evidence was reported by Agha *et al*., (2004) who attributed the significant stimulation of nodules number per plant and nodules dry weight by rhizobium inoculation to the increase in nitrogenase activity. Moreover, high rates of N application should be avoided as also found in other studies (Abdel Wahab and Abd-Alla, 1996)). Such depression of nodulation due to N fertilization is attributed to inhibition of the formation of infection threads (Dadson and Acquaah, 1984; Agha *et al*., 2004) or suppression of nitrogen fixation by nodules (Lindemann *et al*., 2003).

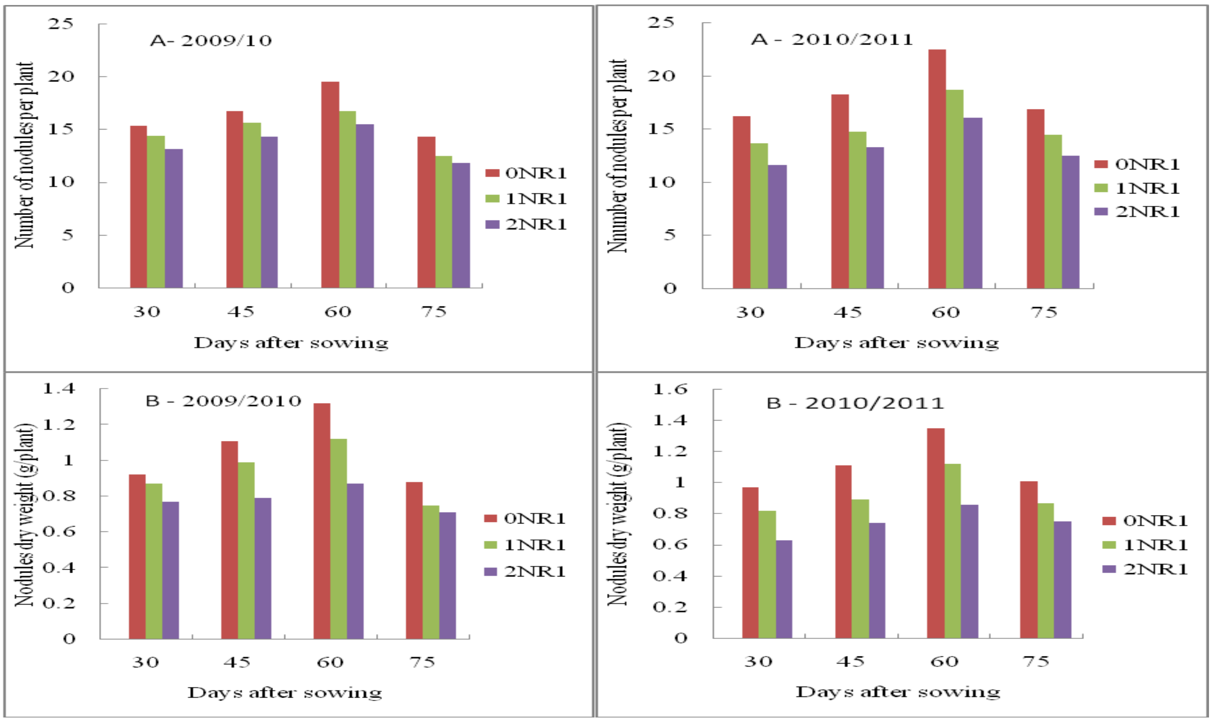

Fig 1. Effect of nitrogen fertilization and rhizobium inoculation on mean number of nodules per plant (A) and nodules dry weight/plant (B) during 2009/10 and 2010/11 seasons (0NR1≡ inoculums only; 1NR1≡ inoculums + 40 kg ha^{-1}N; 2NR1≡ inoculums + 80 kg ha^{-1}N)

Seed yield and yield components

The results of the present investigation reveal that inoculated or fertilized plants significantly increased seed yield relative to uninoculated non-fertilized control plants in both seasons (Table 1). In this regard, inoculation increased seed yield by 83% whereas nitrogen application at a rate of 40 Kg ha^{-1} enhanced grain yield by 89% compared to the control (Table 2). The substantial increase in yield observed in the *Rhizobium* inoculated seeds may be due to the nitrogen fixation potential of soybean. Similar results were reported by Kumawat, *et al.,* (2000) and Patra *et al.,* (2012). However; high dose of nitrogen fertilizer (80 Kg ha^{-1}) significantly depressed the positive effect of inoculums on seed yield relative to uninoculated non-fertilized plants. The high seed yield was associated with significantly higher number of pods per plant and number of seeds per pod (Table 1). Similar results were reported by many workers (Akbari *et al.,* 2001; Agha *et al.,* 2004).

Table 1: Effect of nitrogen levels and rhizobium inoculation on seed yield and yield components of soybean (CV Giza 22) plants grown during 2009/10 and 2010/11 seasons.

