

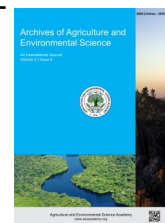


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ORIGINAL RESEARCH ARTICLE



Impact of *Rhizobium* biofertilizer on agronomical performance of lentil (BARI Masur-6) in Bangladesh

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ABSTRACT

Excessive nitrogen fertilizer uses in crop field causes surface water pollution, which has a harmful effect on the ecosystem. The study was conducted to reducing the use of nitrogen fertilizers during cultivation of pulse crop and as an alternative to increase the use of biofertilizers. *Rhizobium* can fix atmospheric nitrogen to the soil; it can be used as an alternative to urea for the cultivation of lentil (BARI Masur-6). The *Rhizobium leguminosarum* was isolated from root nodules of lentil (*Lens culinaris*) plants and cultured in YEMA (Yeast Extract Mannitol Agar) media. The *Rhizobium* was screened on the ground of physiological, biochemical and environmental conditions. Different doses of urea fertilizers (20, 40, 60, 80 kg ha⁻¹) and liquid *Rhizobium* were used in experimental plots. The results indicated that biofertilizer with different chemical fertilizer performed higher than application of several level of urea nitrogen fertilizer in respect of plant height, number, chlorophyll content ($\mu\text{g cm}^{-1}$) and number of nodules plant⁻¹ with variety BARI Masur-6. There was optimum relative growth rate (RGR) also observed. The increase in urea nitrogen levels was the reason for the decline in relative plant growth. Yield and yield contributing characters like number of pod plant⁻¹, number of seed plant⁻¹, 1000-grain weight (g), grain yield (t ha⁻¹), straw yield and biological yield were significantly influenced by biofertilizer application. The assembled application of biofertilizer and chemical fertilizer produced maximum number of harvest index (%) compare to the chemical fertilizers. Significant correlation found with no of nodule, no of seed, seed weight, grain yield and straw yield. Significant correlation also found in chlorophyll content with some yield contributing characters like seed number, seed weight, grain yield and straw biomass. Further significant correlation observed between pod number and seed number, between seed weight and grain yield. *Rhizobium* can fulfill the alternate source of nitrogen that promoted significant growth and yield of lentil and it was much closer to the farmer's conventional amount of urea.

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INTRODUCTION

Rhizobium is a gram-negative bacterium which subsistent in the root nodules of most leguminous crops. *Rhizobium* is a bacterium that can fix atmospheric N₂ after established inside root nodules of legumes. It is one of the most favorable bacteria to agriculture. *Rhizobium* the unique ability to produce substantial amounts of organic nitrogen through symbiotic biological nitro-

gen fixation. Thus, legume rhizobia symbiosis is an easy, affordable way to maintain soil quality and increase soil fertility and crop yields. Symbiosis is any type of a close and long-term biological interaction between two different biological organisms, be it mutualistic, commensaliastic, or parasitic. *Rhizobium* fixes the atmospheric nitrogen by the formation of nodule and associated takes carbohydrate from the associated plant. Symbiosis is based on metabolic exchange for mutual benefit.

Using energy from photosynthesis, the plant supplies carbon for bacterial respiration and the root nodule provides a low-oxygen niche for biological nitrogen fixation. In exchange, *Rhizobium* provides the plant with its own source of fixed nitrogen (Prell and Poole, 2006). Gomare et al. (2013) were noted that 20-60 kg nitrogen remains back which benefits for further plantation of leguminous plants.

