

**EFFECT OF *RHIZOBIUM* INOCULATION, NITROGEN AND PHOSPHORUS
APPLICATION ON THE GROWTH, YIELD AND NODULATION OF
SOYBEAN (*Glycine max* L.)**

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APPLICATION ON THE GROWTH, YIELD AND NODULATION OF
SOYBEAN (*Glycine max* L.)**

by

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CERTIFICATE

This is to certify that the thesis entitled '**EFFECT OF *RHIZOBIUM* INOCULATION, NITROGEN AND PHOSPHORUS APPLICATION ON THE GROWTH, YIELD AND NODULATION OF SOYBEAN (*Glycine max* L.)**' submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE (MS) in SOIL SCIENCE**, embodies the result of a piece of bonafide research work carried out by **MEHERUN NESA LUBNA**, Reg. no. **12-04819** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I, further, certify that any help or source of information, received during the course of this investigation has duly been acknowledged.

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Dedicated To

**My beloved
parents**



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The Author

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Abstract

The experiment was conducted at the farm of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during the period from January 2018 to May, 2018 to study the effect of *Rhizobium* inoculation, nitrogen and phosphorus application on the growth, yield and nodulation of soybean (*glycine max* L.). The experiment comprised of three factors viz. Factor A: *Rhizobium* inoculation; Factors B: Levels of Nitrogen (3 levels)- viz. 0, 25 and 50 kg N ha⁻¹ denoted as N₀, N₂₅ and N₅₀; Factor C: Levels of Phosphorus (2 levels) – viz. 0 and 40 kg P ha⁻¹ denoted as P₀ and P₄₀ P₀: 0 kg P ha⁻¹. The three factors experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. The highest plant height (51.64 cm and 68.18 cm), number of leaves (17.13 and 24.17) and branches plant⁻¹ (4.07 and 10.50) at 30 DAS and 60 DAS respectively were also observed in RI₁N₂P₁ treatment combination. The highest weight of dry shoot (2.93 gm plant⁻¹), dry root (0.66 gm plant⁻¹) and total plant weight (11.88 gm plant⁻¹) were respectively in RI₁N₂P₁ treatment. The application *Rhizobium* inoculant along with N and P showed positive effect on the number of pods plant⁻¹, number of seed pod⁻¹, number of seeds plant⁻¹, 100 seed weight (gm), seed wt. plant⁻¹(gm) grain yield (t ha⁻¹) and stover yield (t ha⁻¹). The maximum number of nodule plant⁻¹ (49.64), size of effective nodule (3.60 mm in diameter) were observed in RI₁N₂P₁ treatment. The highest seed yield ((2.06 t ha⁻¹) was observed from RI₁N₂P₁ treatment which was statistically similar with RI₁N₁P₁ treatment. It may be concluded that application of 25 kg N ha⁻¹ & 90 kg P ha⁻¹ along with *rhizobium* inoculation can be more beneficial for the farmers to get maximum yield.

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CHAPTER I

INTRODUCTION

Soybean (*Glycine max* L.) is one of the most important oil seed legume crops of the world. It belongs to the family Leguminosae, sub-family Papilionaceae. It is considered as an important economic food legume cultivated worldwide because of its higher nutritional and industrial values. Soybean seed is referred to the protein hope of the future because of its high nutritive value containing about 42-45% protein (Rahman, 2001). It builds up the soil fertility by fixing a large amount of atmospheric nitrogen through the root nodules and also through leaf fall on the ground at maturity. The nodulated soybean plants can fix 94 kg nitrogen in a hectare in one season (Satter, 2001). Soybean contains 20% edible oil, 35% carbohydrate, 40% protein, 10% moisture, 4% mineral and 5% ash. In view of the nutritional value of soybean, it is perhaps the best of all other pulses (Kim, 1995).

Soybean was introduced in Bangladesh many years ago but its cultivation as well as yield of soybean is not satisfactory compared to developed countries. The statistical information regarding soybean acreage and production in Bangladesh is 41,388 hectare and 65,158 metric ton. The annual production of soybean in Bangladesh is negligible compared to its huge demand. Despite suitable climatic and edaphic conditions the yield of soybean in the world is about 3.0 t ha^{ix} while that in Bangladesh is only 1.4 t ha⁻¹ (BBS, 2012).

The use of biological nitrogen fixation (BNF) technology in the form of *Rhizobium* inoculants in grain legumes can be an alternative of expensive fertilizer, particularly for improving the production of food legumes in the country. *Rhizobium* can fix atmospheric nitrogen by symbiosis process with roots of legume crops and makes N available to plants. A field trail showed that *Rhizobium* inoculant and different levels of nitrogen increased nodulation, growth and nitrogen uptake of lentil (Mahmud *et al.*, 1997).

Biological nitrogen fixation and seed yield of soybean also significantly increased due to *Rhizobium* inoculant. Generally it is considered that inoculation with rhizobia should be performed in two different situations. (1) in soils which are depleted or contain a low indigenous rhizobial population. (2) when there is an established but inefficient rhizobial population (Araujo *et al.*, 1994).

Nitrogen is the most important nutrient element among the major essential elements. It is the most deficient element in Bangladesh soils limiting crop production. For legume, nitrogen is more useful because it is the main component of amino acid as well as protein. Adequate supply of nitrogenous fertilizer is essential for normal growth and yield of a crop.

No soil can sustain high yields if it is deficient in P (Rao *et al.*, 1999). As an essential plant nutrient, P is involved in a wide range of plant processes from permitting cell division to the development of a good root system to ensuring timely and uniform ripening of the crop. P is needed most by young, fast-growing tissues, and performs a number of functions to growth, development, photosynthesis, and utilization of carbohydrates (Rao, 1996). Because of the importance of P for plant growth and yield, many compound fertilizers (NPK) used to correct major deficiencies in soil contain P as a major element.

In Bangladesh, limited information is available on the role of *Rhizobium* inoculants, nitrogen and phosphorus on the growth, yield and oil content of soybean. With a view to generate information a field experiment containing the treatments of each of *Rhizobium* inoculants, nitrogen and phosphorus was conducted with the following objectives:

1. To find out effective doses of nitrogen and phosphorus in combination of *Rhizobium* inoculants on nodulation, growth and yield of soybean

2. To observe the effects of N and P on the nodulation of soybean plant.

CHAPTER II

REVIEW OF LITERATURE

2.1 Effects of *Rhizobium* inoculation

Effect of *Rhizobium* inoculation on soybean and other legumes have been presented below:

Kumar *et al.* (2001) inoculated soybean with *Bradyrhizobium* (CP-3) and *Azotobacter* (Mac-21) in nutrient deficient sterilized soil using earthen pots. Plants inoculated with *Bradyrhizobium* + *Azotobacter* exhibited highest nodule number and dry weight.

Okere *et al.* (2000) were conducted two field experiments at Akwa, Nigeria Soybean seeds were inoculated with antibiotic mutants of the bradyrhizobia strains before sowing. increased %N and total N of plant were obtained as a result of increased nodulation by the *Bradyrhizobium* strain.

Sobaran *et al.* (1999) conducted a field experiment in Uttar Pradesh, India, with one soybean variety and two levels of N (0 and 15 kg ha⁻¹). three levels of P (0, 30 and 90 kg P ha⁻¹), two levels of K (0 and 30 kg K ha⁻¹) and uniform inoculation with *Bradyrhizobium japonicum* multistrain culture (USDA-I) O-TAL-3 77). The results showed that application of 30 kg phosphorus or potassium or N x K interaction, significantly increased the number of nodules at 60 days (flowering stage) of plant growth.

Kavathiya and Pandey (2000) conducted a pot experiment with *Rhizobium* on mungbean (*Vigna radiata* cv. K 851) and found that nodule plant⁻¹ increased significantly over uninoculated control. They also reported that maximum seed germination (96.6%), plant height (24.6 cm), fresh shoot weight (5.33 g), fresh root weight (4.42 g) and nodulation (69 healthy nodules plant⁻¹) was recorded in the *Rhizobium* treatment.

Narjes *et al.* (2000) conducted an experiment In green house; soybean cv. Maple Glen were soil inoculated with the growth promoting rhizobacteria (PGPR) *Serratia proleamaculans* 1-102 or *S. Liquefaciens* 2-68 with or without inoculation by *Bradyrhizobium japonicum* USDA 110 PGPR strains and genistein in combination increased the number of nodules than uninoculated control.

Navgire *et al.* (2001) carried out an experiment on mungbean cultivars to different *Rhizobium* strains under rainfed conditions. Seeds of mungbean cultivars BM-4, S-8 and BM-86 were inoculated with *Rhizobium* strains M-11-85, M-6-84, GR-4 and M-6-65. Cultivars S-8, BM-4 and BM-86 recorded the highest mean nodulation (16.7), plant biomass (8.29 q ha⁻¹) and grain yield (4.79 q ha⁻¹) during the experimental years. S-8, BM-4 and BM-86 recorded the highest nodulation, plant biomass and grain yield.

Islam *et al.* (2002) conducted an experiment to study the performance of some bradyrhizobial inoculants on soybean at BINA experimental farm, Mymensingh. They found significantly lowest nodule number in uninoculated and highest in inoculated treatments, all the *Bradyrhizobium* inoculation treatments performed better in nodulation of soybean.

Effect of Nitrogen:

Wingeyer *et al.* (2014) conducted an experiment on nitrogen fertilization and supplemental irrigation during soybean (*Glycine max* (L.) Merr.) reproductive stages have gained interest to increase soybean yields. We assessed the effect of N fertilizer (0 and 60 kg N ha⁻¹) applied at the beginning of bloom (R₁) and full pod (R₄) combined with rain-fed (NIrr) and irrigated (Irr) conditions during reproductive stages on crop growth and yield in the southeast of Buenos Aires Province, Argentina. At the beginning of seed filling (R₅), aboveground biomass and plant N accumulation were unaffected by the addition of N fertilizer. Average soybean yields were 4.24 and 3.39 Mg ha⁻¹ for Irr and NIrr treatments, respectively, and were not affected by N fertilization. Our results suggest addition of N fertilizer during soybean reproductive stages was not an effective management practice to increase yields of irrigated or rain-fed soybean plants.

Wilson *et al.* (2014) conducted a field study with soybean cultivars released from 1923 to 2008 in maturity group MG II and MG III was conducted in multiple environments with a non-limiting supply of fertilizer N to examine the main effects and interactions of N supply and release year on seed yield and seed quality. Supplemental N totaled 560 kg N ha⁻¹ with 40% applied at planting and 60% applied at V₅. Seed yield increased with release year in MG II (17.2 kg ha⁻¹ yr⁻¹). Application of N to MG II cultivars increased seed protein by 10 to 19.5 g kg⁻¹ across all release years, but seed yield and seed oil was not affected. Seed yield gains of MG III cultivars fertilized with N was 27.4 kg ha⁻¹ yr⁻¹, which was 20% better than unfertilized (22.8 kg ha⁻¹ yr⁻¹). Application of N to MG III cultivars increased seed mass (11%) across release years with no changes in seed protein and oil. The limited supply of N increased seed protein across all release years in MG II cultivars, and the N supply from the soil and biological N fixation was insufficient to maximize seed yield in modern, MG III cultivars in the tested environment.

