NIGERIAN AGRICULTURAL JOURNAL
ISSN: 0300-368X
Volume 53 Number 1, April 2022 Pg. 337-345
Available online at: http://www.ajol.info/index.php/naj
https://www.naj.asn.org.ng

Influence of Nitrogen and Phosphorus Fertilizers on Nitrogen Fixation, Nitrobacteria and Yield of Soybean under Irrigation in Sudan Savanna of Nigeria

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Abstract
Irrigation farming has boosted grain legume production especially in non-salt affected areas. A field study was undertaken with Soybean (TGX 1904-6F) at the Irrigation Research Farm of Institute for Agricultural Research (IAR), Kadawa, Kano in the Sudan savanna to assess the effect of nitrogen and phosphorus fertilization on biological nitrogen fixation (BNF), nitrifying bacteria population and grain yield. A non-salt affected soil was cultivated. The treatments consist of two levels of nitrogen (0 and 20 kg N ha^{-1}) applied as urea and four levels of phosphorus fertilizers (0, 6.6, 13.2, 26.4 kg P ha^{-1}) applied as single super phosphate, while maize (*Zea mays*) variety SAMMAZ 14 was used as reference crop for estimating biological nitrogen fixation. The treatments were laid out in a randomized complete block design with levels of N and P in a factorial combination and replicated three times. Application of N fertilizer at 6WAP and 8WAP increased N_2 fixed by 21.49% and 12.68% respectively, while % Ndfa was 6.53% and 8.40% respectively. Phosphorus at the rate of 26.4 kg P ha^{-1} at 8WAP increased N_2 fixed (151.17 kg N ha^{-1}) and % Ndfa (75.49%) by 21.84% and 7.47% respectively. Nitrogen fertilizer decreased grain yield by 15%, while Phosphorus at 26.4kg P ha^{-1} increased grain yield by 18.27% over the control. Nitrogen fertilizer increased *Nitrosomonas spp* by 43.64% and *Nitrobacter spp* by 174%, indicating the dominance of nitrite oxidizing bacteria (*Nitrobacter spp*), while phosphorus (26.4 kg P h^{-1}) increased both populations by 8.51% and 51% respectively, indicating the importance of sufficient P in nitrification process. In conclusion, from the present study it shows that increasing levels of nitrogen negatively affected nitrogen fixation, grain yield of soybean, but enhanced nitrobacteria activity; suggesting that the soil N was adequate for the native strains of rhizobia to initiate nodule production on plant roots growing where no N was applied. Whereas, phosphorus at the rate of 26.4 kg P ha^{-1} at 8WAP enhanced N_2 fixation, grain yield and nitrifying activity; indicating the importance of phosphorus in soybean growth and development.

Keywords: Irrigation, nitrogen fixation, nitrobacteria, soybean, Sudan Savanna

Introduction

Irrigation farming has enhanced grain legume yield and production, though it has problem such as salt accumulation in the soil significantly which limits crop production and consequently has negative effects on food security. The consequences are damaging in both socio-economic and environmental terms (Esmail *et al*., 2011). To mitigate this problem and minimize crop loss, scientists searched for and developed salt-tolerant crops through breeding and Legumes such as soybean (*Glycine max* L.) was suggested as appropriate crop for the enhancement of bio-productivity and reclamation of marginal lands; because these plants not only yield nutritious fodder, protein-rich seeds and fruits, but they also enrich soil with nitrogen in symbiotic association with *Bradyrhizobium* (Younesi and Moradi, 2013; Adekunle *et al*. 2013; Maina *et al*., 2012).

Soybean cultivation in Nigeria has expanded as a result of its nutritive and economic importance and diverse domestic use. Given its cash/food values and high potential for soil fertility improvement and *striga* control (Jantar *et al*., 2013); soybean production in the Sudan Savanna of Nigeria is currently on the rise. Soybean requires adequate phosphorus supply for satisfactory nodule production and nitrogen fixation. Phosphorus has consistently increased grain yield of soybean on phosphorus deficient soils and also favors production and retention of more pods per plant (Chiezey, 2001). Biological nitrogen fixation (BNF) is a natural way whereby atmospheric nitrogen is converted to the plant available forms without polluting the environment and reduced to ammonia in the presence of nitrogenase enzyme (Wagner, 2012). The ammonia is further converted to nitrate (NO_3^-) by the action of nitrifying bacteria (Amba *et al*., 2013).