Biofertilizer is a special type of compound that enhances soil nutrient by using microorganisms that establish symbiotic relationships with the plants. Biofertilizers cannot meet the total nutrient needs and are fact one of the inputs that can be used along with other fertilizers. The nitrogen fixing biofertilizers make a net addition to nitrogen supplies by fixing atmospheric nitrogen for the soil-plant system (Juwarkar et al., 2004). Nitrogen is needful for cellular synthesis of enzymes, proteins, chlorophyll, and nucleic acids and consequently important in plant enhancement and the outcome of food and feed. For legume plants, nitrogen is provided through symbiotic fixation of atmospheric N₂ by nitrogenase enzyme in rhizobial bacteria (Senanayake et al., 1987). This way of biological nitrogen fixation (BNF) accounts for 65% of the nitrogen now a days utilized in agriculture, and will sustain to be important in future crop productivity, especially in sustainable systems (Viviene and Dakora, 2003). The application of an effective biofertilizer and the use of an efficient soybean variety can play important roles in promoting plant growth, nodulation, nitrogen fixation, and higher seed yield (Htwe et al., 2019). Biofertilizer's biggest significant is that they do not only increase the yield of farmers, but are also safe and do not negative impact on the soil and environment (Kumar et al., 2019).

Lentil is one of the ancient and popular pulses in Bangladesh. In Bangladesh lentils cover about 33% of the total area under pulses and they are, from the consumer's point of view, the most preferred pulse, popularly known as Masur daal. Pulses have played an important role in maintaining soil fertility in Bangladesh for centuries. Lentil plays a significant role in human and animal nutrition and in maintenance and improvement of soil fertility. Its cultivation enriches soil nutrient status by adding nitrogen, carbon and organic matter which promotes sustainable cereal-based systems of crop production (Sarker and Kumar, 2011). Nitrogen fixation capacity of Lentil is significantly higher when grown in intercropping with other legume species than mono cropping. The crop is generally grown in rotation with cereals to break cereal disease cycles and to maintain soil nitrogen, thus reducing the demand of other cereal crops for nitrogen fertilizers (Fikiru et al., 2007). They can reduce the use of nitrogenous fertilizers and protect the environment. Pulses respond to *Rhizobium* inoculation depending on soil type, crop cultivation and efficacy of *Rhizobium* strain. *Rhizobium* increases the legume crop yield by fixing the atmospheric nitrogen gas and converts it into ammonia that can be used for its growth and development. A suggestive amount of nitrogen remains after harvesting of legume crop that improve soil structure. *Rhizobium* assures attainment in crop productivity and reduces the need for synthetic fertilizers that are expensive and cause environ-

mental matters. Biofertilizers are cheaper sources of nutrients than commercial fertilizers. Inoculation of *Rhizobium* appears to be very important for legume cultivation and sustainable agriculture. The amount of *Rhizobium* in soil is not optimum so if we apply *Rhizobium* to the soil at optimum rate with appropriate variety, it will produce more nodule as well as will supply more nitrogen to soil. *Rhizobium* improves the root nodulation that ultimately enhances the total biomass and growth of lentil crop with augmenting the organic agriculture production (Das et al., 2017).

The objectives of this experiment were to reduce the use of urea and chemical fertilizers, evaluate of growth and yield of lentil using biofertilizer and compare biofertilizer with urea nitrogen for lentil crop.

MATERIALS AND METHODS

Collection of soil sample

Lentil plants with roots were uprooted from the soil and were brought to the lab for the isolation of bacteria from it.

Culture preparation of *Rhizobium*

Guidelines for collecting nodules and preserving them during a collecting trip have been described and discussed by Date and Halliday (1987) and by Somasegaran and Hoben (1994). The *Rhizobium leguminosarum* was isolated from root nodules of lentil plants using YEMA (Yeast extract-1gm, Mannitol-10gm, K₂HPO₄-0.50gm, MgSO₄·7H₂O-0.20gm, NaCl-0.10gm, CaCO₃-1gm, Distilled water-1000ml, Agar-10gm) medium and identified based on morphology, motility and various biochemical and physiological tests. After that the *Rhizobium* was screened in pH (6.8±0.2) temperature (25 F-26 F). Then the screened Rhizobial culture was used for the plant growth and yield study.

Field experiment

Land preparation: Completely Randomized Design (CRD) with three replications was followed as experimental design in growth and yield measurement of lentil. Each plot size was 2m×2m.

