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# Effect of NP Fertilizer Rate and Bradyrhizobium Inoculation on Nodulaton, N-Uptake and Crude Protein Content of Soybean [*Glycine Max* (L.) Merrill], At Jinka, Southern Ethiopia

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**ABSTRACT**
A field experiment was conducted at Jinka Agricultural Research Center to determine the effect of NP fertilizers application rate and bradyrhizobium inoculation on nodulation, N-uptake and crude prtein content of soybean [Glycine max (L.) Merrill] at Jinka under rain fed conditions in 2008. The experiment was conducted with two levels of nitrogen in the form of urea (0 and 46 kg ha$^{-1}$), two levels of bradyrhizobium (0 and Str-TAL-379) and four levels of phosphorous fertilizer in the form of TSP (0, 25, 50 and 75 kg ha$^{-1}$). The experimental design was split-split plot with four replications where, N was arranged as main plot factor, bradyrhizobium and P were arranged as sub and sub-sub plot factors, respectively. Nodulation parameters, N-uptake and crude protein content of soybean were studied. Nitrogen application significantly affected N uptake by soybean except straw N uptake. Application of 46 kg N ha$^{-1}$, inoculation or P fertilization of 25 kg P ha$^{-1}$ resulted in higher net benefit and maximum MRR (%). Therefore, it can be concluded from this result that nitrogen application of 46 kg N ha$^{-1}$, inoculation or phosphorous application at the rate of 25 kg P ha$^{-1}$ is advisable and could be appropriate for soybean production in the test area even though further testing is required to put the recommendation on a strong basis.
**Key words:** Inoculation, Nitrogen fertilizer, Phosphrous fertilizer and Soybea

## 1. Introduction

The soybean [*Glycine max (L.*) Merrill] is a species of legume native to East Asia. It is a member of the Leguminosae family. Soybean [*Glycine max (L.*) Merrill], originally confined to temperate Zones is becoming more important in many tropical regions, especially in Brazil, South America, the Far East and more recently Africa (CIAT, 1987).

Although most parts of the southern regions of Ethiopia have a potential for soybean production, cultivation is limited to only some areas. In addition, farmers around the study area do not yet cultivate soybean. So far, there is no research conducted in the area investigating the response of the crop to NP fertilization and bradyrhizobium inoculation. Thus, for inclusion of the crop in the existing cropping systems and increasing its productivity, some of the packages that could increase productivity of soybean such as NP fertilization and inoculating the crop with specific rhizobium strains should be worked out.

Objectives:-

1. To determine the influence of *Bradyrhizobium japonicum* strain inoculation and NP fertilization on nodulation, yield and yield components of soybean [*Glycine max* (L.) Merrill] cultivar at Jinka, Southern Ethiopia
2. To assess the nodulation and N fixation capacity of soybean cultivar at different levels of NP fertilizers application at the experimental site
3. To identify the optimum rate and combinations of NP fertilizer with the specific bradyrhizobium inoculation for profitable soybean production at Jinka.

## 2. MATERIALS AND METHODS

### 2.1. Description of the Study Area

The experiment was conducted at Jinka Agricultural Research Center located at 5°52' N latitude and 36°38' E longitude. Jinka is situated in south Ethiopia at 750 kms from Addis Ababa, at an altitude of 1450 m above sea level. The average annual rainfall of the area for the last twelve years is 1294 mm with a range of 994.1 to 1675.8 mm, while the average annual minimum and maximum temperatures were 16.1ºc and 27.6ºc, respectively. The main rainy season extends from March to June interrupted by some dry periods in May. The experiment was conducted during the second cropping season (July to October, 2008) under rain fed condition.

Meteorological data such as mean monthly rainfall (mm) and average minimum and maximum temperatures (ºc) of Jinka for the year 2008 are indicated on Figure 1.

### 2.2. Treatments and Experimental Design

Treatments were made from a combination of three factors. Four levels of phosphate fertilizer (0 kg, 25 kg and 50 kg and 75 kg P ha$^{-1}$) in the form of (TSP), two levels of nitrogen fertilizer (0 kg ha$^{-1}$ and 46 kg N ha$^{-1}$) in the form of urea and inoculation (inoculation with strain of bradyrhizobium strain, Str-TAL-379 from national soil research center or without inoculation) were used. One variety of soybean namely Williams, which is early maturing was selected for the study based on its yielding potential and protein content.