Due to increasing human population and insufficient rainfall, irrigation farming in Kadawa Kano in the Sudan Savanna of Nigeria has become the order of the day to improve and sustain the living population conditions and meet the high food demand to achieve food security in Nigeria. With the current challenges confronting sustainable food production under irrigation, there is need for continued research to identify the best soybean variety that has higher N_2 fixing capacity and yield under non-salt affected soil in this region.There is dearth of studies available regarding the effect of nitrogen and phosphorus fertilizers on nitrogen fixation, nitrobacteria and grain yield under irrigated conditions for soybean in this region. The aim of this study therefore, was to assess the effect of nitrogen and phosphorus fertilizers on nitrogen fixation, nitrobacteria and grain yield of soybean.

Materials and Methods

Site Description

This experiment was conducted at the Irrigation Research Station of the Institute for Agricultural Research (IAR) located at Kadawa-Kano (11°56′N, 08°42′E) under non-salt affected field and 500 meters above sea level in the Sudan ecological zone of Nigeria. The mean daily maximum and minimum temperature were 31°C and 21°C respectively. Temperatures fall as low as 15°C between November to January and could rise to 40°C or more between March to May (Maina *et al.*, 2012).

Soil Sampling, Land Preparation and Agronomic Practices

Soil sampling was done at two depths (0-15cm and 15-30 cm). Samples were air-dried, ground and passed through 2mm sieve for routine soil analysis. Following standard recommended methods, Particle size distribution was determined by Bouyoucos hydrometer method (Gee and Dr, 2002). Soil pH was determined in 1:2.5 soil: water ratio and salt (0.01M $CaCl_2$) suspension with a glass electrode pH meter. Organic carbon determined using wet oxidation method of Walkley-Black, total nitrogen by the Kjeldahl method and available P using $Bray^{-1}$ method were determined as described by Okalebo *et al*. (2002). Exchangeable bases were determined as follows: Ca and Mg using Atomic Absorption Spectrophotometer (AAS); K and Na by Flame Emission Photometry after extraction by 1N NH_4OAc (Hesse, 1971). Exchangeable acidity (Al^{3+} + H^+) was determined by titration method after extraction with 1N KCl (Anderson and Ingram, 1993). The total exchangeable bases obtained and the exchangeable acidity was summed up to give effective cation exchange capacity (ECEC). Electrical conductivity (EC) was measured using conductivity meter (Rayment and Higgison, 1992). Sodium Adsorption Ratio (SAR) was computed as follows:

$$\mathrm{SAR} = \frac{\mathrm{Na}^{+}}{\sqrt{\mathrm{Ca}^{2+} + \mathrm{Mg}^{2+}}} \ldots\ldots (1)$$

Where, Na^+=Sodium, Ca^{2+}=Calcium, Mg^{2+}=Magnesium

The field was ploughed, harrowed and ridged 75cm apart and planted with soybean at inter and intra spacing of 75cm × 5cm. Two seeds per hole were sown and at two weeks after germination were thinned down to one plant per stand. All plots were manually weeded using hoe, at three and six weeks after planting (WAP). Fertilizer application was done a week after sowing.

Treatments and Experimental Design

The treatments consist of two levels of nitrogen (0 and 20 kg N ha^{-1}) applied as urea and four levels of phosphorus fertilizers (0, 6.6, 13.2, 26.4 kg P ha^{-1}) applied as single super phosphate and one (1) grain legume variety; soybean (*Glycine max* L. Merr) variety TGX 1904-6F. The treatments were laid out in a randomized complete block design with levels of N and P in a factorial combination and replicated three times. Alleys were allowed 0.5m within plots and 1m between replications on the non-salt affected field. The plot size was 4m by 4.5m ($18m^2$). The two inner ridges were used as net plots, while the two outer ones as sampling plot. At physiological maturity, grains were harvested and threshed from the pods. All the pods from the net plot of each treatment were harvested, threshed and grain yield recorded in kilogram per hectare.