Biofertilizer inoculation

Only nitrogen fertilizers were omitted during the application of basal dose mixed chemical fertilizers during land preparation. *Rhizobium* (50mL kg⁻¹ seed) was subsequently applied as a biofertilizer and the Urea 20, 40, 60 and 8 kg ha⁻¹ doses of urea as an inorganic chemical fertilizer. Basal dose TSP 80 kg ha⁻¹ and MP 30 kg ha⁻¹ were applied during land preparation. *Rhizobium* treated lentil seeds were inoculated with liquid *Rhizobium* by using a sticky solution made by corn flour and the inoculated seeds were dried in the air. Other fertilizers were applied to the plot one week before seed sowing.

Plant material and crop husbandry

Lentil variety BARI Masur-6 was used as plant material. Seeds

were collected from Bangladesh Agricultural Development Corporation (BADC), Rajshahi. The seeds were sown on Robi season. Weeds were controlled by hand, weeding completed at about one and two months after sowing. Irrigation was applied as per the need of the crop. Fungicide was applied during rainy and foggy situation.

Agronomic characteristics

During the experiment, growth and development of plants in the field were carefully observed. Ten plants were collected from each unit of plot. Plant height, number of root nodule and chlorophyll content ($\mu\text{g cm}^{-1}$) for respective treatments were recorded at 60 days old plant for assessing growth character. For enumerate Relative Growth Rate (RGR) fresh weight plant⁻¹ also recorded at 15, 30, 45, 60 and 75 days old plant. Number of pod plant⁻¹, number of seed plant⁻¹, 1000 grain weight (g), yield ton ha⁻¹, straw yield ton ha⁻¹, and biological yield ton ha⁻¹ was measured during postharvest period.

RESULTS AND DISCUSSION

Evaluation of *Rhizobium* and inorganic nitrogen on the growth and growth contributing characteristics

Mean values with standard error having different letters are significant as per DMRT of plant height, number of nodule and chlorophyll contents were shown in Table 1. *Rhizobium* and urea doses were significant on number of nodules plant⁻¹. Highest nodule number plant⁻¹ (17.90) at 60 days found in *Rhizobium* was used instead of urea and it was significantly different among all dose of fertilizers. There was an indicative variation found in the growth of root and root nodule of lentil plant at 15 days after sowing (Figure 1). *Rhizobium* treated lentil root and nodule has the utmost growth then the urea treated lentil plants at the age of 15 days. Conventional practices of urea nitrogen (40 kg ha⁻¹) dose were suitable to increase the nodule number among the urea dose. Significantly lowest nodule number was produced using over dose of nitrogen.

Maximum number of nodules was observed due to use of biofertilizer along with chemical fertilizer, respectively compared to application of other chemical fertilizer. These

results revealed that use of biofertilizer increased the nodule number of lentil. Ansari et al. (2015) investigation that the effect of bio fertilizers was significantly affected the active number of nodules per plants of the lentil crop. This was reported by Asghar et al. (1988) that *Rhizobium* inoculation increased nodule number and root dry weight.

There was significant variation observed among fertilizer doses on Plant height (cm) of BARI Masur-6 when the field was incorporated with different doses and types of fertilizer. The highest plant height (cm) (27.21) was observed where recommended dose of nitrogen was applied. *Rhizobium* treated lentil plant height was also significantly different with other application of different doses of urea. The lowest Plant height (22.07) was found using Urea-80 kg ha⁻¹. Plant height increased by the application of recommended nitrogen dose and biofertilizer. So that biofertilizer increased plant growth. *Rhizobium* with chemical fertilizer accomplished improved result then chemical fertilizers. A similar result was reported by Sultan (1993) observed that *Rhizobium* inoculation resulted significantly higher plant height than in un-inoculated control. There was indicative change observed among *Rhizobium* and different amounts of urea on chlorophyll content ($\mu\text{g cm}^{-1}$) (Table 1) of BARI Masur-6. Maximum chlorophyll content (10.52) was observed using 40 kg ha⁻¹ nitrogen chlorophyll content was also proximate to *Rhizobium* (9.64). The minimum amount of chlorophyll content ($\mu\text{g cm}^{-1}$) (6.57) was observed where Urea-80kg ha⁻¹ was applied.