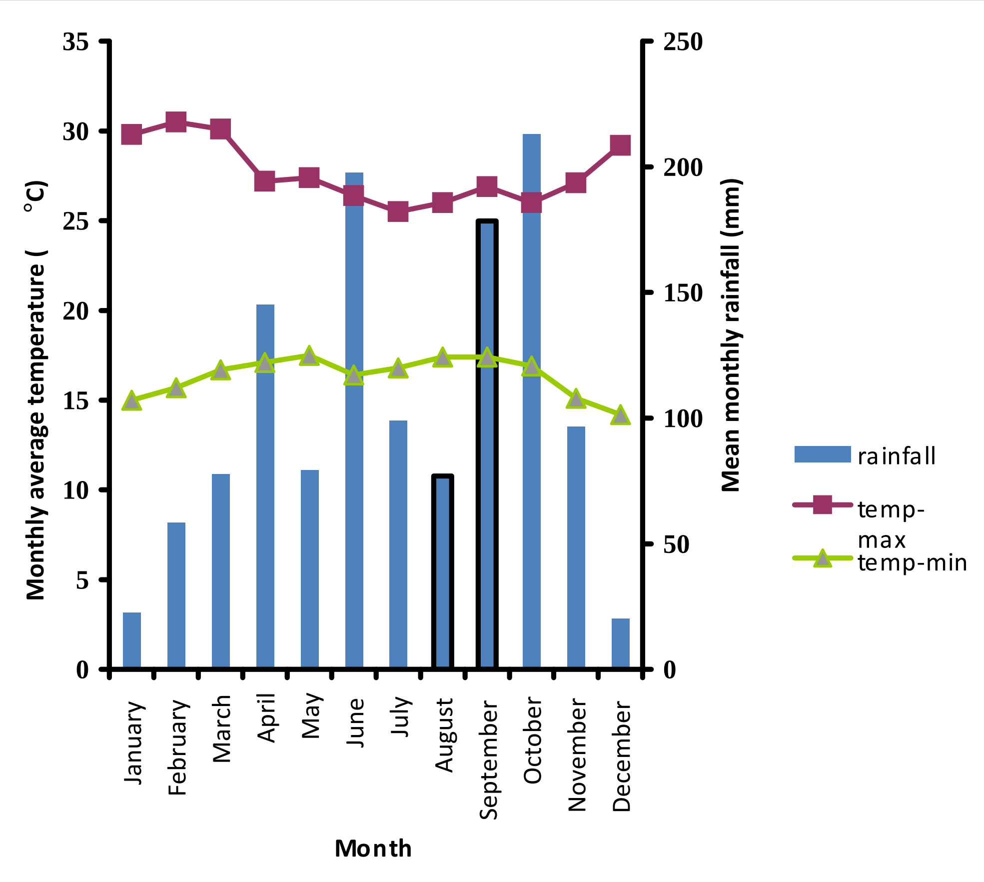


Figure 1: Average Maximum and Minimum Temperature (°c) and Mean Monthly Rainfall (mm) at Jinka, in 2008.

The experiment consisted of 16 treatments with a total of 64 plots. The field experiment was laid out in a split-split-plot design with four replications. Nitrogen levels, inoculation and phosphorous levels were used as main plot, sub-plot and sub-sub plot factors, respectively. The seeds of soybean for the inoculation treatments were inoculated with carrier- based inoculants of *Bradyrhizobium japonicum* (Str-TAL-379) at the rate of 10 g $kg^{-1}$ of seed applied to the moistened seeds. All inoculations were done just before planting under shade to maintain the viability of the Bradyrhizobium bacteria. Seeds were air dried for a few minutes before planting.

2.3. Agronomic Management

Soybean was sown on July1, 2008 in eight rows per plot with spacing of 40 cm between rows and 10 cm between plants within a row. Each plot was 3 m long and 3.2 m wide. Plots receiving no inoculants were planted before others following those receiving inoculants in order to reduce the possibility of contamination. A single person, on a single factor level basis has made the planting for the inoculation factor. Ridges were made between each plot to reduce the movement of bacteria from plot to plots by rainwater.

