Pak. J. Agri. Sci., Vol. 58(X), 975-984;2021
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI: 10.21162/PAKJAS/21.1217
http://www.pakjas.com.pk

COMPARATIVE EFFICACY OF VARIOUS NITROGEN FERTILIZERS IN IMPROVING GROWTH OF CLUSTER BEAN

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Nitrogen is an essential element for plant growth and development because it has special role in various physiological and biochemical processes of plants that improve plant productivity. This study was aimed on exploring the effect of supplementation by various levels (0.4, 0.7, 1 mM) of four inorganic nitrogen fertilizers viz., ammonium sulphate (AS) ammonium nitrate (AN), ammonium chloride (AC), diammonium phosphate (DAP) and an organic nitrogen fertilizer viz., urea in improving growth and development of cluster bean (*Cyamopsis tetragonoloba* L.). Results revealed a marked increase in length, fresh and dry weights of shoot at 0.7 mM of AN, AC and AS respectively, however, DAP improved leaf area at 1 mM level. Nitrogen content in shoot and root was significantly improved by application of AS at 1 mM level. Application of AN proved very effective for improving nitrate reductase activity at 0.7 mM treatment level. All studied yield attributes (nodules fresh and dry weights, number of pods per plant and seed yield) were improved by application of DAP at 1 mM level. Chlorophyll *a*, *b,* total chlorophyll and chlorophyll/carotenoid ratio were improved by application of AS at 0.7 mM concentration, however, carotenoid contents were improved by application of AC at 0.7 mM and chlorophyll *a*/*b* ratio was improved by all nitrogen fertilizers at 0.4 mM level. Photosynthesis (A), transpiration rate (E) and sub-stomatal conductance (g_s) were improved by application of urea at 0.7 mM level, however, internal CO_2 concentration (Ci) and A/E was improved by applying DAP and AS at 0.7 mM respectively. In conclusion, application of a combination of AS and urea at 0.7 mM and DAP at 1 mM concentration is very helpful in improving growth, physiological and yield attributes of cluster bean.

Keywords: Fertilizers, growth, nitrogen, nitrate reductase activity, photosynthesis, yield.

Abbreviations: AS= Ammonium Sulfate (NH_4SO_4); AN= Ammonium nitrate (NH_4NO_3); AC= Ammonium chloride (NH_4Cl); DAP= Diammonium phosphate $(NH_4)_2HPO_4$)

INTRODUCTION

Plants require various nutrients for their optimal growth and development. Nitrogen is one of the essential mineral nutrients that improve growth, development and ultimately plant yield (Brady and Weil, 2008; Maheswari *et al*., 2017). It is most recognized component in plant biomolecules, being part of many structurally and physiologically important molecules such as proteins, nucleic acids, chlorophyll and coenzymes. It is an important component of ATP which helps cells to metabolize energy and plays a significant role in the synthesis of various plant constituents (Taiz and Zeiger, 2015).

Although nitrogen is one of the most abundant elements on earth, however, many agricultural soils are deficient of nitrogen resulting in reduction of crop yield. Nitrogen deficiency is a common nutritional problem causing chlorosis of lower leaves, stunted growth, necrosis of older leaves and ultimately reducing photosynthetic capacity of plants. Moreover, plants cannot efficiently exploit phosphorus, potassium and other elements in soil due to nitrogen deficiency (Duli *et al*., 2005; Onasanya, 2009). This deficiency can be overcome by using various nitrogen fertilizers. Nitrogen fertilizers are not being used efficiently, because a large proportion is lost to atmosphere by ammonia volatilization and denitrification, or leached into the ground or surface waters (Zhang *et al*., 2009). Proper use of nitrogen fertilizers improves and maintains soil health which is very important for sustainability of agricultural system (Zebarth *et al*., 2009; Bijay, 2018).