Determination of N_2 Fixed

Total N difference

The amount of N_2 fixed by Soybean was estimated using the Total Nitrogen Difference (TND) method. This was done by comparing total N of the legume with that of a non-legume (maize- SAMMAZ 14) used as the reference crop (Murray *et al*., 2008). The amount of N fixed was calculated by subtracting total N of the reference crop (maize- SAMMAZ 14) from that of the legume (Soybean), and the difference was assumed as N derived by BNF (N_2 fixed).

Thus, N_2 fixed = Total N in legume -Total N in reference crop.

Where,

$$\text{Total N in plants} = \frac{\text{Dry matter weight (kg/ha) x \% N in plants}}{100\text{g}} \ldots\ldots (2)$$

$$\%\ \text{Ndfa} = \frac{[\text{Total N in legume} - \text{Total N in reference crop}]}{\text{Total N in legume}} \ldots\ldots (3)$$

Where % Ndfa is the percentage of N_2 derived from the atmosphere

Statistical Analysis

Data collected was subjected to analysis of variance (ANOVA) using SAS computer package (SAS, 2011) and differences in means of treatments were determined using Duncan Multiple Range Test at 5% level of significance (Duncan, 1955).

Results and Discussion

Results

Physical and Chemical properties of the Soil

The physical and chemical properties of the sites of study are shown in Table 1. The texture of the soils showed that the soil was sandy loam. The soil reaction was slightly acidic in the non-salt affected field at both depths (0-15 and 15- 30 cm) with pH of 6.59 and 6.61 respectively. The organic carbon for the non-salt affected field (7.76 and 4.98 g kg^{1}) was considered low. The available P (82.71 and 77.0 mg kg^{-1}) was considered high. The total N was 0.7 and 0.14 g $kg^{-1}$and low according to Federal Ministry of Agriculture and Natural Resources (FMANR, 1990). Exchangeable Ca^{2+} (3.7 and 4.0 cmol kg^{-1}) was medium, while Mg^{2+} (2.5 and 1.7 cmol kg^{-1}) was high. Exchangeable K^{+} was 0.13 and 0.08 cmol $kg^{-1}$and low, while Na^{+} was medium (0.14 and 0.26 cmol kg^{-1}) in the soil. Effective cation exchange capacity (ECEC) (7.77 and 6.64 cmol kg^{-1}) was medium according to FMANR (1990). The electrical conductivity (EC) values were generally low (0.81 and 1.15 dSm^{-1}) respectively. Exchangeable sodium percentage (ESP) (1.8 and 3.91%) for non-salt affected field was within safe limits (<15%). The sodium adsorption ratio (SAR) values were 0.08 and 0.15 for both soil depths respectively.

Effect of Nitrogen and Phosphorus fertilizers on nodulation of Soybean on non-salt-affected field

Nodulation of Soybean at 6 and 8 weeks after planting

Results presented in Table 2, showed that nitrogen (N) application had no significant (P>0.05) effect at 6 and 8WAP. Plots that received no N application (0 kg N ha^{1}) gave the highest number of nodules (2.16 and 4.91) at 6 and 8WAP respectively. On the contrary, application of 20 kg N ha^{-1}generally suppressed nodule number. Application of P at 8WAP did not show any significant (P>0.05) difference except at 6WAP. Higher nodule number (4.00 and 5.33) was obtained at P rate of 0 $kgPha^{-1}$ for 6 and 8WAP respectively. Results also showed that P application decreased nodule number. Nitrogen application had no significant ((P>0.05) effect on nodule weight at both weeks. Control recorded the highest mean nodule weight at 8WAP (0.04), while 20 kg N ha^{-1} produced lower mean weight (0.01), but at 6WAP, the nodule weight was at par with 0kg N ha^{-1} and 20 kg N ha^{-1}. Phosphorus application as well did not have significant (P>0.05) effect on nodule weight at both weeks. Though at 6WAP, 0 and 6.6 kg P ha^{-1} (0.02g) was obtained (Table 2), while at 13.2 and 26.4 kg P ha^{-1}, 0.00 g was recorded but at 8WAP; 0.04g was recorded for 0 kg P ha^{-1} and the least 0.00 g at 13.2 kg P ha^{-1}. There was interaction between N and P on nodule number of soybean at 6WAP (Fig. 1).