3.6. Plant Tissue Analysis

At physiological maturity, five plants from the two central rows were randomly selected from each plot. The sampled five plants were composited in to one sample and were partitioned in to grain and straw. The total N in the tissue samples was quantitatively determined by Kjeldahl procedure by treating the plant with $H_2SO_4$ (Sahelemedhin and Taye, 2000). Grain nitrogen content is therefore multiplied by 6.25 (100/16) to give grain crude protein percent (AOAC, 1975). Tissue nitrogen (%) was calculated as the ratio of total nitrogen content of the tissue to total biomass multiplied by 100.

3.8. Economic Analysis

Simple partial budget analysis was employed for economic analysis of fertilizers and inoculum. The price of fertilizers and inoculum and the crop potential response towards the added fertilizers and inoculum were used to determine the economic feasibility of fertilizers and inoculum. To estimate the total costs, mean market price of soybean, N and P fertilizers and inoculum were taken from market assessment at the time of harvesting. The economic analysis was based on the formula developed by CIMMYT (1988).

Adjusted yield (t ha$^{-1}$): the average yield adjusted downward by a 10% to reflect the difference between the experimental yield and yield of farmers.

Gross field benefit: calculated by multiplying field price that farmers receive for the crop when they sale it by adjusted yield and subtracting all costs associated with harvest and sale.

Net benefit: calculated by subtracting the total variable costs from the GFB for each treatment.

Marginal rate of return (MRR%): calculated by dividing change in net benefit by the marginal cost, that is change in cost.

**Results and Discussion**

2. 4. Nodulation Parameters

Nitrogen fertilization had significantly increased nodule number and nodule dry weight (Table 1). Maximum number of nodule (21) was from application of 46 kg N ha$^{-1}$ and minimum (15) was from no N application (Table 2). The highest nodule dry weight (230 mg plant$^{-1}$) was recorded from 46 kg N ha$^{-1}$ and the lowest (90 mg plant$^{-1}$) was from no N application (Table 2). This showed that nitrogen enhances nodulation when applied in small amount as a starter fertilizer during inoculation. This may be probably attributed to the positive effect of N fertilizer to initiate nodulation. Mohammed *et al*. (2001) reported that application of 50 kg N ha$^{-1}$ produced greater number of nodules and more nodule dry weight. However, Dereje (2007) reported that N fertilization did not significantly affect nodule number, nodule dry weight and volume in soybean at Awassa.

Inoculation had significantly affected nodule number and nodule dry weight (Table 1). The maximum number of nodules (34.4) was recorded for inoculated treatment while nodules were almost absent from non inoculated treatments (Table 2). A similar effect was observed on nodule dry weight. Inoculation enhanced nodule formation and development in the roots of soybean. It can be suggested that there were no native rhizobia to infect soybean. This could be attributed to the fact that soybean has not been growing in the area before. Similar results were reported by Neveen (2008), who reported that inoculation of soybean significantly increased the nodule number over the control. The author also stated that inoculation had significantly affected nodule dry weight. Moreover, Islam *et al*. (2004) reported that bradyrhizobium inoculation resulted in greater number of nodules (24.1) in soybean.

Phosphorous fertilizer significantly affected nodule number per plant and nodule dry weight (Table 1). Generally, P improves nodule number and nodule dry weight. The reason might be that phosphorous improves the overall performance of the plant leading to a better nodulation. Ankomah *et al*. (1996) reported that application of P resulted in a significant increase in nodule number on cowpea.

Table 1: Mean Square Values for Nodule Numbers and Nodule Dry Weight of Soybean at Mid Flowering Stage as Affected by Nitrogen Fertilizer, Inoculation and Phosphorous Fertilizer at Jinka, in 2008.

| | | Mean square | |
|---|---|---|---|
| Source | DF | Nodule number plant$^{-1}$ | Nodule dry weight ( gm) |
| Replication(R) | 3 | 16.29$^{ns}$ | 0.006$^{ns}$ |
| Nitrogen (N) | 1 | 650.25*** | 0.29*** |
| Error a | 3 | 8.38 | 0.003 |
| Inoculation (I) | 1 | 17424*** | 1.33*** |
| N*I | 1 | 676$^{ns}$ | 0.29$^{ns}$ |
| Error b | 6 | 8.25 | 0.005 |
| Phosphorous (P) | 3 | 50.42* | 0.009* |
| N*P | 3 | 32.08$^{ns}$ | 0.005$^{ns}$ |
| I*P | 3 | 58.83$^{ns}$ | 0.01$^{ns}$ |
| N*I*P | 3 | 37.17$^{ns}$ | 0.004$^{ns}$ |
| Error c | 36 | 17.57 | 0.005 |

*, **, *** indicate significance at P < 0.05, P < 0.01 and P < 0.001, respectively;
'ns,' not significant.

