Effect of urea, bio-fertilizers and their interaction on the growth, yield and yield attributes of *Cyamopsis Tetragonoloba*

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**ABSTRACT**
A field experiment was conducted at Agriculture Research Station Bahawalpur (Pakistan) during Kharif, 2016 to evaluate the response of cluster bean against bio and chemical fertilizers. The treatments consisted of three levels of nitrogen (urea 75g, 85g and 95g/40g seeds) with three level of *Rhizobium* (1.5g, 2.0g and 2.5g/40g of seeds). The experiment arranged in a randomized complete block design with 16 treatments and three replications. Combinetreatment of urea and *Rhizobium* resulted in maximum plant height, number of pods/plants, number of seeds/pod, 1000 seeds weight, yield/plant, and chlorophyll content. Similarly, application of 2.5g rhizobium and 85g of urea separately resulted in highest number of leaves. Our results concluded that the use of urea and rhizobium in consortium could be a potential agronomic practice for the production of high grain yield in cluster bean.

**Key words:** Cluster bean, Growth, Rhizobium, Seed yield, Urea.

**INTRODUCTION**
Soil, a vital component for plant germination and survival must enrich from all macro and micro nutrients in sufficient and balanced quantities for their growth and nourishment. Chemical fertilizers provide essential plant nutrients, but it also toxic the soil which affect plant and animal health direct and indirectly. Bio fertilizer could address the problem of low soil fertility and improve agricultural productivity and food security. Physical or chemical properties of soils are less responsive to changing soil conditions than biological properties (Kowaljow and Mazzarino, 2007; Melero et al., 2007). Moreover, soil organic matter in a particular soil is greatly influenced by vegetation, climate change, soil reaction and biological conditions (Meena and Sharma, 2016). Microbe are able to grow under harsh conditions and gives some important understandings regarding their ability to increase under the ecological limitations of the environment (Muzamil Shah and Muhammad Zubair, 2018).

The efficient use of nutrients is one of the most important factors in any program designed to achieve an economic increase in agricultural production. Continuous and unbalanced use of nutrients is an important area of concern. Soil degradation and depletion of fertility due to the unbalanced and inadequate use of nutrients is largely responsible for the reduction of crop productivity. Maintaining soil health through balanced nutrition is essential to maintain crop productivity (Meena et al., 2017). Soil fertility can also be regulated by soil microbial enzymatic activities which are responsible for the soil properties and cultivation factors (Corstanje et al., 2007). The depletion of the soil organic matter can be prevented by the biofertilizer particularly rhizobia, which is an alternative source of N-fertilizer (Jeyabal and Kuppuswamy, 2001).

The intimate symbiotic relationship between rhizobia and legumes result in nodule formation by lipochitooligosaccharide (LCO) signals and enhance yield (Lhuissier et al., 2001; Matiru and Dakora, 2004). In order to raise the soil fertility and crop production, bio-fertilizers have been recognized as a substitute to chemical fertilizers in sustainable farming (Manral and Saxena, 2003). Rhizosphere harbors diverse and rich regime of beneficial microorganisms which directly affect plant health and soil fertility in which a significant number of bacterial and fungal species have a mutual association with plants. They are able to bring useful effects in plant growth (Belay et al., 2001). In agricultural practices, use of beneficial microbes was started about 50 years ago. Now there is rising indication that these useful microbial populations can also improve plant opposition to contrary environmental stresses, e.g. heavy metal, impurity of water and nutrient deficiency (Chemining’wa and Vessey, 2006; Verma et al., 2011).

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In oil yielding legumes, minerals and N fertilization are crucial factors which results high yield (Rathke et al., 2005). Deficiency of N is a major limiting factor for high yielding crops all over the world. It has been assessed that the microbial activities of Rhizobium fixed about 40-250 kg N/ha/year by diverse leguminous crops. Yield can be increased up to 10-35 % by the bio-augmentation of Rhizobium (Namvar et al., 2011).