Treatment	Seed yield (ton/ha)		Number of pods/plant		Number of Seeds/pod		100-seed weight (g)	
	2009/10	2010/11	2009/10	2010/11	2009/10	2010/11	2009/10	2010/11
0NR0	0.91 a	1.35 a	50.3 a	57.6 a	2.8 a	2.8 a	12.6a	12.3a
0NR1	1.90 b	2.12 b	68.8 b	80.5 b	3.1 b	3.0 b	12.6a	12.7 a
1NR0	1.74 b	1.96 b	64.8 b	76.1 b	3.0 b	3.0 b	12.5a	12.6 a
1NR1	1.94 b	2.22 b	68.7 b	81.4 b	3.1 b	3.0 b	12.6 a	12.9 a
2NR0	1.83 b	2.14 b	70.3 b	78.5 b	3.1 b	3.0 b	12.4 a	12.6 a
2NR1	1.23 a	1.54 a	52.4 a	60.3 a	2.8 a	2.8 a	12.3a	12.6 a
$LSD_{0.05}$	**0.5 ***	**0.4 ****	**12.8 ***	**12.1***	**0.2 ***	**0.2***	**0.91 NS**	**0.84 NS**
C.V. (%)	**29.7**	**40.5**	**24.4**	**34.3**	**12.6**	**18.3**	**19.3**	**16.9**

Means followed by similar letters in each column are not significantly different according to least significant difference (LSD).

0NR0 ≡ Control; 0NR1≡ inoculums only; 1NR0 ≡ 40 kg ha^{-1} N only; 1NR1≡ inoculums + 40 kg ha^{-1}N; 2NR0 ≡ 80 kg ha^{-1}N only; 2NR1≡ inoculums + 80 kg ha^{-1}N

NS: * and ** Not significant and significant at 0.05 and 0.01 level of probability, respectively.

Table 2. Yield performance of soybean to nitrogen fertilizer and rhizobium inoculation.

Treatment	Yield increase (%)		Average yield increase (%)
	2009/10	2010/11	
0NR0	-	-	-
0NR1	109	57	83
1NR0	91	45	68
1NR1	113	64	89
2NR0	101	59	80
2NR1	35	14	25

0NR0 ≡ Control; 0NR1≡ inoculums only; 1NR0 ≡ 40 kg ha^{-1} N only; 1NR1≡ inoculums + 40 kg ha^{-1}N; 2NR0 ≡ 80 kg ha^{-1}N only; 2NR1≡ inoculums + 80 kg ha^{-1}N

Seed chemical composition

In both seasons, plants developed from seed inoculated with rhizobium or fertilized with nitrogen showed appreciable increase in seed chemical composition (Table 3). In this respect, rhizobium inoculation significantly increased the mean seed protein content compared to uninoculated plants (Table 3). Similarly, Egamberdiyeva *et al.* (2004) and Elsheikh *et al.,* (2008) reported considerable increase in protein content of soybean following the inoculation of *Rhizobium japonicum.* A 22% increase in protein content also has been reported in *Glycine max* inoculated with *Rhizobium* (Morshed *et al.,* 2008). Other workers reported that in soybean, protein and oil content were probably genetically-controlled characters (Wafaa, *et al.,* 2002; Achakzai *et al.,* 2003). High dose of nitrogen fertilizer (2N) either alone or in combination with rhizobium decreased, although insignificant, seed protein and oil content in the two seasons (Table 3). In conclusions, the lack of nodules in non-inoculated plants indicates the absence of *Rhizobium japonicum* in the soil. Therefore, in any future cultivation of soybean the seeds should be appropriately inoculated. The substantial increase in yield and protein content observed in the Rhizobium inoculated seeds may be due to the nitrogen fixation potential of soybean. Therefore, application of small N-fertilizer (40 kg N/ha) in combination with rhizobial inoculation seemed to be an appropriate cultivation practice for increasing the productivity of soybean.

Table 3: Effect of nitrogen fertilization and rhizobium inoculation on seed chemical composition of soybean plants grown during 2009/10 and 2010/11 seasons.

Treatment	Protein content (%)		Oil content (%)	
	2009/10	2010/11	2009/10	2010/11
0NR0	59.9a	39.9 a	18.4 a	13.6 a
0NR1	63.3 b	43.2 b	18.9 a	14.6 a
1NR0	60.7 a	40.2 a	19.2 a	14.4 a
1NR1	61.3 a	41.1 a	18.1 a	14.1a
2NR0	59.5a	38.8 a	17.9 a	13.3 a
2NR1	58.2 a	38.2 a	18.2 a	13.4
LSD $_{0.05}$	**1.8** *	**1.8** *	**1.9**NS	**1.9** NS
C.V. (%)	**19.7**	**16.5**	**17.5**	**19.5**

Means followed by similar letters in each column are not significantly different according to least significant difference (LSD).

0NR0 ≡ Control; 0NR1≡ inoculums only; 1NR0 ≡ 40 kg ha^{-1} N only; 1NR1≡ inoculums + 40 kg ha^{-1}N; 2NR0 ≡ 80 kg ha^{-1}N only; 2NR1≡ inoculums + 80 kg ha^{-1}N

NS: and * Not significant and significant at 0.05 level of probability, respectively

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