Table 2: Nodule Number and Nodule Dry Weight of Soybean at Mid Flowering Stage as Affected by Nitrogen Fertilizer, Inoculation and Phosphorous Fertilizer at Jinka, in 2008.

| Treatments | Nodule Number plant$^{-1}$ | Nodule dry weight (mg plant$^{-1}$) |
|---|---|---|
| **Nitrogen (N kg ha$^{-1}$)** | | |
| 0 | 15b | 90b |
| 46 | 21a | 230a |
| ***LSD 0.05*** | **2.3** | **0.04** |
| **CV%** | **16.13** | **24.23** |
| **Inoculation (I)** | | |
| Non inoculated ($I_0$) | 1.44b | 20b |
| Inoculated ($I_1$) | 34.44a | 310a |
| ***LSD 0.05*** | **1.76** | **0.04** |
| **CV%** | **16.01** | **24.19** |
| **Phosphorus (P kg ha$^{-1}$)** | | |
| 0 | 15.5b | 140d |
| 25 | 17.8b | 150c |
| 50 | 19.13a | 170b |
| 75 | 19.34a | 190a |
| ***LSD 0.05*** | **3.01** | **8.5** |
| **CV%** | **23.37** | **22.99** |

**Note**: Means with the same letters within the columns are not significantly different at $P < 0.05$.

2.5. Effect on N Uptake and Grain Crude Protein

Significant variation existed in grain N uptake, total N and crude protein content of soybean due to nitrogen fertilization whereas the effect on straw N uptake was not significant (Table 3). Grain N, total N and crude protein content were increased by 35, 31 and 35 %, respectively when N application rate was raised from 0 kg N ha$^{-1}$ to 46 kg N ha,$^{-1}$(Table 4). The reason may be that the improved availability of soil N increased N uptake and consequently protein content. According to Hossain *et al.* (2007), applied nitrogen fertilizer influenced N content of seeds. Pongsakul and Jensen (1979) reported that when N application rate was raised from 0 and 112 kg N ha,$^{-1}$ soybean grain crude protein content increased from 129 to 220 g kg$^{-1}$. Fernando and Dobermann (2006) also reported that grain protein content of soybean ranged from 37 to 42 % and N uptake of 61 to 151 kg N ha$^{-1}$ due to varying N fertilization rate from 46 to 92 kg N ha$^{-1}$. The increase in grain protein content due to N fertilization could be attributed to since N is source of protein and it is also mobile nutrient as a result accumulated in grain as the crop got physiological maturity.

There was no significant variation in straw N, grain N, total N and crude protein content of soybean due to inoculation (Table 3). There was some indication that these values seemed to increase under inoculated treatments, though non significant (Table 4). On the other hand, Dereje (2007) reported that there was significant variation existed in grain N and crude protein percent of soybean due to inoculation by bradyrhizobium strains. He also reported an increased grain yield for inoculation with strain- TAL-379. It seems that the strain has not been effective in fixation in this experiment probably due to unfavorable environmental conditions.

Phosphorous fertilization significantly affected straw N and grain N uptake, total N and crude protein content of soybean (Table 3). Application of P increased nitrogen content in all of these observed parameters. However, the variation among the three upper levels of P application was not significant. The result showed that P fertilization enhanced N uptake of the soybean plant. Taylor and Philadelphia (2006) suggested that phosphorus supply increased the amount of N accumulated at both flowering and late pod filling in soybean. Hossain *et al.* (2007)

 

also reported that N uptake of plants was improved by P fertilization. This indicates that phosphorous application not only increases yield but also improves nutritional quality of soybean.