The cluster bean also known as Guar [Cyamopsis tetragonoloba (L.) Taub] is an annual legume that occupies a substantial part of moisture deficient areas of the sub-continent. The main uses include green fertilizers, grains and animal feed (Douglas, 2005). The ideal environment of its optimal germination is hot condition where soil has temperature more than 25°C. Neutral conditions of soils (pH 7) are favourable for the plants while guar grows effectively on soil ranging in pH from 5.3 to 8.3 (Jackson and Doughton, 1982). This legume is a very valued plant within a crop rotation cycle, as it survives in symbiosis with nitrogen-fixing bacteria. In pulses nitrogenous fertilizers application enhance yield (Corstanje et al., 2007). Guar inoculation with Rhizobium increased seed yield, number of nodules, nodules fresh weight, plant dry weight, nitrogen fixation and total nitrogen content (Singh and Singh, 1989). Hence this study was under taken with the objective of effect of chemical fertilizers and rhizobium inoculation on growth, inflorescence and yield attributes of guar [Cyamopsis tetragonoloba (L.) Taub].

MATERIALS AND METHODS

Description of experimental area: A field experiment was conducted at Agricultural Research Station (Bahawalpur, Pakistan) situated at 29°23'44” N 71°41’ 1”E and elevated at 461 m above sea level (ASL). The crop was cultivated during Kharif, 2016. Before sowing, soil samples were taken and subjected to physical and chemical analysis at soil and water testing laboratory Bahawalpur following the method of Estefan et al. (2013). The soil texture of the experimental site was loamy. The maximum minimum, and relative humidity were collected on daily basis from the Pakistan Meteorological Department. Soil analysis and weather data are shown in Tables 1, 2.

Experimental setup, plant material and treatments: The experiment was laid out in a randomized complete block design (RCBD) with three replications. The seeds of guar variety viz. 99BR used in the experiment were obtained from Guar Research Station Bahawalpur. Plants were treated with three different levels of a bio-fertilizer (Rhizobium at 1.5g/40 g seeds, 2.0g/40g seeds and 2.5g/40g seeds), three level of a nitrogen source (urea at 75g/40g seed, 85g/40g seed and 95g/40g seeds) and one treatment without urea and Rhizobium was taken as control. Rhizobium japonicum used in this study was provided by Annex crop science company Multan, Pakistan. Seeds were treated at the time of sowing. Two percent guar gum was applied on overnight soaked seeds, mixed thoroughly in polythene bags with slurry of rhizobium, urea and their mixture and sown immediately in the field. The experimental unit area (plot) was 12.96 m² consisting of 4 rows, 1.8 m width and 7.2 m length and were sown with hand drill method keeping row to row distance of 45 cm. All the excess seedlings were thinned out 15 DAS by maintaining a spacing of 15 cm between two plants in a line. Crop was irrigated four times i.e. first irrigation at flowering stage, 2nd irrigation at flowering stage, 3rd at pod appearance and 4th at grain formation. The crop was harvested on 10th November 2016 when it retained 10 % moisture level.

Data collection: Data was collected from tagged plants in order to record morphological, yield and inflorescence parameters. Plant height, number of leaves/plant, stem diameter (mm), number of pods/plants, number of pods/cluster, number of cluster/plant, length of pod (cm), Chlorophyll contents and size of ovary was measured via stereomicroscope at physiological maturity. Number of seeds/pod, weight of seeds/plant, 1000 seeds weight (g) and yield per plot was recorded from harvested plants. Yield per plot (g/m²) was converted to kg/ha.

Table 1: Soil Analysis Report.

| Depth (cm) | E.C (dSm⁻¹) | pH | Organic Matter (%) | Available Phosphorus (ppm) | Available Potassium (ppm) | Saturation % | Texture | Remarks |
|-----------|-------------|----|--------------------|-----------------------------|---------------------------|--------------|---------|---------|
| 15.3-30.5 | 3           | 8.4 | 0.77               | 7.5                         | 139                       | 48           | Loam    | Normal  |

Table 2: Weather data during experiment period.

| Month | Max Temp °C | Min Temp °C | Humidity (%) | Rain fall (mm) | Cloudy day |
|-------|-------------|-------------|--------------|----------------|------------|
|       | Avg         | Range       | Avg          | Range          |            |
| Jun   | 43          | 40-46       | 27           | 24-30          | 77         | 73-80       | 9        | 4       |
| Aug   | 40          | 38-42       | 28           | 27-390         | 75         | 68-80       | 4        | 2       |
| Sep   | 35          | 31-39       | 24           | 22-27          | 74         | 62-85       | 5        | 2       |
| Oct   | 33          | 29-36       | 21           | 19-25          | 74         | 68-80       | –        | –       |
| Nov   | 26          | 29-38       | 22           | 22-26          | 73         | 63-84       | –        | –       |

Max Temp: Maximum Temperature, Min Temp: Minimum Temperature, Avg: Average.
Statistical analysis: The collected data were subjected to analysis of variance, descriptive analysis and Duncan Multiple Range Test (DMRT) to find out the specific differences between the pairs of means (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp). Correlations of morphological, growth, yield and inflorescence characters were estimated by using Pearson correlations.