Ahmed and Ali (2013) reported that legume crops are not only used as human diet but also enriched soil fertility through biological nitrogen fixation. Micronutrients are imperative module of soil fertility and they manipulate crop productivity. An experiment

was conducted to evaluate their effect on soybean productivity. Treatments comprised of control, *Rhizobium* inoculation alone and along with two levels (2 and 4 kg ha⁻¹) of micronutrients. The performance of *Rhizobium* alone was superior over uninoculated control in all parameters. The results showed that increasing rate of micronutrients along with *Rhizobium* inoculation significantly increased mungbean productivity. *Rhizobium* inoculation along with Mo @ 2 kg ha⁻¹ increased plant height, root length, 100-seed weight, nodule number plant⁻¹ and nitrogen content in seed by 59, 67, 72, 162 and 8%, respectively, over uninoculated control whereas highest number of pods plant⁻¹ and seed yield was obtained along with Zn @ 4 kg ha⁻¹. Number of nodules plant⁻¹ was positively correlated with %N in seed and seed yield. It was concluded that micronutrients application along with *Rhizobium* inoculation enhanced mungbean productivity.

Umeh *et al.* (2011) conducted an experiment to determine the effect of different types and rates of nitrogen (N) fertilizer on growth and yield response of soybean varieties under Umudike ecological conditions, Abia State, Nigeria. Pot experiment was conducted in 2008 outside the green house of Michael Okpara University of Agriculture, Umudike. The response of two varieties of soybean (Tropical Glycine Max-TGX 1440 and TGX 1740) to three different nitrogen fertilizer types (NPK 15 15 15, NPK 20 10 10 and Urea) at four different rates (0, 30, 60 and 90 kg N ha⁻¹) were evaluated. It was observed that plant height, number of branches, number of seed, dry matter weight and dry pod weight differed significantly at ($P < 0.05$) in both varieties using different fertilizer types and at different fertilizer rates. TGX 1440 using NPK 15 15 15 at the rate of 60 kg N ha⁻¹ gave the highest plant height than using NPK 20 10 10 and Urea and at other rates (0, 30 and 90 kg N ha⁻¹) in the same variety and in TGX 1740. Also increased application of nitrogen fertilizer rate increased plant height, dry matter weight, dry pod weight and pod length as in TGX 1440 using NPK 15 15 15 and NPK 20 10 10 but decreased pod number per plant.

Inorganic N inhibits the *Rhizobium* infection process and also inhibits N₂ fixation (Zahran, 1999; Anne-Sophie *et al.*, 2002). The former problem probably results from impairment of the recognition mechanisms by nitrates, while the latter is probably due to diversion of photosynthates toward assimilation of nitrates. The inhibitory effects of mineral N on nodulation and N₂ fixation of soybean are clear at high concentrations (>5 mM), but far less at lower concentrations. Although there are a few reports on positive effects of low nitrate concentrations on N₂ fixation in legume species such as soybean (Gulden and Vessey, 1997), Olsson *et al.* (2005) showed that plants reduce carbon allocation to *arbuscular mycorrhizae* when grown in high compared to low nitrogen agar media. Application of large quantities of fertilizer N inhibits N₂ fixation, but low doses (<30 kg N ha⁻¹) of fertilizer N can stimulate early growth of legumes and increase their overall N₂ fixation. The nodulation process begins when the bacteria infect the root hairs of soybean during the early stages of the crop after planting. At this stage, nitrogen is needed to stimulate early growth of soybean before nodulation begins in soils with low residual N or low soil organic matter. Thereafter, soybean regulates this process - lack of available nitrogen triggers the nodulation process and N₂ fixation. The consensus is that some level of N stress is required for symbiotic relationship to fully develop. In-season application of N is not recommended for soybean as yield increase is rarely economic. The amount of this starter nitrogen must be defined in relation to available soil nitrogen (Graham, 1992).

An experiment was conducted by Wingeyer *et al.* (2014) on nitrogen fertilization and supplemental irrigation during soybean (*Glycine max* L. Merr.) reproductive stages have gained interest to increase soybean yields. They assessed the effect of N fertilizer (0 and 60 kg N ha⁻¹) applied at the beginning of bloom (R1) and full pod (R4) combined with rain-fed (NIrr) and irrigated (Irr) conditions during reproductive stages on crop growth and yield in the southeast of Buenos Aires Province, Argentina. At the beginning of seed filling (R5), above ground biomass and plant N accumulation were unaffected by the addition of N fertilizer. Average soybean yields were 4.24 and 3.39 Mg ha⁻¹ for Irr and

NIrr treatments, respectively, and were not affected by N fertilization. Our results suggest addition of N fertilizer during soybean reproductive stages was not an effective management practice to increase yields of irrigated or rain-fed soybean plants.

Boroomandan *et al.* (2009) conducted an experiment on soybean (var. Williams) as a split-plot based on randomized complete blocks design with three replications. Nitrogen starter fertilizer treatments were arranged in three rates (0, 40, 80 kg ha⁻¹ as main plots and plant density as sub plots arranged with three levels (15, 30, 45 plant m⁻² . Based on similarity treatments and experimental designs, the results of analysis of combined variance and mean comparisons showed significant (528.4 kg ha⁻¹ yield increase as density increased from 30 to 45 plant m⁻² and nitrogen starter fertilizer increased from 0 to 40 kg ha⁻¹ in two years. Seed protein was unaffected by plant densities, but nitrogen application changed it. This experiment showed density of 45 plant m⁻² and application of nitrogen starter fertilizer 40 kg ha⁻¹ are optimum and increase seed yield under condition of our experiment.

Osborne and Riedell (2006) conducted a field experiment using a split-plot design with three replications. Whole plots were tillage [no-tillage (NT) and conventional tillage (CT)] with starter fertilizer rate as the split plot treatments. Nitrogen was band applied at planting as urea (UR), at rates to supply 0, 16, 32 and 64 kg N ha⁻¹ . Analysis of the experiment showed an average yield increase of 16.4% and 12.2% for the 32 kg N ha⁻¹ rate, compared to the no N treatment in CT and N, with no difference in seed N or oil concentration. This research demonstrates that applying N as starter has the potential to increase soybean yield but this may or may not translate into improved seed quality in the unique environments of the northern of Iran.

Taylor *et al.* (2005) conducted a field study to determine the optimum economic rate of N that would stimulate early dry matter accumulation, and thus yield, in late-planted soybean. The effects of three planting dates (mid-June, late-June, and mid-July), two MG

VIII cultivars (Kuell and Prichard), and five N rates (0, 25, 50, 75, and 100 kg ha⁻¹) were studied for 2 year at three Alabama locations (Fairhope, Shorter, and Crossville). Nitrogen application of 60 to 70 kg ha⁻¹ maximized yield and R1 dry matter accumulation. However, N reduced nodule number and mass, but had no effect on R1 plant height, mature plant height, or seed quality, protein and oil content. Yield was reduced linearly by later planting, but there was no interaction between N rate and planting date for yield. At current prices for N and soybean, we concluded that N can be a viable input for double-cropped soybean at an optimal economic rate of 59 kg ha⁻¹.

A field experiment was conducted by Barker and Sawyer (2005) at five locations in Iowa during 1999 and 2000. The study was to determine the impact of N fertilizer applied to the soil at the beginning pod growth stage on soybean yield and seed quality. Additional objectives were to study alternative N fertilizer and application practices that might enhance soybean use of applied N. Nitrogen treatments were urea and polymer-coated urea broadcast and subsurface band placed between the rows at 45 and 90 kg N ha⁻¹ and a no-N control. The study showed few, small, and inconsistent effects of N material, placement, and rate on seed yield and quality components at individual sites or when combined across individual sites. There were no significant effects on seed yield, with only a 39 kg ha⁻¹ increase from applied N. Seed protein, oil, and fiber concentrations were the same with or without N application. Aboveground plant dry matter (DM) at the R6 growth stage was greater with the higher N rate, but plant DM with N application was lower than the no-N control. Nitrogen concentration in plant DM was significantly increased with applied N. In conclusion, N application increased N concentration in R6 soybean plants, but N rate and alternative application practices had no positive effect on plant DM, seed N concentration and removal, seed yield, or seed quality components. It was concluded that growers should not consider fertilizer N applied to soil during early reproductive stages as a method to increase soybean yield or seed quality.

Chang *et al.* (2005) conducted an experiment in Tandojam, Pakistan in 2004 to investigate the effect of *Bradyrhizobium japonicum* inoculation on N accumulation in soybean at various parts (roots, nodules, stems, pods, leaves and seeds) and growth stages (budding, flowering, podding and maturity). Uninoculated plants served as the control. A higher nodulation N content was recorded in inoculated plants compared to uninoculated ones.

Agha *et al.* (2004) conducted a field experiment to Tandojam, Pakistan. The effects of N fertilizer rates (0, 50 and 75 kg ha⁻¹) singly or in combination with the seed inoculation of *Bradyrhizobium japonicum* on the yield components of soybean. The application of N and inoculation of *Bradyrhizobium japonicum* significantly enhanced seed yield and yield components. The highest number of pods plant⁻¹, number of seeds pod⁻¹, seed weight plant⁻¹, number of nodules plant⁻¹ and seed yield were obtained higher with 50 kg N ha⁻¹ + inoculation of *Bradyrhizobium japonicum*.

Egamberdiyeva *et al.* (2004) conducted a field experiment to see the effect of inoculation with S2492 on soybean growth, nodulation and yield in N deficient soil of Uzbekistan. The results revealed positive effects on growth, nodule number and yields of soybean after inoculation with *Bradyrhizobium japonicum*.

Gan *et al.* (2002) conducted a field experiment to study the effects of N application as urea at various stages during the vegetative and reproductive phases on crop biomass, N₂ fixation and yield of two soybean genotypes, Luyuebao and Jufeng. Starter N at 25 kg ha⁻¹ resulted in minimum biomass and pod yield while starter N at 75 kg ha⁻¹ had no significant effect and N top dressing, at either the R₁ or R₅ stage, resulted in increased biomass and pod yield. Maximum biomass and pod yield were obtained when a top dressing of 50 kg ha⁻¹ was applied at the flowering stage. The effects of top dressing on the capacity to fix N₂ were complex. Any topdressing reduced nodulation and phosphorus fix, but increased biomass, so that total N₂ fixed increased for top dressing at the

flowering or pod filling stage. Common farmer's practice of applying 75 kg N ha⁻¹ at the V₄ stage, resulted in a significant reduction in N₂ fixation. To evaluate the application of nitrogen fertilization at various stages of development on growth, nodulation and N₂ fixation in more detail, an experiment in nutrient solution with or without 20 ml MnO₃ conducted with genotype Tidar. The N-free treatment gave the lowest biomass and total N accumulation, as in the field experiment. A continuous nitrate supply resulted in the highest biomass, associated with an increase in total leaf area per plant, maximum individual leaf area, branches and node number plant⁻¹, shoot root⁻¹ ratio and leaf area ratio, compared to the N-free treatment. R₁ was the most responsive stage for nitrate supply as well as for interruption of the nitrate supply. Since the results from the field experiment were in agreement with those from the experiment in nutrient solution in a greenhouse, the latter can be used to predict crop performance in the field.