The application of nitrogen fertilizers improves development of roots which ultimately enhances the supply of other nutrients and water to the growing plant parts that increase photosynthetic area and dry matter accumulation (Ali *et al*., 2012). Different types of nitrogen fertilizers improve grain quality and plant yield. An adequate supply of nitrogen during early crop growth stages is important for initiation of leaves and floral primordia (Naegle *et al*., 2005). The application of nitrogen fertilizer increases shoot length, leaf area and plant yield of spinach (Gulser, 2005). Moreover, the application of an equal ratio of nitrate and ammonium as basal fertilizer and higher ratio of ammonium-nitrogen as top dressing significantly increased photosynthesis, grain-filling and final grain yield in past studies. Nitrogen supply increases water potential of leaf, chlorophyll content, CO_2 assimilation and transpiration of the flag leaf of wheat (Abera *et al*., 2017). Liu *et al.* (2004) reported that plant biomass, grain yield and water use efficiency consistently increased with application of nitrogen but decreased at highest levels. Similarly, Pillai and Naidu (1995) reported that the nitrate reductase activity (NRA) increased with increasing rate of N and P application in soybean.

Cluster bean is commonly known as guar and is mostly grown as forage in many tropical and sub-tropical countries. It has a number of medicinal properties. It acts as appetizer and has laxative, anti-ulcer, anti-secretory, hypolipidemic and hypoglycemic properties. Its beans have many types of phytochemicals such as saponins and flavonoids (Mukhtar *et al*., 2006). Cluster bean is mostly grown in tropical Asia, Africa and America. Pakistan, India and United States are among major cluster bean growing countries (Undersander *et al*., 2006).

Keeping in view the importance of fertilizer applications, it was hypothesized that supplementation of different inorganic ammonium fertilizers and urea should have differential effects in enhancing growth and development of plants. Therefore, the present study was envisaged to study the effect of AS, AN, AC, DAP and urea on various physiological, biochemical and reproductive attributes of cluster bean.

MATERIALS AND METHODS

***Plan of study and treatment application*:** Seeds of cluster bean (*Cyamopsis tetragonoloba* L. Variety BRS-99) were obtained from Agricultural Research Institute (ARI), Bahawalpur and evaluated for the relative efficiency of various nitrogen fertilizers. Five seeds were sown in polyethylene lined earthen pots (30 cm length and 25 cm diameter), containing 7.5 kg clay-loam soil. The pots were placed in a wire house under natural growth conditions with an average photoperiod (15.2 h), photosynthetic photon flux density (*PPFD* 1500 µmol m^{-2} s^{-1}), and relative humidity (RH 40-50%). Seeds were allowed to germinate and thinning was practiced fifteen days after germination to maintain two plants of approximately the same size in each pot. Plants were treated with previously optimized levels of N (0, 0.4, 0.7 and 1 mM) calculated from analar grades of ammonium sulphate [$(NH_4)_2SO_4$)] (90% pure), ammonium chloride (NH_4Cl) (95% pure), ammonium nitrate (NH_4NO_3) (80% pure), diammonium phosphate (DAP) (99% pure) and urea (91% pure). The plants were regularly irrigated with tap water according to the need and allowed to grow for 25 days for recording of various growth and physiological characteristics while tagged plants were grown to maturity for the determination of yield attributes.

***Soil analysis*:** Soil characteristics in relation to changes in soil pH and ECe were analyzed before sowing and after harvesting by using the following methods: 21a for pH, 27a for saturation percentage, and 3a and 4b for electrical conductivity (ECe) as described by USDA in Agriculture HandBook No. 60 (Richard, 1954) (Table 1). The oven dried (at 72 ºC) soil samples were used for the determination of saturation percentage; texture and nitrogen contents in acid digested material (Wolf, 1982) by Micro-Kjeldahl method (Bremner, 1965) prior to sowing.

Table 1. Soil analysis showing changes in pH and EC before sowing and after harvest

Treatments	Conc. (mM)	pH (after harvest)	EC ds m^{-1} (after harvest)
Control	0.0	7.53	1.82
AS	0.4	7.40	1.77
	0.7	7.38	1.83
	1.0	7.36	1.86
AN	0.4	7.48	1.71
	0.7	7.42	1.81
	1.0	7.41	1.76
AC	0.4	7.44	1.85
	0.7	7.41	1.88
	1.0	7.41	1.92
DAP	0.4	7.54	1.68
	0.7	7.41	1.75
	1.0	7.35	1.84
Urea	0.4	7.46	1.82
	0.7	7.45	1.76
	1.0	7.45	1.71