Chlorophyll content of lentil at 60 DAS was shown highest content in farmer's conventional treatment but it was varying to the treatment were used *Rhizobium* place of urea. *Rhizobium* inoculum with inorganic fertilizer showed better performance against only with inorganic fertilizers treatments (Bhuiyan et al., 2015). Groups of microbes that are often used are the microbes that fix Nitrogen from the air, which dissolves microbial nutrient (especially P and K), the microbes that stimulate plant growth (Poonia, 2011). *Rhizobium* will be served as a biofertilizer to enhance plant growth (Rajeshwari et al., 2017). Plant growth promotion was determined by length of shoot and root, count of root nodules formed and by the estimation of chlorophyll content (Thimmaiah,1999).

Table 1. Influence of *Rhizobium* and different urea doses on lentil (BARI Masur-6) growth and development at 60 DAS.

Fertilizers	Plant height (cm)	Number of Nodule/Plant	Chlorophyll content ($\mu\text{g/cm}$)
<i>Rhizobium</i> ₅₀ mL/kg seed	27.21±0.75a	17.90±0.79a	9.64±0.87ab
Urea ₂₀ kg /ha	24.60±0.75ab	13.20±0.79b	7.38±0.87bc
Urea ₄₀ kg /ha	26.20±0.75b	14.80±0.79b	10.52±0.87a
Urea ₆₀ kg /ha	25.90±0.75ab	12.40±0.79b	8.11±0.87abc
Urea ₈₀ kg /ha	22.07±0.75c	12.50±0.79b	6.57±0.87c

* In a column, data are the mean values with standard error having different letters are significant as per DMRT. TSP80kg/ha+MP40kg ha⁻¹ was common for each treatment.



Figure 1. Influence of biofertilizer and urea nitrogen on nodulation and root growth of lentil 15 days after sowing.

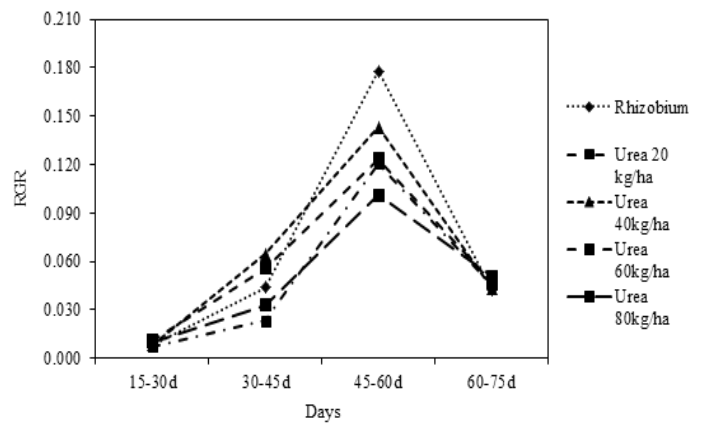


Figure 2. Effect of Rhizobium and different amount of urea on relative growth rate (RGR) of lentil at diversified days after sowing.

Table 2. Influence of *Rhizobium* and urea dose on lentil yield contributing characters at harvest period.

Treatments	Number of Pod/Plant*	Number of Seed/Plant	1000-grain weight (g)
<i>Rhizobium</i> _{50mL/kg seed}	57.60±1.59a	101.50±4.59a	28.18±0.72a
Urea ₂₀ kg /ha	47.90±1.59c	67.39±4.59b	25.09±0.72b
Urea ₄₀ kg /ha	57.60±1.59a	96.20±4.59a	27.22±0.72a
Urea ₆₀ kg /ha	54.10±1.59ab	72.00±4.59b	23.92±0.72b
Urea ₈₀ kg /ha	51.80±1.59bc	71.50±4.59b	24.35±0.72b

*In a column, data are the mean values with standard error having different letters are significant as per DMRT.