El-Desouky and Atawia (1998) conducted two field experiments in Aswam, Egypt during 1999 and 2000 to assess the effect of single or mixed inoculations with *Bradyrhizobium japonicum* alone or with selected Rhizobacterial strains on field grown soybean (cv. Clark) and the effect of inoculant application on the nodulation, growth and yield of soybean. Results showed that nodulation and vegetative growth were greatly promoted by inoculation with *Bradyrhizobium japonicum* alone compared with uninoculated control.

Podder *et al.* (1999) conducted a field experiment at Old Brahmaputra Flood plain soil to evaluate the effect of seed inoculation with 8 bradyrhizobial strains on shoot length of soybean. The result showed that significantly higher shoot length obtained in the inoculated treatments compared to the uninoculated control.

Islam *et al.* (1999) carried out an experiment and reported that significantly higher nodules were observed in inoculated treatment and lower number of nodules were observed in uninoculation. All the *Bradyrhizobium* inoculation treatments performed better in nodulation, dry matter and straw yield of soybean.

Gowda and Kaul (1998) conducted a field trial in Egypt to study the effects on soybeans with or without seed inoculation with *Bradyrhizobium japonicum* and found that soybean yield increased with inoculation over uninoculated control.

Mitra *et al.* (1998) carried out a pot experiment where inoculation of soybean seed with *Bradyrhizobium japonicum* was done. Seed inoculation increased root nodulation and biomass yield.

Dubey (1997) revealed that seed inoculation with different strains of *Bradyrhizobium japonicum* enhanced symbiotic traits and yield attributes. compared with uninoculated control and found significantly increased nodulation in inoculated plots.

Vera *et al.* (2002) inoculated Gujrat soybean-I seeds with *Bradyrhizobium* and obtained the yield of 1293 kg ha⁻¹ against the lower yield of 1197 kg ha⁻¹ from with no inoculation.

A field experiment was carried out by Hasan *et al.* (2007) with soybean at BAU farm and found that *Bradyrhizobium* inoculation produced marked effect on growth and yield of soybean.

Panwar *et al.* (1998) conducted an experiment to study the effect of N concentration of cultural solution, *Bradyrhizobium* inoculation and plant density on the growth and yield of soybean cultivars. They observed that *Bradyrhizobium* inoculation increased the stem length of soybean.

Uslu *et al.* (1997) conducted a field trial where soybean that were inoculated with *Bradyrhizobium japonicum*, or not inoculated. They found that nodules per plant were increased by inoculation.

Thanausont and Vilhay (1996) carried out a pot experiment and observed that *Bradyrhizohium* inoculant increased nodulation and N₂-fixation of soybean. Ranjit and Pal (2004) reported that soybean seed inoculated with *Bradyrhizobium* had significant effects on nodulation and nodule distribution along with roots.

An experiments was conducted by Dwivedi *et al.* (1996) and reported that supplemental N will increase yields in plants under drought stress. When water stress occurs during the critical time of seed filling, carbon and N can be remobilized from plant leaf tissue and translocate to the seeds, resulting in faster declines in photosynthetic rates. This leads to premature leaf senescence, and lowered yields through reduced seed size and seed number.

In an additional greenhouse experiment, Purcell and King (1996) also observed that through supplemental N fertilization, the effects of drought were partially reversed when compared to plants which received full water and no additional N, since the N application compensated for the low N fixation of the *Bradyrhizobium*. Supplemental late season N applications offer the potential benefits of increasing yield under optimum moisture conditions as well as during times of inadequate moisture.

Rahman *et al.* (1982) carried out an experiment to study the response of applied N to soybean (cv. Davis). They found that N applied @ 100 kg ha⁻¹ increased plant growth but drastically reduced the nodule number and nodule weight. The combined application of inoculum + urea (25 kg N ha⁻¹) showed good performance with respect to number of nodules and their dry weight.

Welch *et al.* (1973) reported that Pre plant applied N at 134 kg ha⁻¹ on a silt loam soil in Nebraska to irrigated soybeans did not consistently alter yield (Slater, et al., 2001). Similarly, on a silty clay loam in Illinois N applications from 0 to 900 kg ha⁻¹ yr⁻¹ elicited no yield effect, except for slight yield reductions at the higher N rates.

One solution for overcoming this situation of supplying needed N for plant growth without impeding the development of *Bradyrhizobium* is foliar N applications during reproductive stages of plant growth. Yield increases from foliar fertilization using fertilizer grade urea applied at rates of 45, 90, and 135 kg ha⁻¹ at R₄ (Fehr and Caviness, 1977) were reported (Xiong and Liu, 1993). Increases over the control yield of 2640 kg ha⁻¹ for the two years averaged 123, 160, and 243 kg ha⁻¹ for the three treatments, respectively. In a series of experiments conducted at eight sites over a 2-year period on irrigated soybean, applications of N at 22 and 44 kg ha⁻¹ increased yield at six of the eight sites for an average increase of 464 kg ha⁻¹ or 11.8% (Wesley et al., 1998). Yields at the two unresponsive sites were less than 3360 kg ha⁻¹ and the authors suggested that responses from N might only be realized under high yield potentials.

Similarly, greenhouse and field studies by Yoshida (1979) reported yield increases with N applications. The greenhouse study utilized five N rates from 15 to 150 ppm NO₃-N in complete hydroponic solutions and a control containing no N. Total seed yield increased from the control of 0.9 g to 81.6 g per pot with 50 ppm N, while the highest N rate, 150 ppm, yielded 76.7 g per pot. In the field study, N applications from 30 to 90 kg ha⁻¹ applied from seedling emergence to R₁ increased yields by 720 kg ha⁻¹, averaged across all treatments. Yield benefits of up to 148 kg ha⁻¹ over the control of 2856 kg ha⁻¹ have been reported in Kentucky. In this work, liquid UAN (28% N) was dribbled beside the rows between the R₂ to R₃ development stages at rates of 29 kg ha⁻¹ and 36 kg ha⁻¹ in 1996 and 1997, respectively.

2.3 Effect of phosphorus on soybean

Phosphorus is one of the essential nutrients for legume growth and BNF (Giller and Cadisch, 1995; Whitbread *et al.*, 2004). Phosphorus deficiency can limit nodule number, leaf area, and biomass and grain development in legumes. Symbiotic nitrogen fixation

has a high phosphorus demand because the process consumes large amounts of energy (Schulze *et al.*, 2006) and energy generating metabolism strongly depends upon the availability of P (Plaxton, 2004). Phosphorus affects root development and hence uptake of nutrients and water. Phosphorus, apart from its effect on the nodulation process and plant growth, has also been found to exert some direct effects on soil *Rhizobia* (Singleton *et al.*, 1992). Singh and Sale (2000) reported that P fertilization stimulates root growth, photosynthesis and increases hydraulic conductivity of roots.

Phosphorus fertilizer application to soybean is an important step in attaining high yield under low soil P (<10 mg kg⁻¹-Bray-1) (Aune and Lal 1997; Martin, 2005). Soybean plant requires an application of 20-30 kg P₂O₅ ha⁻¹ during the growing season to sustain a with 0.5 t/ha extra grain yield than unfertilized plots. Fertilizer application increased biomass of these legumes. Soybean fertilized with Phosphorus had 1.5 t ha⁻¹ of biomass on top of the unfertilized treatment. Similar studies by Khonje (1994) reported that the population of *Bradyrhizobium* species and *Rhizobium* species in soils of Malawi are not uniform such that nodulation of promiscuous soybean will also depend on initial levels of indigenous populations of these nodule-forming bacteria. However, the study failed to ascertain why soybean variety surprisingly reduced nodulation after application of phosphate fertilizer at one of the sites in Malawi as compared to other sites used in the study which showed positive response to phosphate fertilizer application. In spite of these inconsistencies, the importance of P in soybean cultivation has been determined by many scientists (Vance, 2001; Mahamood *et al.*, 2009; Shahid *et al.*, 2009; Sharma *et al.*, 2011). Studies in Nigeria savanna showed that uninoculated soybean required 24-39 kg P ha⁻¹ at low soil P levels below critical limits to produce higher yields (Pal *et al.*, 1989) and *Rhizobium* inoculation increased the yield of promiscuous soybeans, particularly in soils having a low population of indigenous *Bradyrhizobia* (Olufajo, 1990).

CHAPTER III

METHODS AND MATERIALS

In this chapter includes a brief description of the methods and materials that were used in the experiment. This section discuss about soil of the experimented field, variety of soybean, land preparation, experimental design, treatments cultural operations collection of soil and plant samples etc. and analytical methods followed in the experiment to study the effects of nitrogen and phosphorus with or without *Rhizobium* inoculation on the growth, yield and nodulation of soybean.

3.1 Soil description of experimented area

The Research work was done to study the effects of Rhizobium inoculation, nitrogen and phosphorus fertilization on growth, yield and nodulation of soybean at Sher-e-Bangla Agricultural University Farm, Dhaka 1207 during rabi Season 2017-18. The experimental

location situated at 23⁰77 N and 90⁰33 E longitude with an elevation of 8.2 from sea level.

3.2 Description of soil

Soil of the experimented field in belongs to the Tejgaon series under the Agro ecological Zone, AEZ-28 (Madhupur Tract). In this series soil types are in general is Deep Terrace Soils. A composite sample was made by collecting soil from several spots of the field at a depth of 0-15 cm before the initiation of the experiment. The collected soil was oven-dried, ground and passed through 2 mm sieve and analyzed for some important physical and chemical parameters. The morphological characteristics of the experimental field and initial physical and chemical characteristics of the soil are presented in Table 3.1, 3.2 and 3.3 respectively.

Table 3.1 Morphological characteristics of experimental field

Morphological features	Characteristics
Location	Sher-e-Bangla Agricultural University
AEZ No. and name	AEZ-28, Madhupur Tract
General soil type	Shallow Red Brown Terrace Soil
Soil series	Tejgaon
Topography	Fairly leveled
Depth of inundation	Above flood level
Drainage condition	Well drained
Land type	Medium high land

Table 3.2 Physical characteristics of the initial soil of the experimental field

%Sand(2-0.02 mm)	31.14
%Silt(0.02-0.002 mm)	36.98
%Clay(<0.002 mm)	31.88
Textural Class	Clay Loam

Table 3.3 Chemical characteristics of the initial soil of the experimental field

pH	6.48
Organic Matter(%)	0.86
Total N(%)	0.079
Total P (ppm)	15
Exchangeable K (meq/100 g dry soil)	0.12
Available S (meq/100 g dry soil)	0.119