Saturation percentage: 39%; Texture: Silty loam; NO_3-N: 4.68 mg kg^{-1}, pH before sowing: 7.78; ECe before sowing: 1.88 ds m^{-1}

***Growth attributes*:** Data for relative growth rate was started from the day of treatment at 14-day interval. Plants of approximately same height were selected and tagged. The height was measured in centimeters with the help of measuring tape from the base of stem to shoot apex. Relative growth rate (RGR) (g day^{-1}) was calculated using Radford's formula (1967). The leaf area of plants in each treatment was calculated following the method of Carleton and Foote (1965). Plants were uprooted carefully, and soil from the roots was removed by gentle washing of water, avoiding damage to the root system and nodules of plants. Plant height was determined from the base to the shoot tip. Shoots and roots were separated and used for the determination of shoot fresh weights. For the determination of dry weight, the harvested plants were wrapped in paper bags and oven dried at 65 ºC for 72 h to constant dry weight. The nodules formed on the root system were detached and their fresh and dry weights were determined.

***Yield attributes*:** The seeds formed in all pods in each plant were separated and yield attributes were determined by counting the total number of pods formed on each plant and yield per plant was calculated.

Nitrogen contents: The oven dried leaf, shoot and root samples were used for the determination of nitrogen content. Samples were grinded separately to pass through a 2 mm sieve. The dried material (0.5 g) was digested with sulphuric acid and hydrogen peroxide, according to the method of Wolf (1982) and nitrogen was estimated by Kjeldahl method following Bremner (1965) using micro-Kjeldahl ammonia distillation unit (Behr Labor-Technik GmbH, Behrotest Inkjel, Germany).

***Nitrate reductase activity*:** Nitrate reductase activity was estimated according to the method of Sym (1984). A 5 mL of 0.02 M phosphate buffer [(pH 7) containing 0.02 M KNO_3] was added to finely cut fresh leaf segments (0.3 g) and incubated in the dark at 32 °C for 1 h. 1 mL of the above extract was taken and 0.5 ml reagent (1% sulphanilamide in 3*N* HCl) and 0.5 mL reagent mixture [0.02% N (1-Naphthyl)-ethylene diamine dihydrochloride] was added. The samples were incubated at room temperature for 20 minutes. Then 5 mL of distilled water was added to dilute the color of the samples. Optical density was read at 542 nm using spectrophotometer (Hitachi, Model-U 2001, Japan). The nitrate reductase activity was represented as µmol NO_2 h^{-1} g^{-1} fresh weight of leaves.

***Photosynthetic pigments*:** Chlorophyll and carotenoid determination was carried by the method as described by Arnon (1949) and Davis (1976), respectively. 0.2 g of fresh leaves was chopped into small pieces and extracted with 80% acetone. The extract was examined for absorbance read at 645 nm and 663 nm (for chl. *a*, *b* and total chlorophyll) and at 480 nm (for carotenoids) by using a double beam spectrophotometer (Hitachi, Model-U 2001, Japan). These observations were used for the calculations for chl. *a*, chl. *b*, total chl. and carotenoids.

***Photosynthetic and gas exchange attributes*:** Data for photosynthetic and gas exchange attributes including CO_2 assimilation rate (A) (µmol m^{-2} s^{-1}), transpiration rate (E) (mmol m^{-2} s^{-1}), stomatal conductance (g_s) (mmol m^{-2} s^{-1}), sub-stomatal conductance (C_i) (µmol mol^{-1}), water use efficiency (A/E) (µmol CO_2/mmol H_2O) was determined by using Open System LCA-4 ADC Portable Infrared Gas Analyzer (IRGA) (Analytical Development Company, Hoddeson, England). All the measurements were made from 10 a.m. to 12 a.m. on 2nd leaf from top of each plant with the following specification/adjustment of equipment: leaf chamber area 6.25 cm^2, ambient CO_2 concentration (C_{ref}) 290.1 µmole $mole^{-1}$, temperature of leaf chamber (Tch) varied from 41 to 43.8° C, leaf chamber gas flow rate (V) 394 mL min^{-1}, leaf chamber gas flow rate (U) 256.6 µ mol s^{-1}, ambient pressure (P) 98.9 k Pa, water vapour pressure (e_{ref}) into chamber ranged from 4.4 to 6.6 mbar, molar flow of air per unit leaf area (Us) 410.6 mol m^{-2} s^{-1}, PAR (Q leaf) at the leaf surface was maximum up to 1948 µmol m^{-2} s^{-1}.