The relative growth rate represents the amount of increased fresh matter relative to the initial weight during a time period. In other words, the RGR specifies that each gram of the fresh weight has increased by how much in every day. As seen in Figure (2) in all treatments the RGR is slow at first but afterwards the curve vastly goes upward and then gets slow again and faces a slide and *Rhizobium* treated plants showed highest RGR at 45-60 days. Therefore, the amount of fresh matter produced in every day is more than the last one and following the plant's weight increases with the time passing. But after at maturity do not have a production role. In other words, at first all the plant's weight and cells have a productive role but with time passing the dead tissue and cells that don't produce also increase. Therefore, the amount of production in each day in comparison to the day before decreases and the RGR reaches a descending trend. The plants RGR immediately after germination is normally slow, followed by a vast increase in the curve and then slowed down (Gardner et al., 1985). At first all the plant's weight and cells have a productive role but with time passing the dead tissue and cells that don't produce also increase (Bagher et al., 2013).

Effect of *Rhizobium* on lentil yield contributing characters

Biofertilizer and inorganic nitrogen dose had significant effect on pod number, seed number and 1000-grain weight. Significantly different and maximum number of pod was produced by conventional practices of fertilizer and biofertilizers. There was significantly highest number of seed plant⁻¹ emanated with the application of *Rhizobium* biofertilizer. Even microbial fertilizer also produced significantly highest 1000-grain weight compared to all dose of inorganic nitrogen. Most of the yield contributing characters was significantly reduced using over doses of nitrogen (Table 2).

The highest 1000-grain weight was observed for treatment *Rhizobium* which indicates that the biofertilizer application helped supplying the more nutrients to plants and helped to gain the highest amount of grain weight than untreated plants. A similar result was reported by Podder et al. (1989), they reported that in a field experiment with 6 isolates of lentil significantly increase in 1000 seeds weight due to *Rhizobium* inoculation. Another result was nitrogen concentration in tops and grain; number and weight of nodules on roots; and increased acetylene reduction rate of the nodules (Jessop et al., 1989; Idris et al., 1989; Yahya et al., 1995).

Influence of biofertilizer on lentil yield

Biofertilizer and inorganic nitrogen influence was significant on lentil yield (Table 3). *Rhizobium* treated seed produced significantly highest grain, straw and biological yield. Farmers practices (40 kg ha⁻¹) was proximate to all types of yield. Grain yield, straw yield and biological yield was significantly alleviated exclusive inorganic urea. According to Podder et al. (1989) 6 isolates of lentil significantly increase in hay yield due to *Rhizobium* inoculation. *Rhizobium* inoculation along with chemical fertilizer increased grain and hay yield of lentil significantly compared to un-inoculated control that means use of biofertilizer increased biological yield of lentil. Another work was the dual inoculation of *Azotobacter* and *Rhizobium* significantly influenced all the crop characters including N contents, N uptake by seed and shoot as well as protein content of seed (Hossain and Suman, 2005). A similar result was reported by Hossain (2018), he observed that *Rhizobium* inoculation alone increased grain and hay yield of lentil significantly compared to un-inoculated control and 50 kg urea ha⁻¹ treated lentil.

Table 3. Influence of *Rhizobium* and different amounts of urea on lentil yield at harvest time.

Fertilizer	Grain yield (t /ha)	Straw yield (t /ha)	Biological yield (t /ha)
<i>Rhizobium</i> _{50mL/kg seed}	2.08±0.11a	1.72±0.05a	3.88±0.16a
Urea ₂₀ kg /ha	1.33±0.11b	1.44±0.05bc	3.56±0.16ab
Urea ₄₀ kg /ha	2.04±0.11a	1.53±0.05b	2.91±0.16cd
Urea ₆₀ kg /ha	1.39±0.11b	1.42±0.05bc	3.34±0.16bc
Urea ₈₀ kg /ha	1.29±0.11b	1.33±0.05c	2.83±0.16d

* In a column, data are the mean values with standard error having different letters are significant as per DMRT.

Table 4. Correlation matrix among different growth and yield contributing characters.