RESULTS AND DISCUSSION

Growth parameters: Among the treatment combination (Table 3), combined application of urea 95 g + R. japonicum 2.0 g showed maximum plant height, while a lower dose (urea 75 g + R. japonicum 1.5 g) showed the highest stem diameter. Highest number of leaves recorded for alone R. japonicum 2.5 g. It may be due to the enhanced R. japonicum inoculation and more availability of soluble nitrogen to the crop. It also reveals that 2.0 g to 2.5 g R. japonicum has better effects on number of leaves per plant. The results indicated that morphological characters like plant height and stem diameter are significantly increased by combine treatments of R. japonicum and urea as compared to control and other treatments. Number of leaves also showed good results in combine treatments but significantly enhanced by the alone treatment of R. japonicum as compared to control and other treatments. Some parameters such as plant height and stem diameter show both the maximum and minimum results in different doses of combined treatment of bio and chemical fertilizers. It might be due to the environmental effect or ineffective R. japonicum inoculation. Said-Al Ahl, (2005) reported that plant height, number of branches, plant fresh and dry weights, umbels number and fruits yield increased with nitrogen and bio-fertilizer treatment in Anethum graveolens, with the high dose of nitrogen producing the highest values. Nushair et al., (2018) also reported in Pigeon pea, Sweet pea and Chick pea that bio fertilizer application significantly increased plant height pod and seed weight. Hassan et al., (2012) stated that bio-fertilizer treatments enhanced plant height, branch number/plant and plant dry weight. Growth parameters of guar can be much improved with application of nutrients in balanced ratio (Deshmukh et al., 2014). Nitrogen boosts new cell formation, promotes plant vigor and root growth, hastens leaf development which helps in harvesting more solar energy and extended utilization of nitrogen which can be attributed for higher plant height and branches/plant.

Chlorophyll contents: It is clear from the data (Table 3) that chlorophyll contents significantly enhanced by the combine application of urea and rhizobium (urea 75g + R. japonicum 2.5g). Highest chlorophyll contents recorded for treatment 10 and 11 (urea 75,85g +1.5, 2.5g). The results indicated that combined fertilizers (urea + R. japonicum) had positive effect on chlorophyll contents as compared to control and other treatments. Significant increase in chlorophyll content is an indication of higher N fixation due to R. japonicum application. Afridi et al., (2019) stated that the application of K.rhizophila and C. sakazakii enhanced chlorophyll contents up to 17% in screening wheat genotypes against salt stress. Nitrogen can increase photosynthesis process in plants. Alam and Haider, (2006) stated that increasing photosynthetic rate with N fertilization could be attributed to increasing amount of chlorophyll pigment, since N was one of the main components of chlorophyll. Shu et al., (2012) reported that nitrogenous compounds play a major role in protein and chlorophyll synthesis and thus increase the photosynthetic ability and consequently dry matter production.

Table 3: Effects of rhizobium inoculation and Urea fertilizers on Morphology and chlorophyll contents of cluster bean.

