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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. Combine treatment 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 effect 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 set up, 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 contro1. *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 m^2 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 after 35 days, 2nd irrigation at flowering stage, 3rd at pod appearance and 4th at grain formation. The crop was harvested on 10th of 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/m^2) 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 %age	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	Avg	Range		
Jul	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 cells 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_2 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 ih	5.7 a	33.3 bc	28.7 b	0.83 f	0.33 e	1.16 bcde
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 bcde
T3: Seed + Urea 85 g	85.9 jk	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 bc	1.07 bcd
T7: Seed + Rhizobium 2.5 g	82.6 gh	5.8 a	37.3 fg	40.8 j	0.9 g	0.25 bc	1.15bcde
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	76.8 bc	5.8 a	32.4 b	35.5 e	1.34 j	0.41 fg	1.76 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 l	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.

Table 4: Effects of rhizobium inoculation and Urea fertilizers on seed yield and yield contributing parameters of cluster bean.

Treatment	No Of Pod/P	No of Pod/C	No of C/P	No of S/Po	No of L/P	Yield/P (gm)	1000 SW (gm)	Yield (kg/ha)
T1: Seed (Control)	190.9 a	18.1 a	13.6 a	8.5 a	13.8 a	21.8 ab	27.4 c	669.5 ab
T2: Seed + Urea 75 g	193.9 a	18.4 a	15.2 a	8.5 a	14.5 abc	25.7 ab	28.1 d	787.9 ab
T3: Seed + Urea 85 g	162.4 a	13.5 a	14.3 a	8.5 a	14.1 abc	17.5 a	29.3 gh	537.5 a
T4: Seed + Urea 95 g	185.6 a	17.0 a	13.1 a	8.6 a	13.6 a	23.6 ab	29.6 h	725.9 ab
T5: Seed + Rhizobium 1.5 g	173.7 a	15.4 a	15.5 a	8.8 a	15.3 abcd	24.1 b	30.8 ij	736.6 ab
T6: Seed + Rhizobium 2 g	154.3 a	17.2 a	13.2 a	8.7 a	15.4 abcd	23.9 ab	22.6 a	734.3 ab
T7: Seed + Rhizobium 2.5 g	215.7 a	18.2 a	16.8 a	8.6 a	16.7 d	31.8 ab	27.9 d	918.5 ab
T8: Seed + Urea 75 g + Rhizobium 1.5 g	185.8 a	14.3 a	19.7 a	9.1 a	15.7 bcd	20.5 ab	26.2 b	630.2 ab
T9: Seed + Urea 75 g + Rhizobium 2 g	180.2 a	17.5 a	15.4 a	8.5 a	15.1 abcd	25.7 ab	28.1 d	790.2 ab
T10: Seed + Urea 75 g + Rhizobium 2.5 g	181.8 a	15.1 a	17.1 a	8.9 a	14.4 abc	22.3 ab	30.2 i	684.9 ab
T11: Seed + Urea 85 g + Rhizobium 1.5 g	173.7 a	19.4 a	14.4 a	8.7 a	14.9 abc	21.9 ab	22.6 l	671.9 ab
T12: Seed + Urea 85 g + Rhizobium 2 g	198.6 a	16.8 a	16.6 a	8.6 a	15.0 abc	25.4 ab	30.4 i	780.3 ab
T13: Seed + Urea 85 g + Rhizobium 2.5 g	162.5 a	16.8 a	16.1 a	8.5 a	15.8 cd	21.4 ab	29.0 fg	656.2 ab
T14: Seed + Urea 95 g + Rhizobium 1.5 g	181.6 a	17.6 a	14.2 a	8.6 a	14.1 ab	22.1 ab	31.2 j	678.2 ab
T15: Seed + Urea 95 g + Rhizobium 2 g	161.3 a	15.7 a	18.2 a	8.6 a	14.6 abc	19.7 ab	28.4 de	603.7 ab
T16: Seed + Urea 95 g + Rhizobium 2.5 g	221.2 a	16.1 a	19.9 a	8.7 a	15.0 abcd	29.9 b	28.7 ef	977.6 b

Note: The different superscript letters indicate statistically significant differences by a Duncan's multiple range test at P = 0.05; Po/P: Pods per Plant, Po/C: Pods per Cluster, L/P: Leaves per Plant, C/P: Clusters per Plant, S/Po: Seeds per Pod, Wt: Weight, S/P: Seeds per Plant, 1000 SW: 1000 Seed Weight, kg/ha: kilogram per hectare.

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 95g + *R. japonicum* 2.5g and Urea 85g + *R. japonicum* 1.5g 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 *BradyR. 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* 2.5g. Application of Urea 85g + *R. japonicum* 2.0g 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, biological yield and harvest index was obtained in barley by treatment of highest level of nitrogen fertilizers, in contrast, to control which was similar to the findings of Mirshekari *et al*., (2012). Application of urea 95 g + rhizobium 2.5 g showed highest yield (977.6 kg/ha) while minimum yield exhibited by application of alone urea 85 g/ 40 g seeds. Our results were supported by the findings of Hossain *et al*., (2011). These results were also in confirmation with the results of Jatav *et al*., (2016) and Singh *et al*., (2014). Ibrahim *et al*., (2011) stated that yield parameters were significantly increased by the inoculation of *BradyR. japonicum* strain in guar cultivars. The results indicate that various combine treatments of biotic fertilizers (*R. japonicum*) and chemical fertilizer (urea) have positive effect on yield parameters as compare to control and other treatments. Saritha *et al*., (2013) conducted a study on the effect of certain organic manures on the seed germination and seedling growth of Cluster bean. It was found that best seedling and yield of the crop in cluster bean is possible by the treatment with panchagavya (or) bio fertilizer (*R. japonicum*).

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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