Table 3: Mean Square Values for Straw N, Grain N, Total N and Crude Protein of Soybean at Jinka, in 2008.

| Source | DF | Straw N (kg ha$^{-1}$) | Grain N (kg ha$^{-1}$) | Total N (kg ha$^{-1}$) | Crude protein (kg ha$^{-1}$) |
|---|---|---|---|---|---|
| Replication(R) | 3 | 2.26$^{ns}$ | 237.86$^{*}$ | 277.52$^{ns}$ | 9291.31$^{ns}$ |
| Nitrogen (N) | 1 | 0.04$^{ns}$ | 1947.95$^{***}$ | 1964.55$^{***}$ | 76091.98$^{***}$ |
| Error a | 3 | 3.02 | 245 | 302.18 | 9571.31 |
| Inoculation (I) | 1 | 4.58$^{ns}$ | 143.39$^{ns}$ | 199.82$^{ns}$ | 5622.09$^{ns}$ |
| N*I | 1 | 8.79$^{ns}$ | 0.07$^{ns}$ | 10.42$^{ns}$ | 2.72$^{ns}$ |
| Error b | 6 | 0.81 | 84.26 | 99.91 | 3291.54 |
| Phosphorous (P) | 3 | 10.66$^{***}$ | 1175.14$^{***}$ | 1377.76$^{***}$ | 45903.75$^{***}$ |
| N*P | 3 | 1.72$^{ns}$ | 380.55$^{ns}$ | 370.53$^{ns}$ | 14865$^{ns}$ |
| I*P | 3 | 0.8$^{ns}$ | 10.98$^{ns}$ | 9.61$^{ns}$ | 429.07$^{ns}$ |
| N*I*P | 3 | 6.58$^{ns}$ | 102.17$^{ns}$ | 153.93$^{ns}$ | 3991.19$^{ns}$ |
| Error c | 36 | 1.56 | 76.43 | 91.69 | 2985.48 |

*, **, *** indicate significance at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively; 'ns,' not significant.

Table4: Nitrogen Uptake of Soybean as Affected by Nitrogen, Inoculation and Phosphorous Fertilizer at Jinka, in 2008.

| Treatments | Straw N (kg ha$^{-1}$) | Grain N (kg ha$^{-1}$) | Total N (kg ha$^{-1}$) | Grain crude protein (kg ha$^{-1}$) |
|---|---|---|---|---|
| **Nitrogen (N kg ha$^{-1}$)** | | | | |
| 0 | 4.64a | 31.29b | 35.93b | 195.51b |
| 46 | 4.69a | 42.32a | 47.01a | 264.51a |
| ***LSD 0.05*** | **NS** | **8.45** | **10.83** | **47.84** |
| **CV%** | **17.21** | **22.53** | **21.92** | **21.62** |
| **Inoculation (I)** | | | | |
| Non inoculated ($I_0$) | 4.39a | 35.31a | 39.70a | 220.65a |
| Inoculated ($I_1$) | 4.93a | 38.30a | 43.23a | 239.40a |
| ***LSD 0.05*** | **NS** | **NS** | **NS** | **NS** |
| **CV%** | **19.27** | **24.94** | **24.10** | **24.93** |
| **Phosphorous (P kg ha$^{-1}$)** | | | | |
| 0 | 3.36c | 24.07b | 27.70b | 150.44b |
| 25 | 5.57a | 40.82a | 46.39a | 255.14a |
| 50 | 4.51bc | 39.73a | 44.25a | 248.34a |
| 75 | 4.95ab | 42.59a | 47.54a | 266.16a |
| ***LSD 0.05*** | **0.89** | **6.27** | **6.87** | **39.18** |
| **CV%** | **26.74** | **23.75** | **23.09** | **23.75** |

**Note:** Means with the same letters within the columns are not significant

Phosphorous application significantly affected straw N, grain N, total N and grain crude protein content of soybean. There was an improvement in N uptake by soybean with rising P level.

Based on the above findings, application of 46 kg $ha^{-1}$ N fertilizer, inoculation with another suitable strain or checking the viability of the strain before conducting the experiment and P fertilization at the rate of 25 kg P $ha^{-1}$ could be considered as an alternative by small farmers of the experimental area for increasing productivity of soybean. However, there is a need to repeat the experiment to account for weather variability preferably by including the other growing season (March to June).

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