Effect of Nitrogen and Phosphorus fertilizers on BNF and Grain yield of Soybean on non-salt-affected field

Biological nitrogen fixation of Soybean at 6 and 8 weeks after planting (Table 3) indicates that application of N and P had no significant (P>0.05) effect on amount of N_2 fixed and % Ndfa at 6 and 8WAP, but application of 20 kg N ha^{-1} gave higher amount of N_2 fixed (134.43 and 145.40 kg N) and % Ndfa (73.21 and 73.56%) at 6 and 8WAP respectively. At 6WAP, plots that did not receive P (0 kg P ha-^{1}) gave higher amount of N_2 fixed (145.94 kg N) compared to the plots that received (6.6, 13.2 and 26.4 kg P ha-^{1}). But, at 8WAP, plots that received no P gave the lowest amount of N_2 fixed (118.16 kg N), while those that received 13.2 kg P ha^{-1} gave the highest (152.50 kg N). At 6WAP, plots that received no P gave the highest % Ndfa (75.56%) compared to all other treatments, while at 8WAP, application of 26.4 kg P ha^{1} gave the highest %Ndfa (75.49%). There was no significant interaction between N and P on amount of N_2 fixed and percentage of N derived from the atmosphere (% Ndfa). Results obtained showed that the application of N did not have significant (P>0.05) effect on grain yield of soybean. It was observed that the plots without N gave higher grain yield (1184.25 kg ha^{-1}) compared to the plots that received N fertilizer (1029.69 kg ha^{-1}). Application of P fertilizer did not have significant (P>0.05) effect on grain yield of soybean. However, application of P at the rate of 26.4 kg P ha^{-1} gave the highest grain yield of 1231.3 kg ha^{-1}. There was significant interaction (P>0.01) with grain yield. Application of 26.4 kg P ha^{-1} under 0 kg N ha^{-1} gave the highest grain yield as shown in Fig 2.

Effect of Nitrogen and phosphorus on Nitrifying bacteria population on non-salt-affected field

Results obtained showed that N applied had significant (P<0.05) effect on population of *Nitrosomonas spp* (Ammonia oxidizing bacteria in soils, while there was no significant (P<0.05) effect on population *of Nitrobacter sp.* (Nitrite oxidizing bacteria). The 20 kg N ha^{-1} consistently gave the highest population of nitrifying bacteria, 0.55×10^{3} cell g/soil and 2.25×10^{3} cell g/soil for *Nitrosomonas spp* and *Nitrobacter spp* respectively. Nitrogen application increased *Nitrosomonas spp* by 43.64% while *Nitrobacter spp* was increased by 174% showing more presence of nitrite oxidizing bacteria (*Nitrobacter spp*). Phosphorus application did not have significant (P<0.05) effect on *Nitrosomonas sp* and *Nitrobacter sp* populations. However, 26.4 kgP h^{-1} applications significantly increased their population in the soil by 0.47×10^{3} and 2.7×10^{3} respectively. From the result obtained, population of *Nitrobacter sp* was much more compared to that of the *Nitrosomonas sp.*

Effect of Nitrogen and Phosphorus on nodulation of Soybean on non-salt-affected field

Application of 20 kg N ha^{-1} suppressed nodulation of soybean compared to fields that received no N application (1.08 and 1.08) for 6 and 8WAP respectively. This finding agrees with the report of Eche (2011) and Fagam *et al.* (2007) who observed that application of 20 kg N ha^{-1} depressed nodulation and N_2 fixation in grain legumes like soybean and cowpea where the soil N is adequate for the nodule bacteria to initiate nodule production. Nodule number was decreased at 6WAP and 8WAP by 50% and 78% respectively, while nodule dry weight was decreased by 75% at 8WAP. Sanginga (2003) observed that 20 kg N

$ha^{-1}$was the best rate to obtain good grain yield without affecting nodulation.