***Statistical analysis*:** The experiment was arranged in completely randomized design with three replicates. The data so collected for all the above mentioned characteristics was subjected to a two-way analysis of variance and mean values were compared with the least significance difference (LSD) test at $P \leq 0.05$ with a COSTAT computer package (COHORT Software, 2003, Monterey, California). Statisix 8.1 was used to find the difference in treatments (Steel *et al*., 1996).

RESULTS

The results revealed that with an increase in rate of nitrogen treatment, relative growth rate was increased at 0.7 mM after which a slight decrease was noted at the highest level (1 mM) for all nitrogen sources. During the 1st interval, maximum growth rate was observed at 0.7 mM of DAP showing a percentage increase of 52.91% over control, whereas, 51.22% and 47. 85% increase was reported at 0.4 mM and 0.7 mM application level. The order of effectiveness was DAP > AN > AS > AC > urea. In the 2nd and 3rd intervals a similar trend was noted. Moreover relative growth rate also increased across the intervals for all sources. The relative effectiveness of various N-sources were DAP > AN > AS > urea > AC. The 3rd interval showed a similar trend as for the 1st interval and maximum growth rate was recorded for DAP at 0.7 mM level. An overall interpretation of data indicated that DAP was the most superior N source regarding increase in relative growth rate (Fig. 1).

Application of different inorganic and organic nitrogen fertilizers had a significant impact on growth attributes of cluster bean. It was observed that shoot fresh and dry weights, shoot length, and leaf area consistently increased up to 0.7 mM of AC, AS, AN and DAP respectively. Thereafter, a little reduction was observed in all these parameters at the highest level of all fertilizers. At 1 mM urea application showed least effect on leaf area in comparison with control and other fertilizers. At 0.7 mM of all fertilizer treatments, the maximum increase in leaf area was observed by DAP (144%) which was followed by AN (113%), AS (101%) and AC (77%), respectively (Fig. 2).

The application of different fertilizer treatments had a non-significant effect on yield attributes of cluster bean. Fresh and dry weights of nodules increased consistently with the increase in nitrogen fertilizer application up to 0.7 mM after which a decrease was observed in fresh and dry weight of nodules. Among all fertilizer treatments, DAP showed a maximum increase (44%) in fresh and dry weight of nodule, number of pods per plant and seed yield at 0.7 mM followed by AC (110%), AN (67%), AS (64%) and urea (67%). The similar effect was observed at the highest (1 mM) and the lowest (0.4 mM) levels of fertilizer application except for dry weight of nodules where urea showed a maximum increase (61%) at 0.4 mM (Fig. 3).

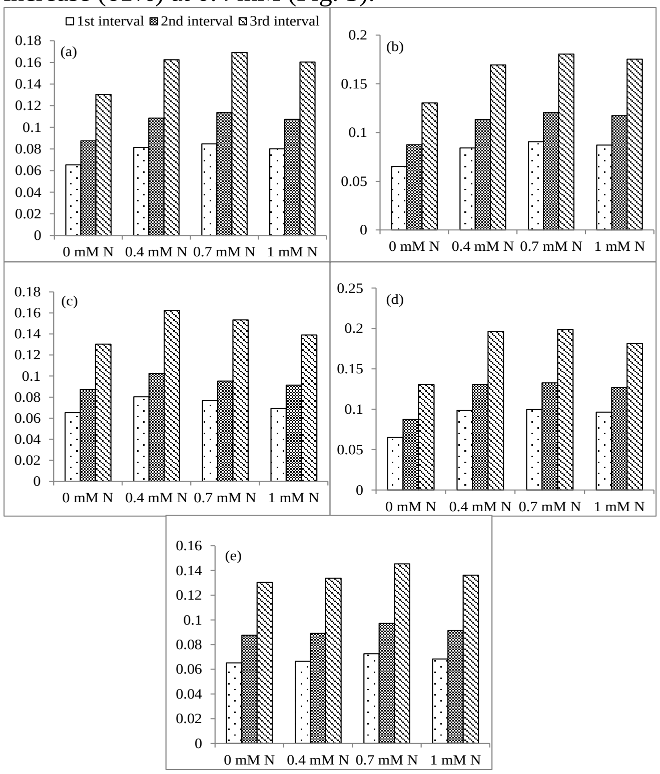

Figure 1. Relative growth rate (cm day^{-1}) of cluster bean (*Cyamopsis tetragonoloba* L.) applied with ammonium sulfate (a), ammonium nitrate (b), ammonium chloride (c), diammonium phosphate (d), and urea (e) at different intervals.