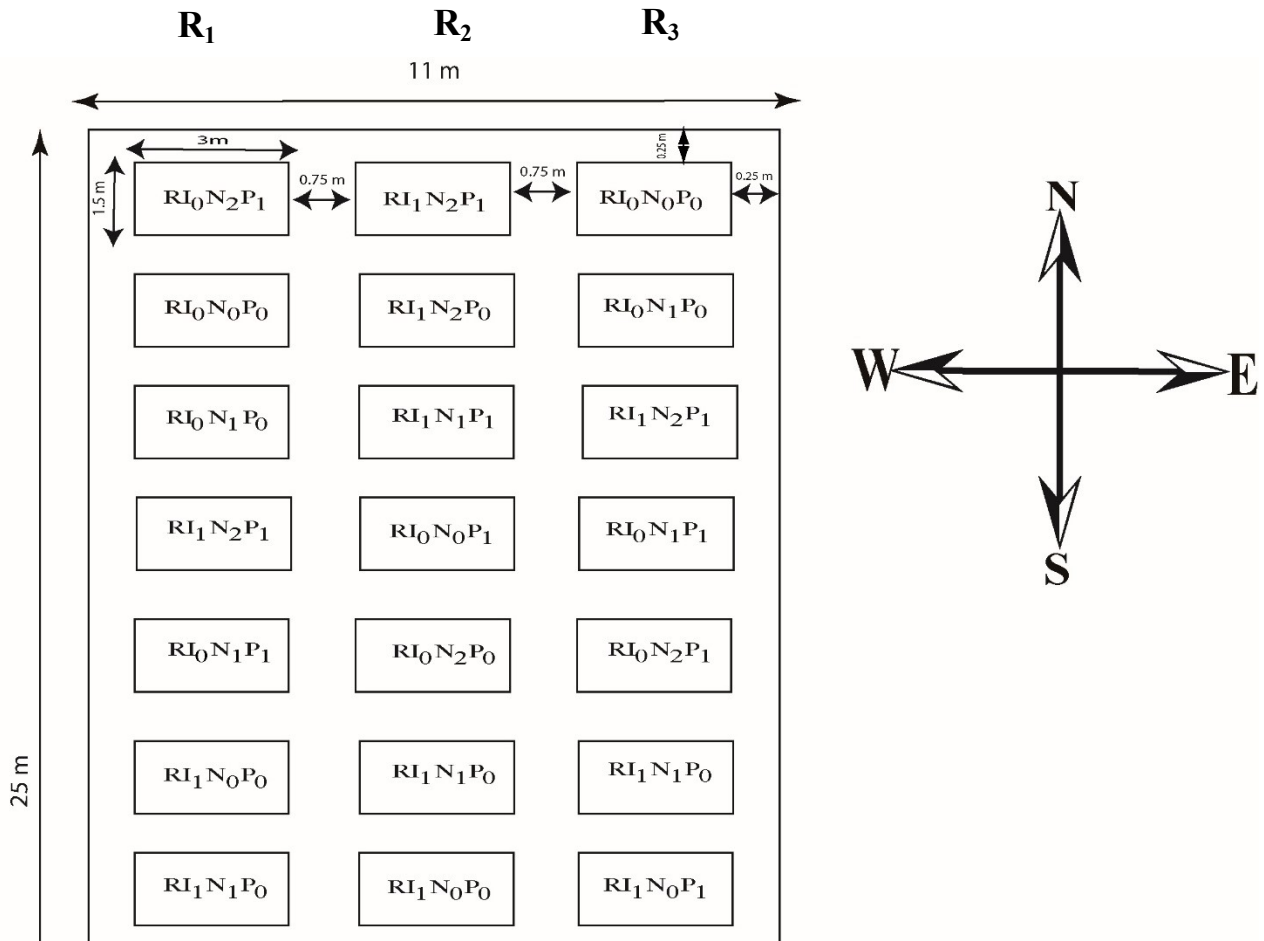
3.3 Description of Soybean Variety

In the experiment BARI Soybean-6, a high yielding variety was cultivated as test crop. This variety was released in 2009 by Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur. It is generally 50-55 cm long having 50-55 capsule per plant and 2-3 seed per capsules. On an average 100g seed weight is around 10-12 g.

The duration of life cycle of this variety is 100-110 days. Generally planted in rabi and kharif season throughout the country. Mid December to Mid-January is suitable time for sowing in rabi season and in kharif season, July is suitable time for sowing. The yield of BARI soybean-6 is 1.80-2.10 t/ha which is higher than BARI Soybean-5. BARI Soybean-6 is resistance to yellow mosaic virus. Vigorous, healthy and well matured seed was selected to sow in the experimented field.

3.4 Preparation of the field

The selected plot for the experiment was opened by power tiller driven rotovator on the 20th December, 2017. Afterwards several times the land was ploughed and cross-ploughed followed by laddering to obtain a good tilt. Weeds and stubbles were removed, and the large clods were broken into smaller pieces to obtain a desirable tilt of soil for sowing of seeds. Finally, the land was leveled and the experimental plot was partitioned into the unit plots in accordance with the experimental design mentioned in the following section (fig 1). The experiment was laid out in a Randomized Complete Block Design with three replications. The total numbers of plots were 36, each measuring 3 m x 1.5 m (4.5m^2).



3.5 Treatments

There were 12 treatment combinations. The following treatments combinations will be comprised for the experiment.

- I. Control [T₁]
- II. *Rhizobium* [T₂]
- III. 40 kg P ha⁻¹ + N₀ [T₃]
- IV. 25 kg N ha⁻¹ + P₀ [T₄]
- V. 50 kg N ha⁻¹ + P₀ [T₅]
- VI. *Rhizobium* + N₀ + 40 kg P ha⁻¹ [T₆]
- VII. *Rhizobium* + 25 kg N ha⁻¹ + P₀ [T₇]
- VIII. *Rhizobium* + 50 kg N ha⁻¹ + P₀ [T₈]

- IX. 25kg N ha⁻¹ + 40 kg P ha⁻¹ [T₉]
- X. 50 kg N ha⁻¹ + 40 kg P ha⁻¹ [T₁₀]
- XI. *Rhizobium* + 25 kg N ha⁻¹ + 40 kg P ha⁻¹ [T₁₁]
- XII. *Rhizobium* + 50 kg N ha⁻¹ + 40 kg P ha⁻¹ [T₁₂]

3.6 Application of fertilizers:

Recommended doses of K, S, Zn and B (40 kg K ha⁻¹ from MoP, 20 kg S ha⁻¹, 2 kg Zn ha⁻¹ from ZnO and 1 kg B ha⁻¹ from Boric acid, respectively) were applied. The whole amounts of MoP, TSP, gypsum, Boric acid and half of the urea fertilizer were applied as basal dose during final land preparation. The remaining half of urea was top dressed after 22 days of germination.

3.7 Seed sowing

Soybean seeds were sown on the 12 January, 2018 in lines following the recommended line to line distance of 30 cm and plant to plant distance of 5 cm.

3.8 Weeding and thinning

Weeds of different types were controlled manually and removed from the field. The weeding and thinning were done after 24 days of sowing, on February 5, 2018. Care was taken to maintain constant plant population per plot.

3.9 Irrigation

Irrigation was given at three times. The first irrigation was given in the field on January 22, 2018 at ten days after sowing (DAS) through irrigation channel. Second irrigation was given in the field on March 08, 2018 at 45 days after sowing (DAS) before flowering. The third irrigation was given at the stage of pod formation (70 DAS) on April 23, 2018.

3.10 Pest Management

To rescue the plant from the infested Cutworm at the seedling stage and application of Dursban-25EC @ 2.5ml liter⁻¹ was done twice on February 2 and 5, 2018. Special care was taken to protect the crop from birds especially after sowing and germination stages.

3.11 Harvesting

The crop was harvested at maturity on 6th May 2018. The harvested crop of each individual plot was bundled separately. Grain and stover were recorded plot wise and the yields were expressed in ton ha⁻¹.

3.12 Collection of samples

3.12.1 Plant sample

From every individual plot plant samples were collected for laboratory analysis at the harvesting stage of the crop. Five plants were collected randomly from each plot by cutting above the ground level. The plant samples were washed first with tap water and then with distilled water several times. The plant samples were dried in the electric oven at 70°C for 48 hours. After that the samples were ground in an electric grinding machine and stored for chemical analysis. The plant samples were collected by avoiding the border area of the plots.

3.13 Collection of data

Five (5) plants from each plot were selected at random and were tagged for the data collection. Data collections were done on the following parameters:

Plant height (cm)

Number of leaves plant⁻¹
Number of primary branches plant⁻¹
Number of Nodule plant⁻¹
Weight of dry shoot (g plant⁻¹)
Weight of dry root (g plant⁻¹)
Total weight of plant (g plant⁻¹)
Number of pods plant⁻¹
Number of seeds pod⁻¹
Number of seeds plant⁻¹
100 seed weight (g)
Seed weight plant⁻¹(g)
Grain yield (t ha⁻¹)
Stover yield (t ha⁻¹)

3.13.1 Plant height

The plant height was measured from the ground level to the top of the plant. Five plants were selected randomly from each plot during harvest. Plant height was measured and averaged.

3.13.2 Number of leaves plant⁻¹

Numbers of leaves were counted at ripening stage. Five plants were selected randomly from each plot and number of leaves were counted and averaged.

3.13.3 Number of primary branches plant⁻¹

Five plants were selected randomly from each plot at ripening stage. Number of primary branches were counted and averaged.

3.13.4 Number of nodule plant⁻¹

Five plants were selected randomly from each plot at ripening. Number of nodule plant⁻¹ were counted and averaged.

3.13.7 Weight of dry shoot

Five plants were selected randomly from each plot at the flowering stage. Weight of dry shoots were measured in gram and averaged.

3.13.8 Weight of dry root

Five plants were selected randomly from each plot at the flowering stage. Weight of dry roots were measured in gram and averaged.

3.13.9 Total weight of plant

Five plants were selected randomly from each plot at the flowering stage. Dry weight of plants were measured in gram and averaged.

3.13.10 Number of pods plant⁻¹

Pods were counted at the ripening stage. Five plants were selected randomly from each plot. Number of pods were counted and averaged.

3.13.11 Number of seeds plant⁻¹

It was done after harvesting. Five plants were selected randomly from each plot. At first, number of seeds plant⁻¹ were counted and averaged. Then it was multiplied with number of pods plant⁻¹ and averaged.

3.13.12 Hundred seed weight

Hundred seed of soybean were counted randomly and then weighed plot wise.

3.13.13 Grain yield

Grains obtained from 1 m² area from the center of each unit plot was dried, weighed carefully and then converted into t ha⁻¹.

3.13.13 Stover yield

Stover remained after collection of grain (1 m² of each individual plot) was dried, weighed carefully and the yield was expressed in t ha⁻¹.

3.14 Measurement of Soil Initial Parameters

a) Organic carbon

Soil organic carbon was determined by Walkley and Black's Wet Oxidation Method as outlined by Jackson (1973) from the samples collected before sowing and also after harvesting the crop.

b) Organic matter

The organic matter content was determined by multiplying the percent organic carbon with Van Bemmelen factor 1.73 (Piper, 1950).

c) Total nitrogen

Total nitrogen of soil samples were estimated by Micro-kjeldahl method where soils were digested with 30% H₂O₂ conc. H₂SO₄ and catalyst mixture (K₂SO₄:CuSO₄.5H₂O Selenium powder in the ratio 100: 10: 1, respectively). Nitrogen in the digest was determined by distillation with 40% NaOH followed by titration of the distillate absorbed in H₃BO₃ with 0.01N H₂SO₄ (Jackson, 1973).

d) Available Phosphorous

Available phosphorous was extracted from the soil by Bray-I method (Bray and Kurtz, 1945). Phosphorous in the extract was determined by ascorbic acid blue color method (Murphy and Riley, 1962) with the help of a Spectrophotometer (LKB Novaspec, 1949).

e) Available Potassium

Available potassium in the soil sample was extracted with 1N neutral ammonium acetate and the potassium content was determined by flame photometer.

f) Available Sulphur

Available sulphur was extracted from the soil with Ca(H₂PO₄)₂.H₂O (Fox *et al.*, 1964). Sulphur in the extract was determined by the turbidimetric method as described by Hunt (1980) using a Spectrophotometer (LKB Novaspec. 4049).

g) Soil pH

Soil pH is an important factor for plant growth, as it affects nutrient availability, nutrient toxicity, and has a direct effect on the protoplasm of plant root cells (Alam *et al.*, 1999). It also affects the abundance and activity of soil organisms (from microorganisms to arthropods) responsible for transformations of nutrients (Nicol *et al.*, 2008). Soil pH was determined by pH meter.

h) Soil Texture

The texture of the soil samples was determined by hydrometer method (Bouyoucos, 1927).