Similar trend was observed for P application. Application of P had significant (P<.05) effect on nodule number at 6WAP on soybean. The control plot (0 kg P ha^{-1}) gave the highest nodule number and nodule dry weight compared to the other rates, suggesting there was adequate inherent available P in the study soil. Application of P at 6WAP and 8WAP decreased nodule number by 91.75% and 47% respectively, and nodule dry weight by 100% and 50% also. Increasing application of P above 6.6 kg P ha^{-1} suppressed nodulation, this agrees with the finding of Udofot (2017) and Tsvetkova and Georgier (2003) who observed that application of P above requirement decreases plant growth, nodulation and acetylene reduction in soybean. This is contrary to the reports of Yakubu *et al*. (2010), Eche, 2011 and Amba *et al*. (2013) who observed that P application increased nodule number of cowpea and attributed it to low level of available P in the soil. In this study, the lack of P response could be due to the high level of native P in the soil according to the ratings of FPDD (2002). In the interaction between N and P on nodule number at 6WAP, it was observed that plots without N and P fertilization gave the best treatment combination for nodule number. With the control recording higher nodule weight than 20 kg N ha^{-1}, the increase could be attributed to the higher nodule number recorded and the negative impact of increased N fertilizer rate on nodulation. This agrees with the findings of Agah (2014) who observed increase in nodule weight in the control over application with 30 kg N ha^{-1} and attributed it to the effect of high N fertilizer application on nodulation.

Effect of Nitrogen and Phosphorus on Biological Nitrogen Fixation (BNF) and Grain yield of Soybean on non-salt- affected field

The application of N did not have significant (P>0.05) effect on N_2 fixed and Ndfa at both 6 and 8 WAP, but 20 kg N ha^{-1} gave higher N_2 fixed and Ndfa suggesting the need for a starter dose and that the mineral N could have positively imparted on the activities of nitrogenase enzyme complex responsible for nitrogen fixation in legumes. This finding agrees with Hardarson *et al.* (1984) who observed soybean genotypes (Ada, Altonia and Kalilur) fixed higher N_2 at 20 kg N ha^{-1}. The average amount of N_2 fixed was within the range of 106 – 145 kg N ha^{-1}. These values fall within the range of 55 – 188 kg N ha^{-1} reported by Giller (2001). However, this is contrary to the findings of Sanginga *et al*. (2001) and Yusuf *et al*. (2006) who reported ranges of 38-126 kg N ha^{-1} and 4-50 kg N ha^{-1} respectively. The large range of these estimates could be due to differences in N –fixing ability of the genotypes, the fertility status of the soil (Sanginga *et al*., 1997), indigenous *Bradyrhizobium sp* and crop management (Okogun *et al*., 2005). The high amount of fixed N_2 showed a higher potential to alleviate soil N deficiency. The average amount of % Ndfa obtained was between 68- 74% and falls within the range of 26- 875 reported by Sanginga *et al*. (1997). This suggests that the main contribution of grain legumes to soil fertility improvement lies in their ability to fix atmospheric N as observed by Yusuf *et al*. (2008). Hence, genotypes that derive high amounts of their N_2 from atmospheric fixation will be highly suitable for N deficient soils. From the study, application of N fertilizer at 6WAP and 8WAP increased N_2 fixed by 21.49% (134.83 kg N ha^{-1}) and 12.68% (145.40kgNha^{-1}) respectively, while % Ndfa was 6.53% (73.21%) and 8.40% (73.56%) also.