| Treatments | PH (cm) | Pod L (cm) | SD (mm) | OS (µm) | Chl a (mg/g Fw) | Chl b (mg/g Fw) | Total Chl (mg/gFw) |
|------------|--------|------------|---------|---------|----------------|----------------|--------------------|
| T1: Seed (Control) | 84.1 hj | 5.7 a | 33.3 bc | 28.7 b | 0.83 f | 0.33 e | 1.16 bcd |
| T2: Seed + Urea 75 g | 86.7 k | 5.7 a | 35.9 e | 36.7 f | 0.59 b | 0.23 b | 0.82 bcd |
| T3: Seed + Urea 85 g | 85.9 kj | 5.8 a | 36.1 ef | 26.6 a | 0.63 bc | 0.24 bc | 0.88 ab |
| T4: Seed + Urea 95 g | 84.5 ij | 5.7 a | 37.1 fg | 41.1 j | 0.66 cd | 0.24 bc | 0.91 abc |
| T5: Seed + Rhizobium 1.5 g | 82.9 ghi | 5.8 a | 36.1 ef | 30.3 c | 0.54 a | 0.18 a | 0.73 a |
| T6: Seed + Rhizobium 2 g | 78.2 cd | 5.7 a | 34.6 de | 40.4 i | 0.82 f | 0.25 | 1.25 bcd |
| T7: Seed + Rhizobium 2.5 g | 82.6 gh | 5.8 a | 37.3 fg | 40.8 j | 0.9 g | 0.25 | 1.15 bcde |
| T8: Seed + Urea 75 g + Rhizobium 1.5 g | 81.7 fg | 5.8 a | 40.8 h | 45.0 k | 0.68 d | 0.22 ab | 0.9 abc |
| T9: Seed + Urea 75 g + Rhizobium 2 g | 72.1 a | 5.9 a | 30.1 a | 39.1 h | 0.9 g | 0.28 cd | 1.19 cde |
| T10: Seed + Urea 75 g + Rhizobium 2.5 g | 75.9 b | 5.7 a | 34.7 de | 39.2 h | 1.34 j | 0.44 g | 1.78 g |
| T11: Seed + Urea 85 g + Rhizobium 1.5 g | 75.6 bc | 5.8 a | 32.4 b | 35.5 e | 1.34 j | 0.41 fg | 1.36 g |
| T12: Seed + Urea 85 g + Rhizobium 2 g | 78.6 de | 5.9 a | 34.0 cd | 38.2 g | 1.19 i | 0.39 f | 1.59 fg |
| T13: Seed + Urea 85 g + Rhizobium 2.5 g | 70.3 a | 5.8 a | 34.1 cd | 34.4 d | 0.75 e | 0.23 b | 0.98 abc |
| T14: Seed + Urea 95 g + Rhizobium 1.5 g | 80.2 ef | 5.8 a | 36.1 ef | 36.8 f | 1.06 c | 0.31 de | 1.37 ef |
| T15: Seed + Urea 95 g + Rhizobium 2 g | 122.6 1 | 5.8 a | 37.6 g | 39.5 h | 0.73 e | 0.23 b | 0.96 abc |
| T16: Seed + Urea 95 g + Rhizobium 2.5 g | 87.6 k | 5.9 a | 37.4 fg | 35.8 e | 0.91 g | 0.33 e | 1.24 de |

Note: The different superscript letters indicate statistically significant differences by a Duncan’s multiple range test at P = 0.05; PH: Plant Height, Po L: Pod Length, SD: Stem Diameter, OS: Size of Ovary, Chl: Chlorophyll.
Yield and yield contributing attributes: Data presenting in Table 4 demonstrate the impact of sixteen treatments of bio (R. japonicum) and chemical (urea) fertilizers on yield parameters. As regards number of pods per plant and number of pods per cluster, application of Urea 95 g + R. japonicum 2.5 g and Urea 85 g + R. japonicum 1.5 g recorded the highest values for both the parameters respectively. It indicates that combine application have positive effect on number of pods per plant. These results were supported by the findings of Prasanna et al., (2014). Our results were also confirmed by the finding of Tahir et al., (2009) who reported that application of N along with P and Brady R. japonicum resulted up to 94% increase in pod number per plant. The highest number of cluster per plant and seeds weight per plant recorded for Urea 95 g + R. japonicum 2.5 g. Application of Urea 85 g + R. japonicum 2.0 g recorded highest pod length while application of Urea 75 g + R. japonicum 1.5 g showed highest number of seeds per plant. These results were in confirmatory to the study of Rajput and Singh, (1996). Thousand seed weight was ranged from 22.64 to 31.21 g being highest for treatment T14 (Urea 95 g + R. japonicum 1.5 g) whereas minimum for T6 (R. japonicum 2.0 g). The maximum grain yield 1000 seed weight, 1000 Seed, kg/ha: kilogram per hectare.

CONCLUSION

The results in this study clearly indicated that chemical fertilizer with rhizobium inoculation significantly enhanced growth, yield components, and seed yield of cluster bean. The application of urea 95 g and 75 g with the inoculation of 1.5 g and 2.5 g R. japonicum respectively recorded higher growth (plant height, branches/ plant, and dry matter); yield attributes (pods/plant, seeds/pod and 1000-seed weight) and grain yield/ha as compared to control and other treatments. Whereas application of rhizobium 2.5 g alone is found to be more effective for some growth characters (leaf number) of cluster bean. So, it was concluded that the co-inoculation of rhizobia and urea fertilizers with appropriate doses may be recommended for obtaining the higher yield of cluster bean in the region.

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CONFLICT OF INTEREST

The authors declare that we have no conflict of interest.
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