3.15 Statistical analysis

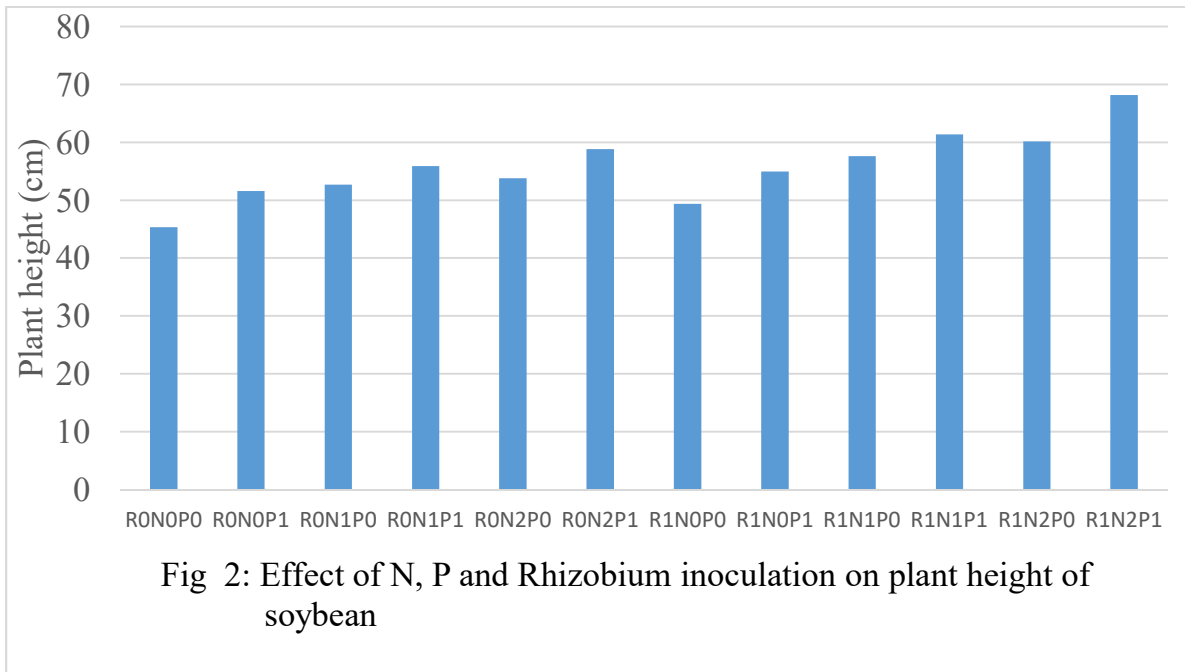
The data obtained from the experiment were analyzed statistically to find out the significance of the difference among the treatments. The mean values of all the characters were evaluated and analysis of variance was performed by the 'F' (variance ratio) test. The significance of the differences among pairs of treatment means was estimated by the least significant difference (LSD) test at 5% and 1% level of probability and DMRT was calculated (Gomez and Gomez, 1984).

CHAPTER IV RESULTS AND DISCUSSION

The results on different yield attributes, yield content and nutrients concentrations in the plants and availability of different nutrients in the soil after harvest of soybean are presented in this chapter.

4.1 Plant height

Result in table 4.1 showed the significant increase of plant at height at harvest with the increase dose of N and P with *Rhizobium* inoculation. In the table 4.1, the tallest plant (69.51 cm) was found in T₁₂ (*Rhizobium* inoculant + 50 kg N ha⁻¹ + 40 kg P ha⁻¹) treatment. It was also observed (Table 4.1) that the shorter plant (51.34 cm) was found in control (zero fertilizer treatment). From the figure 2, it was observed that the plant height increased with the increasing dose of N, P with *Rhizobium* inoculation.



4.2 Number of primary branch plant⁻¹

Result in Table 4.1 showed the significant increase of primary branch plant⁻¹ with the increase dose of N and P with *Rhizobium* inoculation. In the Table 4.1, the highest value of primary number plant⁻¹ (10.33) was found in T₁₂ (*Rhizobium* inoculant + 50 kg N ha⁻¹ + 40 kg P ha⁻¹) treatment combination which was statistically similar (10.20) with T₁₀ (*Rhizobium* inoculant + 25 kg N ha⁻¹ + 40 kg P ha⁻¹) treatment combination. From the (Table 4.1) it was observed that the primary branch number plant⁻¹ increased with the increasing dose of N, P with *Rhizobium* inoculation.

4.3 Number of leaves plant⁻¹

The effect of *Rhizobium*, nitrogen and phosphorus application (table 4.1) on number of leaves plant⁻¹ is positive. The highest value of number of leaves plant⁻¹ (24.17) was observed in T₁₂ (*Rhizobium* inoculation + 50 kg N ha⁻¹ + 40 kg P ha⁻¹) treatment combination which was statistically similar with T₁₀ (*Rhizobium* inoculation + 25 kg N ha⁻¹ + 40 kg P ha⁻¹). The lowest number of leaves plant⁻¹ (14.53) were observed in control (Zero treatment combination).

4.4 Nodulation

4.4.1 Number of nodule plant⁻¹

Rhizobium inoculation had a significant positive effect on the formation of nodules. Seed inoculation with *Rhizobium* markedly increased nodule number as compared to that of the non-inoculated plants of soybean (Table 4.2). The highest number of nodule plant⁻¹ (30.20) was obtained in T₁₂ (*Rhizobium* inoculation + 50 kg N ha⁻¹ + 40 kg P ha⁻¹) treatment combination which are statistically similar with T₁₀ (*Rhizobium* inoculation + 25 kg N ha⁻¹ + 40 kg P ha⁻¹) and T₁₁ (*Rhizobium* inoculation + 50 kg N ha⁻¹ + 0 kg P ha⁻¹) treatment. Increasing dose of N and P with *Rhizobium* inoculant results increased number of nodules (Table 4.2 & Figure 3). These results are in agreement with Sarker *et al.* (1993) and Chowdhury *et al.* (1998). They reported that phosphorus application at the rate of 60 kg P₂O₅ kg ha⁻¹ significantly increased nodulation. Khandaker *et al.* (1985) reported that nodules per plant were increased significantly in blackgram (*Vigna mungo*) due to P application at 40 days after germination. Similar results were reported by Satter and Ahmed (1992). Sharma *et al.* (1995) reported that seed inoculation with *Rhizobium* and application of 40 kg P ha⁻¹ in chickpea (*Cicer arietinum*) either alone or in combination enhanced nodulation over un-inoculated control. Rahman *et al.* (1994) also reported the similar result. Chowdhury *et al.* (1998) found that 50 kg P ha⁻¹ with other fertilizers increased 245% nodule number over control. Khanam *et al.* (1993) also found the similar results in lentil.

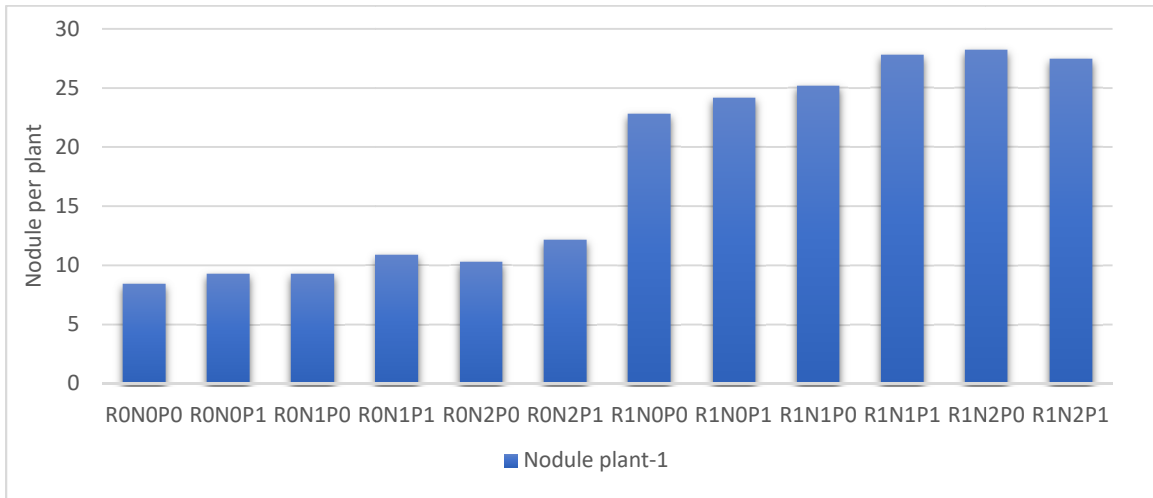


Figure 3: Effects of *Rhizobium* inoculation, nitrogen and phosphorus application on nodulation of soybean

Table 4.1: Effect of *Rhizobium*, nitrogen and phosphorus application on plant height, number of primary branches plant⁻¹ and number of leaves plant⁻¹ of soybean

Treatment	Plant height (cm)	Number of primary branches plant ⁻¹	Number of leaves plant ⁻¹
T ₁	51.34 ^d	2.93 ⁱ	14.53 ^f
T ₂	55.28 ^{b-d}	4.73 ^g	17.30 ^e

T ₃	60.38 ^{a-d}	6.33 ^f	17.83 ^{de}
T ₄	64.92 ^{a-c}	8.63 ^d	19.53 ^{cd}
T ₅	55.43 ^{b-d}	8.02 ^e	18.33 ^{de}
T ₆	66.48 ^{ab}	9.33 ^c	20.70 ^{bc}
T ₇	54.03 ^{cd}	5.03 ^h	14.33 ^f
T ₈	60.63 ^{a-d}	8.17 ^d	18.57 ^{de}
T ₉	61.84 ^{a-d}	8.7 ^d	20.60 ^c
T ₁₀	60.36 ^{a-d}	10.20 ^a	22.53 ^{ab}
T ₁₁	64.83 ^{a-c}	9.83 ^b	21.23 ^{bc}
T ₁₂	69.51 ^a	10.33 ^a	24.17 ^a
LSD _{0.05}	5.81	0.1	0.96
CV(%)	11.77	7.27	10.33

Table 4.2: Interaction effect of Rhizobium, nitrogen and phosphorus application on number of nodule plant⁻¹ of soybean

Treatment	Number of nodule plant ⁻¹
T ₁	3.40 ^g
T ₂	3.87 ^{fg}
T ₃	4.96 ^{ef}
T ₄	6.20 ^{de}

T ₅	7.60 ^d
T ₆	7.50 ^d
T ₇	27.43 ^c
T ₈	27.83 ^{bc}
T ₉	28.50 ^{bc}
T ₁₀	30.00 ^a
T ₁₁	29.10 ^{ab}
T ₁₂	30.20 ^a
LSD _{0.05}	1.49
CV(%)	12.76

4.5 Dry weight of shoot plant⁻¹

Significant effect of *Rhizobium* inoculant, nitrogen and phosphorus on shoot dry weight of soybean was observed (Table 4.3). The highest shoot dry weight (2.93 g/plant) was recorded in T₁₂ (*Rhizobium* inoculation + 50 kg N ha⁻¹ + 40 kg P ha⁻¹), which was significantly higher than other treatments. *Rhizobium* inoculation with N dose performed better than *Rhizobium* inoculation with P dose. The lowest dry weight of shoot was recorded in control treatment. *Rhizobium* inoculation increased dry weight of shoot significantly over control. Eusuf Zai *et al.* (1999) showed that *Rhizobium* inoculant significantly increased shoot dry weight of chickpea compared to un-inoculated control. Hoque and Hashem (1993) stated that *Rhizobium* as biofertilizer was remarkably beneficial on shoot weight and total dry weight of soybean and groundnut. Mahmud *et al.* (1997) reported that weight of shoot in lentil significantly increased due to inoculation compared to control treatment. Dry weight of shoot remarkably increased in inoculated mungbean over control as reported by Solaiman (1999).