Generally, phosphorus application did not significantly (p<0.05) affect N_2 fixation and % Ndfa at both 6WAP and 8WAP but 26.4 kg P ha^{-1} at 8WAP gave the highest N_2 fixed (151.17 kg N ha^{-1}) and % Ndfa (75.49%) indicating that 8WAP is the best time for N to be fixed in the soil and also %Ndfa for the benefit of soil fertility improvement. This agrees with the findings of Yakubu *et al*.(2010) and Sanginga *et al*. (1996) who reported that application of P at 40 kg P_2O_5 ha^{-1} to soybean and cowpea varieties increased N_2 fixation and % nitrogen derived from atmosphere (%Ndfa). Nitrogen fixation and % Ndfa at 6WAP was decreased by 28.64% and 10.30% respectively, but was increased at 8WAP by 21.84% and 7.47% also.

The result showed that application of 20 kg N ha^{-1}decreased grain yield by 15% compared to the control though not significant. Several studies have shown inconsistent response of soybean to nitrogen application making it difficult to draw conclusion about soybean response to N fertilizer (Pulver *et al*., 1985; Barker and Sawyer, 2005). Result of this study agrees with the findings of Ncho *et al*. (2013) who observed that fertilization of soybean with inorganic N in the Northern Guinea savanna resulted in low grain yield compared with soybean inoculated with rhizobium inoculants. Similarly, application of P had no significant (P<0.05) effect on grain yield of soybean, though indicating the importance of P in soybean growth and development. This agrees with the findings of Yakubu *et al*. (2010) and Eche, (2011) who observed increase in grain yield due to increase in P application up to 40 kg P ha^{-1}. There was significant (P<0.01) interactive effect of N and P on grain yield with the result showing that application of 26.4 kg P ha^{-1} with no N gave the highest grain yield than the other level of interaction confirming the importance of P fertilizer application to soybean.

Effect of Nitrogen and Phosphorus on Nitrifying bacteria population on non-salt-affected field

Results obtained showed that N applied had significant (P<0.05) effect on population of *Nitrosomonas sp* (Ammonia oxidizing bacteria)in soils while there was no significant (P<0.05) effect on population *of Nitrobacter sp.* (Nitrite oxidizing bacteria). The significant increase on *Nitrosomonas* bacteria could be due to the decrease in soil pH as *Nitrosomonas* bacteria are known to strive better in acidic soils. The 20 kg N ha^{-1} consistently gave the highest population of nitrifying bacteria, 0.55×10^3 cell g/soil and 2.25×10^3 cell g/soil for *Nitrosomonas sp* and *Nitrobacter sp* respectively. The higher population of *Nitrobacter sp* obtained suggests higher soil nitrite accumulation which acts as a substrate for the nitrite oxidizing bacteria, hence rapid conversion to nitrate resulting in the increase in soil pH. *Nitrosomonas sp* was increased by 43.64% while *Nitrobacter sp* was increased by 174%, indicating the high presence of nitrite oxidizing bacteria (*Nitrobactersp*). Increase in nitrifying bacteria with application of organic N fertilizers was also reported (Tisdale and Nelson, 1970).

Phosphorus application did not have significant (P<0.05) effect on *Nitrosomonas sp* and *Nitrobacter sp* populations. However, 26.4 kg P h^{-1} application significantly increased their population in the soil by 8.51% and 51% respectively indicating the importance of sufficient P in nitrification process. This is contrary to the findings of Sahrawat (2008) who reported that addition of P did not have effect on nitrification in soils. The population of *Nitrobacter sp* was however far more compared to that of the *Nitrosomonas sp.*

Conclusion

It may be concluded from the present study that increasing levels of nitrogen negatively affected nitrogen fixation, grain yield of soybean, but enhanced nitrobacteria activity suggesting that the soil N was adequate for the native strains of rhizobia to initiate nodule production on plant roots growing where no N was applied. On the other hand, P application at 26.4 kg P ha^{-1} gave the highest N_2 fixation, grain yield and nitrifying bacteria population indicating the importance of phosphorus in the overall performance of grain legumes such as soybean.