Correlation							
	No of nodule/plant	Chlorophyll content (µg/cm)	No of pod/plant	No of seed/plant	Seed weight (g/plant)	Grain yield (kg/m ²)	Straw yield (kg/m ²)
No of nodule/plant	1						
Chlorophyll content (µg/cm)	0.17	1					
No of pod/plant	0.368**	0.254	1				
No of seed/plant	0.421**	0.342*	0.903**	1			
Seed weight (g/plant)	0.307*	0.297*	0.126	0.251	1		
Grain yield (kg/m ²)	0.319*	0.316*	0.113	0.238	0.986**	1	
Straw yield (kg/m ²)	0.292*	0.319*	0.186	0.198	0.213	0.247	1

Pearson correlation; level of significance, * = 5%, ** = 1% and *** = 0.1%.

Better yield response found in lentil plant with *Rhizobium* instead of urea. In the experiment every yield attributing character showed highest quantity used of *Rhizobium* in lieu of urea. Hoque and Hoq (1994) and Nadeem et al. (2004) found that number of pods/ plants was significantly affected by seed inoculation. *Rhizobium* inoculum can be used as the substitute of urea for lentil cultivation (Bhuiya et al. 2015). *Rhizobium* inoculation gave 64-68% higher seed yield in lentil Khanam et al. (1993). In another study, Khanam et al. (1999) found 46% higher seed yield in lentil at Meherpur, 30% at Faridpur and 33% at Jessore districts of Bangladesh due to *Rhizobium* inoculums. Sekhon et al. (2002) recorded that *Rhizobium* inoculation resulted higher seed yield over no inoculation.

The harvest index was significantly varied due to *Rhizobium* and different amount of urea which had been shown through bar graph in Figure 3. The graph shows the highest value (0.52) on the plot with 40 kg urea and the closest value (0.51) of *Rhizobium* plot. The lowest value (0.46) was found in the bar graph at urea 80kg treated plot. Hossain (2018), he observed that *Rhizobium* inoculation alone increased grain and hay yield of lentil significantly compared to un-inoculated control and 50 kg urea treated lentil. The combined application of biofertilizer with other chemical fertilizer produced maximum number of harvest index (%) compare to the other application of chemical fertilizer.

Correlation among different growth and yield contributing characters

The statistical relationship between different parameter of

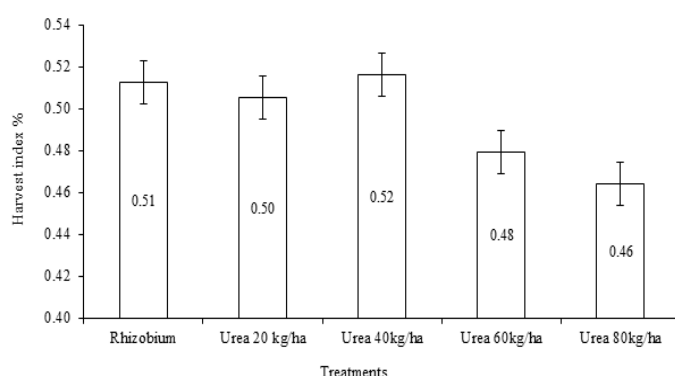


Figure 3. Influence of *Rhizobium* and different amount of urea on harvest index of lentil. The Bar graph presents mean with standard error.

lentil has been calculated. The correlation co-efficient scatter plots of several parameters are also shown in Table 4 and Figure 4 and 5 along with the equation. No of nodule significantly correlated with no of seed, seed weight, grain yield and straw yield. From these observations, it was known that the nodulation formation of lentil plants had a positive effect on yield and yield-related characteristics. There was significant correlation found in chlorophyll content with some yield contributing characters like seed number, seed weight, grain yield and straw biomass. Further significant correlation observed between pod number and seed number, between seed weight and grain yield. These correlations pretend that pod plant⁻¹; seed plant⁻¹ and seed weight plant⁻¹ exhibited high direct effects on grain yield m⁻² (Abo-Hegazy, 2012).