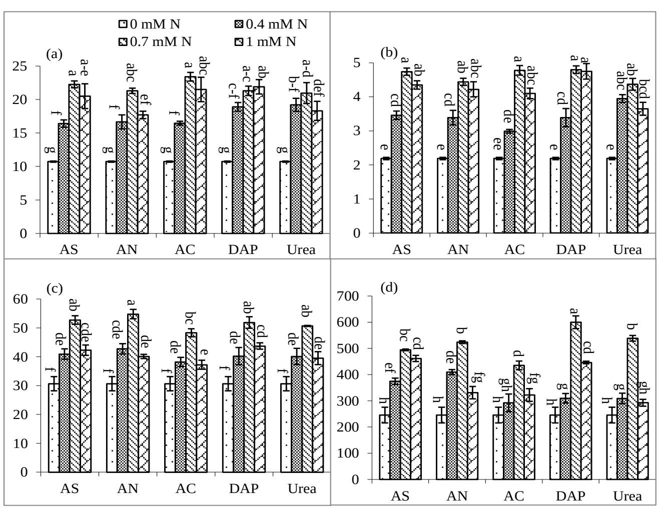

Figure 2. Effect of various concentration of nitrogen fertilizers (ammonium sulfate (AS), ammonium nitrate (AN), ammonium chloride (AC), diammonium phosphate (DAP) and urea) on shoot fresh weight (g) (a), shoot dry weight (g) (b), shoot length (cm) (c) and leaf area (cm^2) (d) of cluster bean (*Cyamopsis tetragonoloba* L.)

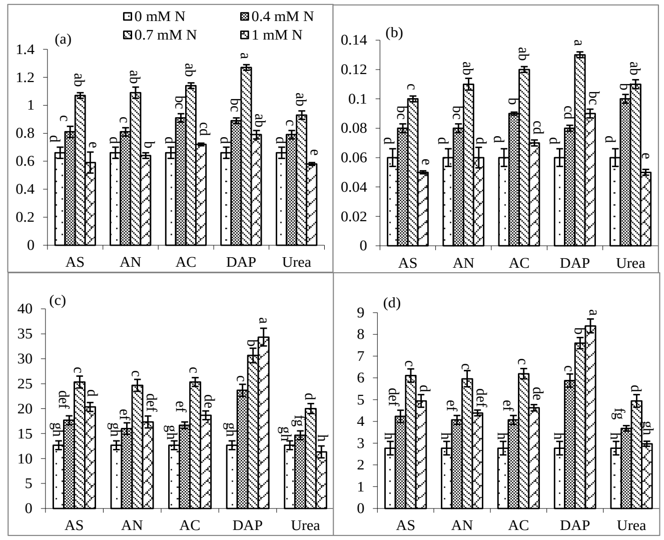

Figure 3. Effect of various concentration of nitrogen fertilizers (ammonium sulfate (AS), ammonium nitrate (AN), ammonium chloride (AC), diammonium phosphate (DAP) and urea) on fresh weight of nodule (g) (a), dry weight of nodule (g) (b), number of pods per plant (c) and seed yield (g plant^{-1}) (d) of cluster bean (*Cyamopsis tetragonoloba* L.)

At 0.7 mM, AS (120%), AC (100%), and AN (94%) showed a parallel behavior while the lowest fertilizer level (0.4 mM) had the least effect on yield parameters. In comparison to inorganic nitrogen fertilizers, application of organic nitrogen fertilizer showed least fluctuations in both these attributes at all levels of nitrogen fertilizer (Fig. 3).