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Table1: Physical and chemical properties of the soil (Non-salt affected soil) of the experimental site

Parameters	0-15cm	15-30cm
Sand(g/kg)	660	654
Silt(g/kg)	194	153
Clay(g/kg)	146	193
Textural Class	Sandy loam	Sandy loam
pH(H_2O)	6.59	6.61
pH($CaCl_2$)	6.38	6.46
Org. C(g/kg)	7.76	4.98
Total N(g/kg)	0.7	0.14
Avail. P(mg/kg)	82.71	77.0
Total P(mg/kg)	704.5	1000
Exchangeable Bases (cmol/kg)		
Ca^{2+}	3.7	4.0
Mg^{2+}	2.5	1.7
K^+	0.13	0.08
Na^+	0.14	0.26
EA(H^++Al^{3+})	1.3	0.6
ECEC	7.77	6.64
EC(dSm^{-1})	0.81	1.15
ESP(%)	1.8	3.91
SAR	0.08	0.15

Org. C=Organic Carbon, Avail. P= Available Phosphorus, ECEC=Effective Cation Exchange Capacity, ESP=Exchangeable Sodium Percentage, SAR= Sodium Adsorption Ratio. EA=Exchangeable acidity, EC=Electrical conductivity

Table 2: Effect of Nitrogen (N) and Phosphorus (P) fertilizer on nodulation of Soybean at 6 and 8WAP on non-salt-affected field

Treatments	NN6	NN8	NDW6	NDW8
Nitrogen (kg N/ha)				
0	2.16	4.91	0.01	0.04
20	1.08	1.08	0.01	0.01
LS	NS	NS	NS	NS
SE(±)	0.609	1.868	0.007	0.017
Phosphorus (kg (P/ha)				
0	4.00[a]	5.33	0.02	0.04
6.6	1.50[ab]	3.00	0.02	0.03
13.2	0.66[b]	0.83	0.00	0.00
26.4	0.33[b]	2.83	0.00	0.02
LS		NS	NS	NS
SE(±)	0.862	2.641	0.010	0.025
Interaction				
N*P	*	NS	NS	NS

[abc] implies having the same letter within the same column and treatment are not significantly (P>0.05) different. NN6 =Nodule number at week 6, NN8= Nodule number at week 8, NDW6=Nodule dry weight at week 6, NDW8=Nodule b weight at week 8. SE=Standard error, LS=Level of significance (* at 5% and ** at 1%), NS=Not significant •P<0.05)

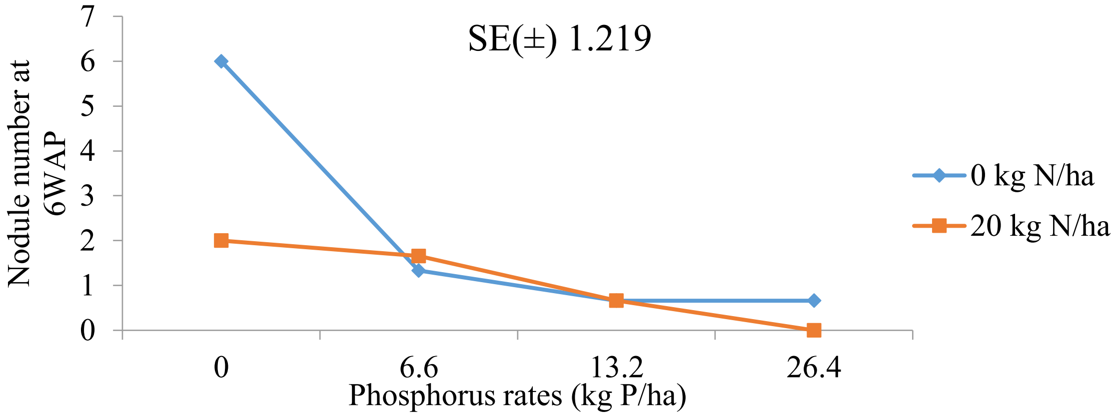

Figure 1: Interaction between N and P fertilizer rates on nodule number of Soybean at 6WAP on non-salt-affected field