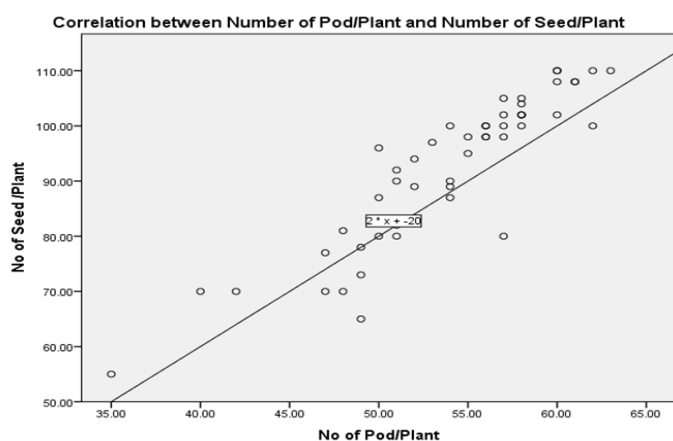


Figure 4. Correlation between no. of pod/plant and no. of seed/plant.

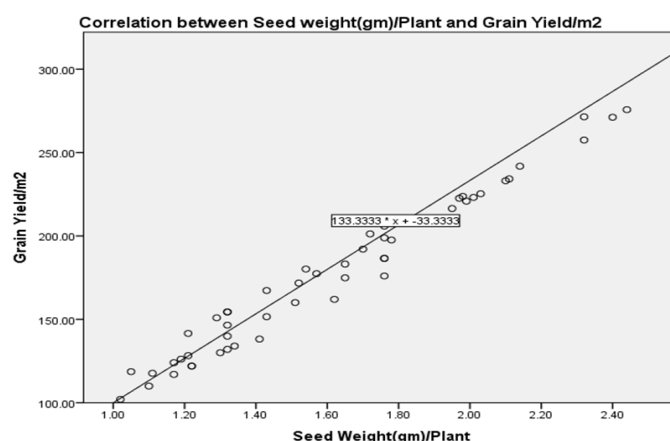


Figure 5. Correlation between seed weight (g) and no. of grain yield m^{-2} .

Correlation studies between agronomic traits such as yield and its components would help plant breeders and agronomists to enhance crop growth and yield attributes (Ezzat and Ashmawy, 1999). Luthra and Sharma (1990) reported in a two-year study on lentil genotypes that number of pod $plant^{-1}$ was highly significantly and positively correlated with the seed yield $plant^{-1}$. Jain et al. (1991) in their multiple correlation and regression studies in lentil indicated that seed $plant^{-1}$ and pod $plant^{-1}$ were the best combination of characters for the improvement of yield. Saraf et al. (1985) found that number of pods $plant^{-1}$ was highly significantly correlated with the seed yield. Rajput and Sarwar (1989) reported a positive and highly significant correlation between number of pods $plant^{-1}$ and seed yield. Begum and Begum (1996) confirmed the highly positive and significant ($P < 0.01$) association of yield components to seed yield in lentil.

The positive correlation coefficient having equation $Y = 2*x + -20$ was shown in Figure 4 indicates that number of pod $plant^{-1}$ and number of seed $plant^{-1}$ are positively correlated i.e., if the number of pod $plant^{-1}$ increases seed weight $plant^{-1}$ will increase. The positive slope of correlation having equation $Y = 133.33*x + -33.33$ was shown in Figure 5 indicates seed weight/plant and grain yield m^{-2} are positively correlated and they have strong correlation i.e., if the seed weight $plant^{-1}$ increases grain yield will increase.

Conclusion

The entire results pointed out that every yield contributing components and yield of lentil were significantly highest by biofertilizer (*Rhizobium*) application. Application of *Rhizobium* predispose the number of nodules, relative growth rate, yield and yield attributes of lentil and excess use of urea nitrogen reduced yield. This study showed that significant plant growth and yield was released with the application of *Rhizobium* instead of conventional practices 40 kg ha^{-1} urea.

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