4.6 Dry weight of root plant⁻¹

The effect of *Rhizobium* inoculant, nitrogen and phosphorus significantly increased dry weight of root plant⁻¹ of soybean compared to control (Table 4.3). In the treatment T₁₂ (*Rhizobium* inoculation + 50 kg N ha⁻¹ + 40 kg P ha⁻¹) results highest dry wt. (0.66 g/plant). Study showed that Application of fertilizer with or without inoculation of *Rhizobium* results no significant different but it was also observed that comparatively higher dry weight of root was obtained when N and P fertilization was applied with inoculation of *Rhizobium*. dry weight of root positively correlated with total number of nodules (Table 4.3).

4.7 Total Dry weight of soybean plant

The effect of *Rhizobium* inoculation and nitrogen and phosphorus application on total dry weight of soybean plant was positive. The significant variation was observed (Table 4.3) with different level of N and P application with or without inoculation. The highest dry wt. per plant was recorded (11.88 g/plant⁻¹) at T₁₂ (*Rhizobium* inoculation + 50 kg N ha⁻¹ + 40 kg P ha⁻¹) treatment combination which was statistically similar with (11.44 g/plant⁻¹) at T₁₀ (*Rhizobium* inoculation + 25 kg N ha⁻¹ + 40 kg P ha⁻¹) treatment combination. The lowest value 5.19 g/ plant⁻¹ was observed in control treatment.

Table 4.3: Effect of *Rhizobium*, nitrogen and phosphorus application on dry shoot wt. plant⁻¹, dry root wt. plant⁻¹, and dry wt. plant⁻¹ of soybean

Treatment	Dry shoot wt. plant ⁻¹ (g)	Dry root wt. plant ⁻¹ (g)	Dry wt. plant ⁻¹ (g)
T ₁	0.99 ^h	0.14 ^b	5.19 ⁱ
T ₂	1.18 ^{f-h}	0.19 ^{ab}	5.98 ^h
T ₃	1.25 ^{f-h}	0.48 ^{ab}	6.55 ^g

T₄	1.52 ^{d-f}	0.45 ^{ab}	7.83 ^{de}
T₅	1.35 ^{e-h}	0.27 ^{ab}	7.10 ^f
T₆	1.89 ^{cd}	0.31 ^{ab}	9.10 ^c
T₇	1.12 ^{gh}	0.30 ^{ab}	5.56 ^{hi}
T₈	1.43 ^{e-g}	0.63 ^{ab}	7.35 ^{ef}
T₉	1.66 ^{de}	0.37 ^{ab}	8.25 ^d
T₁₀	2.31 ^b	0.63 ^{ab}	11.44 ^a
T₁₁	2.22 ^b	0.27 ^{ab}	10.63 ^b
T₁₂	2.93 ^a	0.66 ^a	11.88 ^a
LSD_{0.05}	0.38	0.50	0.47
CV(%)	13.60	16.25	11.49

4.8 Pod length plant⁻¹

Pod length due to the application of *Rhizobium* inoculation, nitrogen and phosphorus, is presented in Table 4.4. Pod length varied significantly over control treatment. The highest pod length of 4.36 cm was obtained with the treatment T₁₂ phosphorus was applied at 40 kg ha⁻¹ along with 50 kg N ha⁻¹ and *Rhizobium* which was statistically similar with T₁₀ (*Rhizobium* inoculant + 25 kg N ha⁻¹ + 40 kg P ha⁻¹) treatment combination. The lowest pod length of 3.13 cm was found with control. In treatment T₁₂ pod length increased to 39% over control treatment (Table 4.4). The positive effect of N application was also observed by Ahmad and Ali (2013).

4.9 Pods Plant⁻¹

From the Table 4.4 the highest number of pod plant⁻¹ (44.53) was observed in T₁₂(*Rhizobium* inoculation + 50 kg N ha⁻¹ + 40 kg P ha⁻¹) treatment combination . (Table 4.4) It was also observed that different level of treatment combination was showed significant variation on pod number plant⁻¹. It was also observed that increasing dose of

N and P application with inoculation results increasing of pods plant⁻¹. Malik *et al.* (2002 and 2003). Podder *et al.* (1999). They reported that the number of pods per plant of mungbean increased with *Rhizobium* inoculant in association with P application. The result obtained from the present study was in conformity with the findings of Ahmad and Ali (2013), Umeh *et al.* (2011) and Soomru *et al.* (2005). A positive correlation between pods plant⁻¹ and number of nodule plant⁻¹ were also observed in Fig 4.

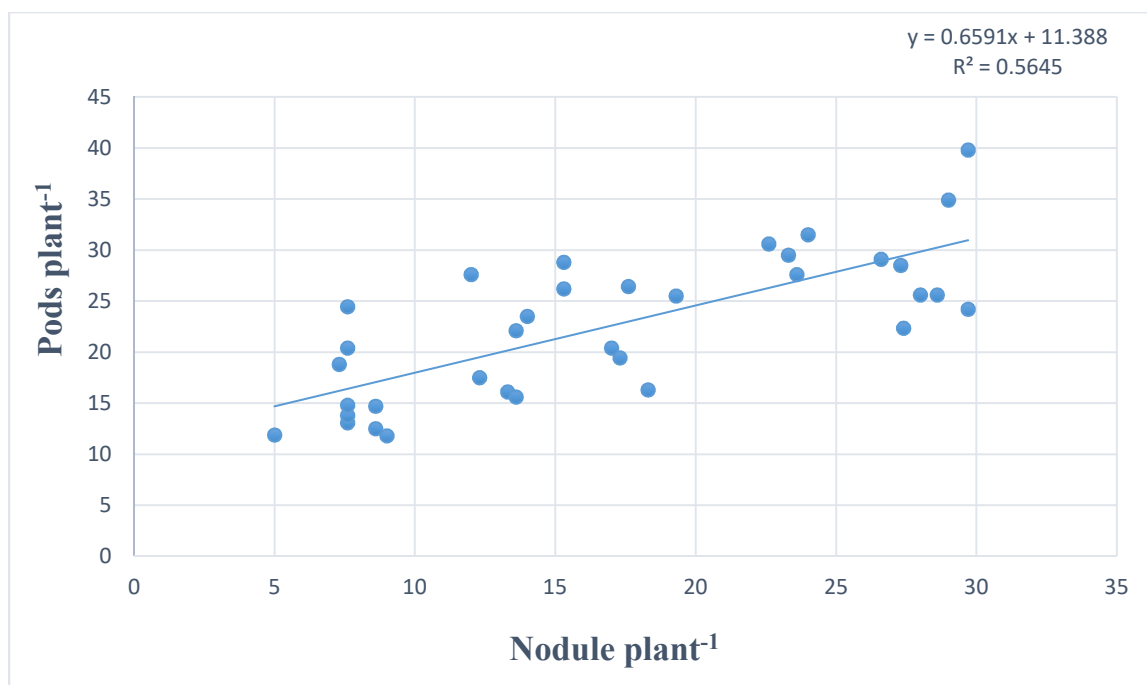


Fig 4: Linear relationship between number of nodule plant⁻¹ and pods plant⁻¹

4.10 Number of seeds pod⁻¹

Rhizobium inoculant, N and P played a significant role on the number of seeds pod⁻¹. Data regarding number of seeds pod⁻¹ are presented in Table 4.4. Number of seeds pod⁻¹ increased upto N levels at 50 kg ha⁻¹ along with *Rhizobium* and P level upto 40 kg ha⁻¹. The highest number of seeds per pod (4.77) was found in T₁₂ (*Rhizobium* inoculation + 50

kg N ha⁻¹ + 40kg P ha⁻¹) treatment combination. The lowest number of seeds per pod (2.50) was found in uninoculated control treatment. It was observed that *Rhizobium* inoculant in association with N led to increase the number of seeds per pod of soybean. Similar results were also reported by Landge *et al.* (2002) and Jana *et al.* (1990). In the present study, number of seeds per pod had a positive correlation with seed yield plant⁻¹.

4.11 Weight of seeds plant⁻¹

The significant variation was observed (Table 4.4) on weight of seeds plant⁻¹ when treated with *Rhizobium* inoculation and nitrogen application. The highest value of pod number plant⁻¹ (135.00 g) was found in *Rhizobium* inoculant + 50 kg N ha⁻¹ + 40 kg P ha⁻¹ marked as T₁₂. It was also observed that comparatively lower number of seeds plant⁻¹ was observed in un-inoculated *Rhizobium* treatment. The lowest number of seeds plant⁻¹ (56.40 g) was observed in control treatment.

4.12 100g seeds wt.

Rhizobium inoculant along with N and P significantly increased 100g seed wt. compared to control (Table 4.4). Treatments with *Rhizobium* inoculant and N @ 50 kg ha⁻¹ and P @ 40 kg ha⁻¹ results the highest value of 100g seed weight (18.00 g) compared to *Rhizobium* inoculant alone and. Significant variation was observed between the treatment with or without inoculum (Table 4.4). The 100g seed weight increased with the application of inoculant, N and P over control. This finding is in agreement with Solaiman (1999). Sarker *et al.* (1993) reported that *Rhizobium* inoculant along with phosphorus increased 100g seed weight of green gram in a pot experiment. Malik *et al.* (2002) reported that seed inoculation with *Rhizobium* significantly increased 100g grain weight (42.27 g) of mungbean. Similar results were observed by Podder *et al.* (1999) and Chowdhury *et al.* (1998).