Table 3: Effect of Nitrogen and Phosphorus fertilizers on BNF and Grain Yield of Soybean on non-salt-affected field

Treatments	N^2fixed6	N_2fixed8	Ndfa6	Ndfa8	Grain Yield (kg ha^{-1})
		kg N ha^{-1}		%	
Nitrogen (kg N/ha)					
0	105.86	126.96	68.43	67.38	1184.25
20	134.83	145.40	73.21	73.56	1029.69
LS	NS	NS	NS	NS	NS
SE(±)	11.349	23.794	2.056	4.073	60.421
Phosphorus (kg P/ha)					
0	145.94	118.16	75.56	69.85	1006.30
6.6	128.82	122.89	73.00	69.44	1131.00
13.2	102.49	152.50	66.92	67.09	1059.40
26.4	104.15	151.17	67.78	75.49	1231.30
LS	NS	NS	NS	NS	NS
SE(±)	16.050	33.650	2.908	5.760	85.448
Interaction					
N*P	NS	NS	NS	NS	[redacted]

Means within the same column having the same letter are not significantly (P>0.05) different. N_2fixed6=Nitrogen fixed at week6, N_2fixed8=Nitrogen fixed at week 8, %Ndfa6=percent nitrogen derived from the atmosphere at week 6, %Ndfa8= percent Nitrogen derived from the atmosphere at week 8. SE=Standard error, LS=Level of significance (* at 5% and at 1%), NS=Not significant (P<0.05).

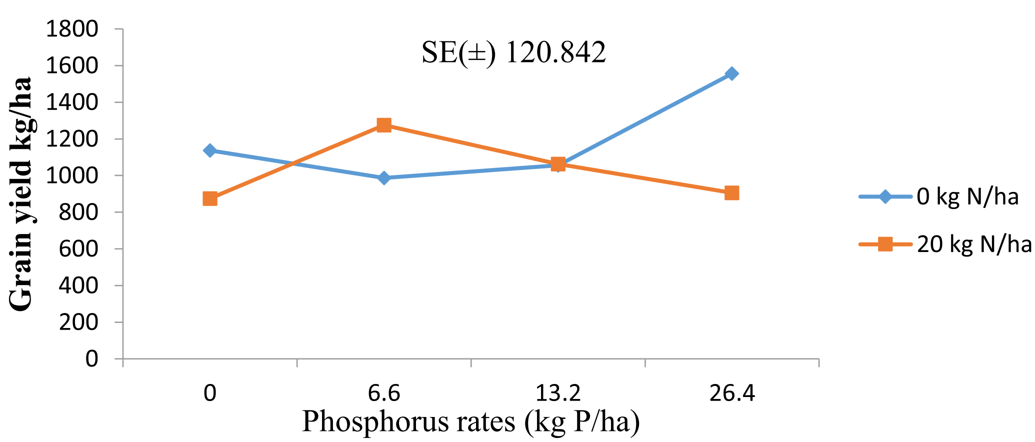

Figure 2: Interaction between N and P fertilizer rates on grain yield of Soybean on non-salt-affected field

Table 4: **Effect of Nitrogen and Phosphorus on Nitrifying bacteria population in soils on non-salt-affected field**

Treatments	*Nitrosomonas*	*Nitrobacter*
Nitrogen (kg N/ha)		
0	0.31×10^{3b}	1.14×10^{3}
20	0.55×10^{3a}	2.25×10^{3}
LSD	**	NS
SE(±)	0.02	0.50
Phosphorus (kg P/ha)		
0	0.43×10^{3}	1.32×10^{3}
6.6	0.45×10^{3}	1.28×10^{3}
13.2	0.38×10^{3}	1.49×10^{3}
26.4	0.47×10^{3}	2.7×10^{3}
LS	NS	NS
SE(±)	0.02	0.50

[abc] Means within the same column having the same letter are not significantly (P>0.05) different. SE=Standard error, LS=Level of significance (* at 5% and ** at 1%), NS=Not significant (P<0.05)