Table 4.4: Effect of *Rhizobium* inoculation, nitrogen and phosphorus application on pods plant⁻¹, pod length plant⁻¹, number of seeds pod⁻¹, 100 seed weight plant⁻¹, seed weight plant⁻¹ soybean

Treatment	Pods plant ⁻¹	Pod length plant ⁻¹ (cm)	Number of seeds of pod ⁻¹	100 seed wt. (gm)	Seed wt. plant ⁻¹ (gm)
T ₁	24.36 ^K	3.13 ⁱ	2.50 ⁱ	9.67 ^h	56.40 ^k
T ₂	26.48 ^j	3.60 ^{gh}	3.03 ^h	12.67 ^f	66.83 ^{ij}
T ₃	27.96 ⁱ	3.63 ^{f-h}	3.33 ^g	13.67 ^e	72.30 ^{hi}
T ₄	30.05 ^h	3.90 ^{de}	3.50 ^f	15.00 ^d	85.57 ^{ef}
T ₅	32.11 ^g	3.70 ^{fg}	3.57 ^f	14.00 ^e	78.20 ^{gh}
T ₆	32.84 ^g	4.00 ^{cd}	3.77 ^e	16.00 ^c	94.67 ^{cd}
T ₇	33.96 ^f	3.47 ^h	3.97 ^d	11.67 ^g	61.20 ^{jk}
T ₈	35.86 ^e	3.80 ^{ef}	4.10 ^c	15.00 ^d	82.57 ^{fg}
T ₉	37.66 ^d	3.90 ^{de}	4.17 ^c	16.00 ^c	89.43 ^{de}
T ₁₀	39.26 ^c	4.20 ^{ab}	4.40 ^b	17.00 ^b	116.97 ^b
T ₁₁	41.40 ^b	4.17 ^{bc}	4.50 ^b	16.00 ^c	98.37 ^c
T ₁₂	44.53 ^a	4.36 ^a	4.77 ^a	18.00 ^a	135.00 ^a
LSD _{0.05}	0.92	0.18	0.13	0.48	6.15
CV(%)	7.61	12.82	9.94	8.95	14.20

4.13 Grain yield

Data regarding grain yield of soybean are presented in Table 4.5. The highest grain yield (2.06 t ha⁻¹) was obtained in treatments with *Rhizobium* inoculant and N @ 50 kg ha⁻¹ and P @ 40 kg ha⁻¹ regarding as T₁₂ which was statistically similar with T₁₀ (*Rhizobium* inoculant + 25 kg N ha⁻¹ + 0 kg P ha⁻¹) treatment combination. It was also observed that (Table 4.5) with the inoculation of *Rhizobium* increased dose of N results higher yield of grain of soybean. Satish *et al.* (2003) found that P at 40 and 60 kg ha⁻¹ increased grain yield over the control in mungbean. Yadav and Jakhar (2001) also found similar result on mungbean. Maurya and Rathi (2000) reported that grain yield of soybean was maximum with the application of 60 kg P ha⁻¹. Pandey and Gupta (2013) and Quddus *et al.* (2011) also found that increased dose of N results increasing of grain yield of soybean. The grain yield increased at 132% at T₁₂ treatment over control. A positive correlation was observed

(Fig 5) between nodule plant⁻¹ and grain yield. In(Fig 6), a positive correlation was also observed between pods plant⁻¹ and grain yield.

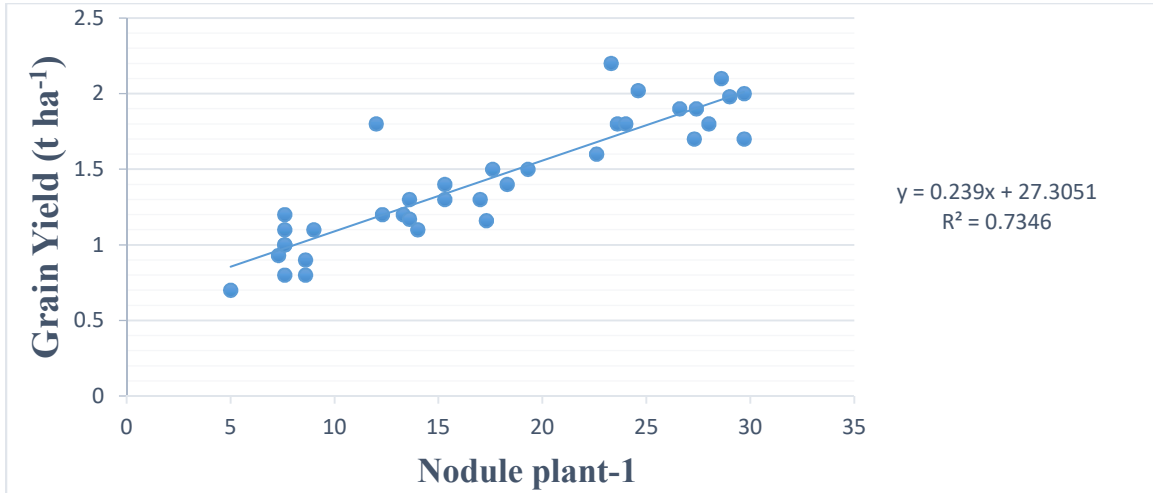


Fig 5: Linear relationship between number of nodule plant⁻¹ and grain yield of soybean

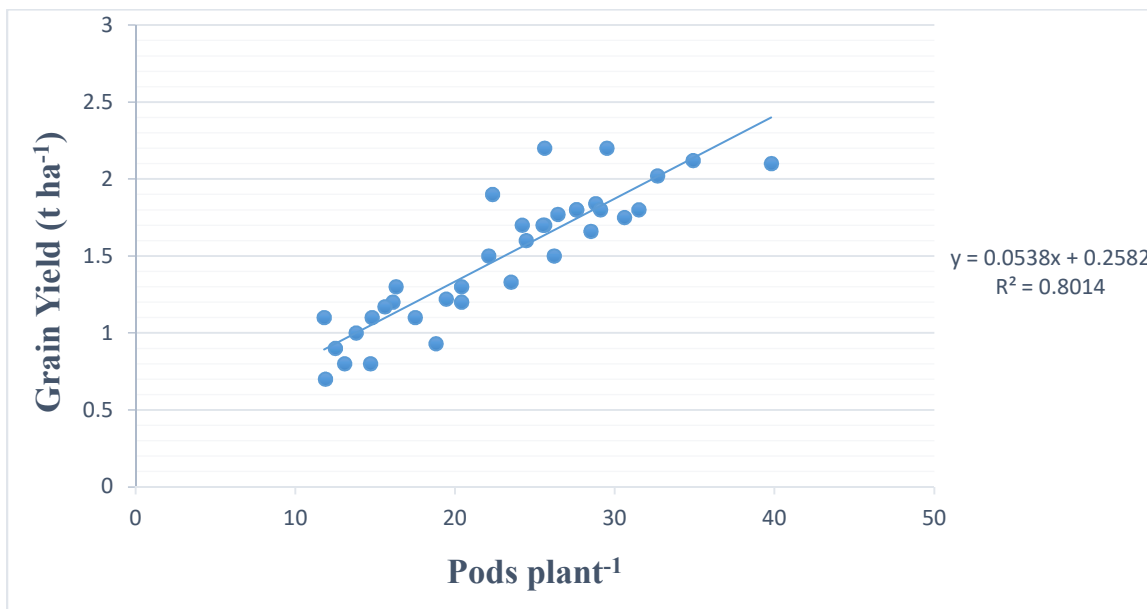


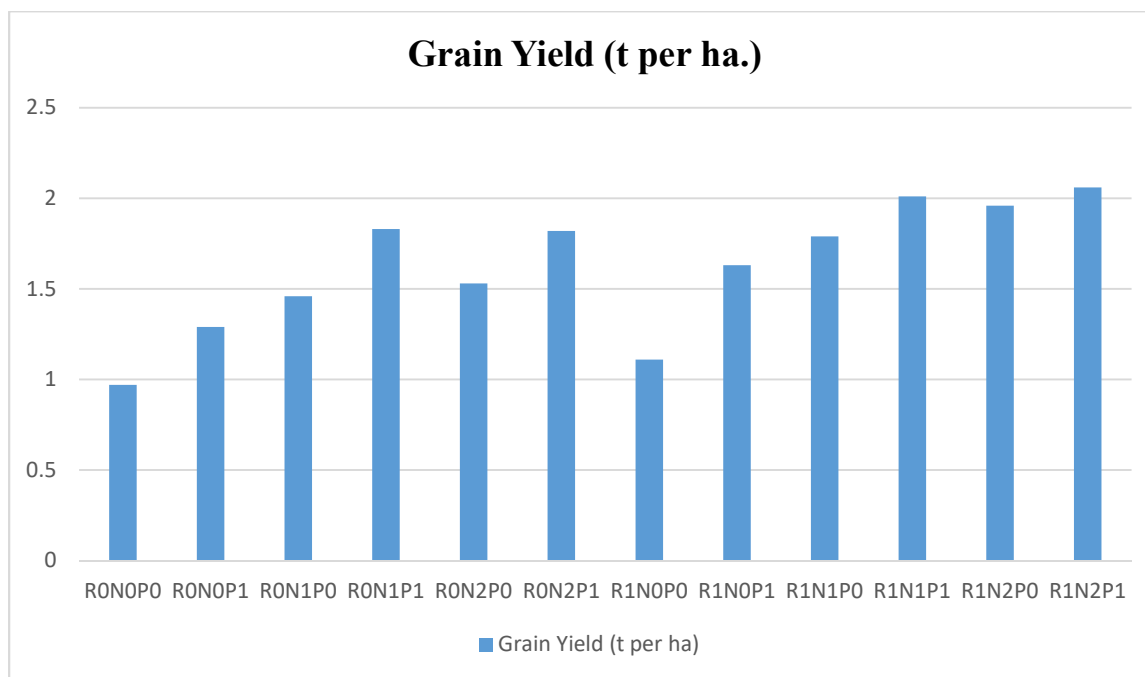
Fig 6: Linear relationship between pods plant⁻¹ and grain yield of soybean

4.14 Stover yield

Rhizobium inoculation, nitrogen and phosphorus influenced significant on the stover yield of soybean (Table 4.5). The highest Stover yield (2.11 t ha⁻¹) was obtained (Table 4.5) in T₁₂ (*Rhizobium* inoculation + 50 kg N ha⁻¹ + 40 kg P ha⁻¹) treatment combination. Comparatively lower yield was observed (table 4.5) in *Rhizobium* un-inoculated plots. It was also observed that (Table 4.5) with the inoculation of *Rhizobium* increased dose of N and P results higher yield of grain of soybean. The findings for these characters agree with the results obtained by Khandkar *et al.* (1985) and Sacchindanand *et al.* (1980) who observed that straw yields of soybean increased with the application of phosphorus @30 kg P ha⁻¹. The lowest stover yield was recorded 0.79 t ha⁻¹ in control treatment. Manpreet *et al.* (2004) and Singh *et al.* (2001) also observed the similar trend in mungbean.

Table 4.5: Effect of Rhizobium inoculation, nitrogen and phosphorus application of soybean

Treatment	Grain yield (t ha ⁻¹)	Stover yield (t ha ⁻¹)
T ₁	0.97 ^j	0.79 ⁱ
T ₂	1.29 ^h	1.04 ^h
T ₃	1.46 ^g	1.13 ^g
T ₄	1.83 ^d	1.27 ^f
T ₅	1.53 ^f	1.20 ^{fg}
T ₆	1.82 ^c	1.80 ^d
T ₇	1.11 ⁱ	0.98 ^h
T ₈	1.63 ^e	1.21 ^{fg}
T ₉	1.79 ^d	1.59 ^e
T ₁₀	2.01 ^a	1.99 ^b
T ₁₁	1.96 ^b	1.89 ^c
T ₁₂	2.06 ^a	2.11 ^a
LSD _{0.05}	0.05	0.08
CV(%)	12.38	13.51



CHAPTER V

SUMMERY AND CONCLUSION

A field experiment was carried out during *rabi* season of 2018 with soybean variety BARI Soybean-6, laid out in randomized complete block design with three replications at the Sher-e-Bangla Agricultural University Farm Dhaka-1207 (Tejgaon series under AEZ No.28). All the treatments were inoculated with *Rhizobium* strains (BARI RVr-2005) except control. The experiment was laid out following three factors randomized complete block design with 12 treatments having unit plot size of There were 12 treatment combinations. The following treatments combinations will be comprised for the experiment: Control [T₁], *Rhizobium* [T₂], 40 kg P ha⁻¹ + N₀ [T₃], 25 kg N ha⁻¹ + P₀ [T₄], 50 kg N ha⁻¹ + P₀ [T₅], *Rhizobium* + N₀ + 40 kg P ha⁻¹ [T₆], *Rhizobium* + 25 kg N ha⁻¹ + P₀ [T₇], *Rhizobium* + 50 kg N ha⁻¹ + P₀ [T₈], 25kg N ha⁻¹ + 40 kg P ha⁻¹ [T₉], 50 kg N ha⁻¹ + 40 kg P ha⁻¹ [T₁₀] , *Rhizobium* + 25 kg N ha⁻¹ + 40 kg P ha⁻¹ [T₁₁] , *Rhizobium* + 50

kg N ha⁻¹ + 40 kg P ha⁻¹ [T₁₂] (Without inoculation, N and P application). The objectives of this study were to find out effective doses of nitrogen and phosphorus in combination of *Rhizobium* inoculants on nodulation, growth and yield of soybean. Another objective was to determine the effect of *Rhizobium* inoculants, nitrogen and phosphorus on nodulation, growth and yield of soybean. Five plants were collected randomly to record the data plot wise for plant height (cm), number of primary branches plant⁻¹, number of leaves plant⁻¹, pod length, number of pods plant⁻¹, number of seeds plant⁻¹, dry weight of shoot, dry weight of root, total dry weight of plant, number of nodule, 100g seed weight (g), grain yield (t ha⁻¹) and stover yield (ha⁻¹). All the data were statistically analyzed following F-test and the mean comparison was made by DMRT. From the experiment, the highest plant height, number of leaves and branches per plant were 69.51 cm, 24.17 and 10.33 respectively which were produced by the inoculated plants with nitrogen and phosphorus application at the rate of 50 kg ha⁻¹ and 40 kg ha⁻¹ respectively. In the same combination of treatment the number of nodules was also significantly higher. The maximum nodule was recorded 30.20 which was obtained in T₁₂ treatment. Weight of dry shoot, dry root and total plant were also significantly higher where N and P was applied with inoculation of *Rhizobia*. The highest weight of dry shoot, dry root and total plant were 2.93 g plant⁻¹, 0.66 gm plant⁻¹ and 11.88 g plant⁻¹ respectively. The application *Rhizobium* inoculant along with N and P showed positive effect on the number of pods plant⁻¹, number of seed pod⁻¹, number of seeds plant⁻¹, 100 seed weight (g), seed wt. plant⁻¹(gm) grain yield (t ha⁻¹) and stover yield (t ha⁻¹). All the plant characters increased with increasing levels of nitrogen and application of *Rhizobium* along with phosphorus. *Rhizobium* inoculants, N and P application influenced significantly on grain yield of soybean like all other plant characters. Grain yield was increased with the inoculation of *Rhizobia* and application of N along with P. The highest grain yield (2.06 t ha⁻¹) was found in plants receiving N @ 50 kg ha⁻¹ along with *Rhizobium* inoculation and P @ 40 kg ha⁻¹ and the lowest was 0.97 t ha⁻¹ recorded in control. In the present investigation the following conclusions may be drawn: Effect on growth, yield and nodulation of soybean by *Rhizobium* inoculation had shown positive.

Application of nitrogen upto 50 kg ha⁻¹ increased nodulation and progressively enhanced growth, yield and yield contributing characters of soybean. Application of phosphorus boost up significantly on nodulation, growth, yield and yield attributes parameters of soybean and zero phosphorus application comparatively decreased these parameters significantly. The present study also showed that *Rhizobium* inoculation with nitrogen and phosphorus fertilization gave better yield and was maximum with 50 kg N and 40 kg P ha⁻¹.

CHAPTER VI

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CHAPTER VII
APPENDICES

Appendix I: Analysis of Variance Table for plant height at 30 DAS

Source	DF	SS	MS	F value	P value
Replication	2	12.170	6.085		
Factor A	1	233.427	233.427	953.94	0.0000
Factor B	2	462.991	231.496	946.04	0.0000
Factor C	1	188.650	188.650	770.95	0.0000
ABC	2	2.547	1.273	5.20	0.0141
Error	22	45.383	20.245		

Appendix II: Analysis of Variance Table for plant height at 60 DAS

Source	DF	SS	MS	F value	P value
Replication	2	15.36	7.680		
Factor A	1	279.67	279.670	309.70	0.0000
Factor B	2	610.46	305.232	338.01	0.0000
Factor C	1	254.40	254.430	281.72	0.0000
ABC	2	5.12	2.562	2.84	0.0801
Error	22	42.87	26.903		

Appendix III: Analysis of variance table for branch at 30 DAS

Source	DF	SS	MS	F value	P value
Replication	2	0.2422	0.12111		
Factor A	1	4.4100	4.41000	281.67	0.0000
Factor B	2	8.2756	4.13778	264.28	0.0000
Factor C	1	3.8678	3.86778	247.04	0.0000
ABC	2	0.0800	0.04000	2.55	0.1005
Error	22	0.3444	0.01566		

Appendix IV: Analysis of variance table for branch at 60 DAS

Source	DF	SS	MS	F value	P value
Replication	2	1.143	0.5713		
Factor A	1	32.281	32.2813	467.01	0.0000
Factor B	2	83.915	41.9576	606.99	0.0000
Factor C	1	31.528	31.5282	456.11	0.0000
ABC	2	2.155	1.0773	15.59	0.0001
Error	22	1.521	0.0691		

Appendix V: Analysis of variance table for leaves at 30 DAS

Source	DF	SS	MS	F	P
Replication	2	0.534	0.2669		
Factor A	1	60.554	60.5543	22.39	0.0001

Factor B	2	99.221	49.6104	18.35	0.0000
Factor C	1	40.301	40.3013	14.90	0.0008
ABC	2	4.253	2.1263	0.79	0.4679
Error	22	59.489	2.7040		

Appendix VI: Analysis of Variance Table for Number of Nodule plant⁻¹

Source	DF	SS	MS	F	P
Replication	2	2.21	1.11		
Factor A	1	4867.39	4867.39	6300.83	0.0000
Factor B	2	53.53	26.76	34.65	0.0000
Factor C	1	5.29	5.29	6.85	0.0157
ABC	2	0.65	0.32	0.42	0.6631
Error	22	16.99	0.77		

Appendix VII: Analysis of Variance Table for Effective nodule plant⁻¹ (%)

Source	DF	SS	MS	F	P
Replication	2	108.58	54.29		
Factor A	1	1706.99	1706.99	29.92	0.0000
Factor B	2	33.26	16.63	0.29	0.7500
Factor C	1	77.58	77.58	1.36	0.2560
ABC	2	2.46	1.23	0.02	0.9787
Error	22	1255.07	57.05		

Appendix VIII: Analysis of Variance Table for Ineffective nodule plant⁻¹ (%)

Source	DF	SS	MS	F	P
Replication	2	108.58	54.29		
Factor A	1	1706.99	1706.99	29.92	0.0000
Factor B	2	33.26	16.63	0.29	0.7500
Factor C	1	77.58	77.58	1.36	0.2560
ABC	2	2.46	1.23	0.02	0.9787
Error	22	1255.07	57.05		
	35	3270.11			

Appendix IX: Analysis of Variance Table for Pod length plant⁻¹

Source	DF	SS	MS	F	P
Replication	2	0.07722	0.03861		
Factor A	1	0.93444	0.93444	80.27	0.0000
Factor B	2	2.00389	1.00194	86.07	0.0000
Factor C	1	0.87111	0.87111	74.83	0.0000
ABC	2	0.01167	0.00583	0.50	0.6126
Error	22	0.25611	0.01164		

Appendix X: Analysis of Variance Table for Pods plant⁻¹

Source	DF	SS	MS	F	P
Replication	2	0.00109	0.00054		
Factor A	1	0.02890	0.02890	402.97	0.0000
Factor B	2	0.14474	0.07237	1009.10	0.0000
Factor C	1	0.00001	0.00001	0.15	0.6977
ABC	2	0.00395	0.00198	27.54	0.0000
Error	22	0.00158	0.00007		

Appendix XI: Analysis of Variance Table for weight of 100 seed

Source	DF	SS	MS	F	P
Replication	2	0.88889	0.44444		
Factor A	1	40.1111	40.1111	496.38	0.0000
Factor B	2	97.7222	48.8611	604.66	0.0000
Factor C	1	40.1111	40.1111	496.38	0.0000
ABC	2	0.16667	0.08333	1.03	0.3732
Error	22	1.77778	0.08081		

Appendix XII: Analysis of Variance Table for Seed pod⁻¹

Source	DF	SS	MS	F	P
Replication	2	0.1667	0.08333		
Factor A	1	3.1211	3.12111	572.20	0.0000
Factor B	2	7.6717	3.83583	703.24	0.0000
Factor C	1	2.8900	2.89000	529.83	0.0000
ABC	2	0.0117	0.00583	1.07	0.3604

Error	22	0.1200	0.00545		
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Appendix XIII: Analysis of Variance Table for Stover yield

Source	DF	SS	MS	F	P
Replication	2	0.03304	0.01652		
Factor A	1	1.60867	1.60867	651.83	0.0000
Factor B	2	3.46187	1.73094	701.37	0.0000
ABC	1	0.83114	0.83114	336.77	0.0000
Error	2	0.16244	0.08122	32.91	0.0000
	22	0.05429	0.00247		

Appendix XIV: Analysis of Variance Table for Yield

Source	DF	SS	MS	F	P
Replication	2	0.03611	0.01805		
Factor A	1	1.28444	1.28444	775.72	0.0000
Factor B	2	3.20382	1.60191	967.45	0.0000
Factor C	1	1.17361	1.17361	708.78	0.0000
ABC	2	0.12962	0.06481	39.14	0.0000
Error	22	0.03643	0.00166		
	35	5.90216			

Appendix XV: Analysis of Variance Table for Seed weight plant⁻¹

Source	DF	SS	MS	F	P
Replication	2	223.9	111.97		
Factor A	1	4196.9	4196.88	318.06	0.0000
Factor B	2	7652.0	3825.99	289.95	0.0000
Factor C	1	3950.1	3950.12	299.36	0.0000
ABC	2	32.8	16.40	1.24	0.3081
Error	22	290.3	13.20		
	35	17663.2			