Application of various levels of nitrogen fertilizers caused a significant effect on nitrogen contents in various organs (root, shoot, leaf) of cluster bean plants. Nitrogen content in roots, shoots, and leaves increased consistently with the increasing application of all nitrogen fertilizers. However, nitrate reductase activity (NRA) was increased up to 0.7 mM followed by a reduction at 1 mM (Fig. 3). The maximum increase in root, shoot, and leaves nitrogen content was observed by AS (70%), while almost parallel results were shown by DAP (55%), urea (44%), AC (52%), and AN (49%), respectively as compared to control. Nitrate reductase activity (NRA) was increased by AN at all levels of fertilizer application. The lowest fertilizer level (0.4 mM) was least effective in increasing in all recorded nitrogen assimilation attributes (Fig. 4).

Photosynthetic pigments (chlorophyll *a*, *b*, chlorophyll *a*/*b*, total chlorophyll, carotenoid and total chlorophyll /carotenoid ratio) showed a differential response to various organic and inorganic nitrogen fertilizers. Chlorophyll *a* showed consistent but non-significant rise with increasing the levels of all fertilizers as compared to control.

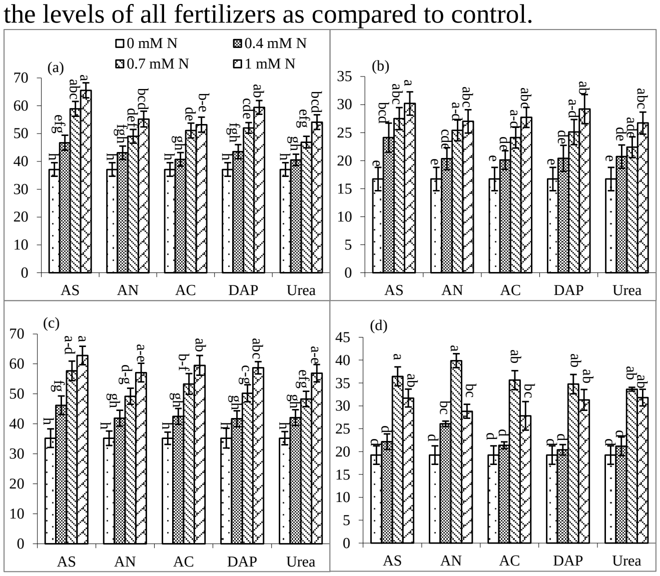

Figure 4. Effect of various concentration of nitrogen fertilizers (ammonium sulfate (AS), ammonium nitrate (AN), ammonium chloride (AC), diammonium phosphate (DAP) and urea) on root nitrogen content (mg g^{-1}) (a), shoot nitrogen content (mg g^{-1}) (b), leaf nitrogen content (mg g^{-1}) (c) and nitrate reductase activity (NRA) (mmol NO_2 h^{-1}g^{-1} f.wt.) (d) of cluster bean (*Cyamopsis tetragonoloba* L.)

The maximum rise in chlorophyll *a* contents was observed by application of AS at 0.7 mM level. Chlorophyll *b* contents were improved by a decreasing order of AS > AC > DAP > AN > urea at 0.7 mM level. Chlorophyll a/b ratio was improved by all nitrogen fertilizers at 0.4 mM level. However, total chlorophyll contents were increased by AS and urea at 0.7 mM after which there was a gradual decrease was observed. In contrast, application of organic and inorganic nitrogen fertilizers had non-significant impact on carotenoid content. Total chlorophyll/carotenoid ratio was slightly increased at all fertilizer levels as compared to control (Fig. 5a, b). Overall, the application of all studied nitrogen fertilizers improved the chlorophyll contents as compared to control. The maximum improvement in chlorophyll contents were observed at 0.7 mM of all studied nitrogen fertilizers.

The application of various types of fertilizer treatments showed a significant effect on photosynthetic and gas exchange attributes [net assimilation rate (*A*), transpiration rate (*E*), and water use efficiency (*A*/*E*)], however, stomatal conductance (g_s) and internal CO_2 concentration (C_i) were not much affected. The application of urea (0.7 mM) proved very effective in improving *A*, *E* and g_s in cluster bean. Although, the impact of other fertilizers on *E* showed non-significant effects as compared to control except for AC which showed a consistent increase even up to 1 mM level. For *A*, the application of AC as well as DAP showed a parallel increase.

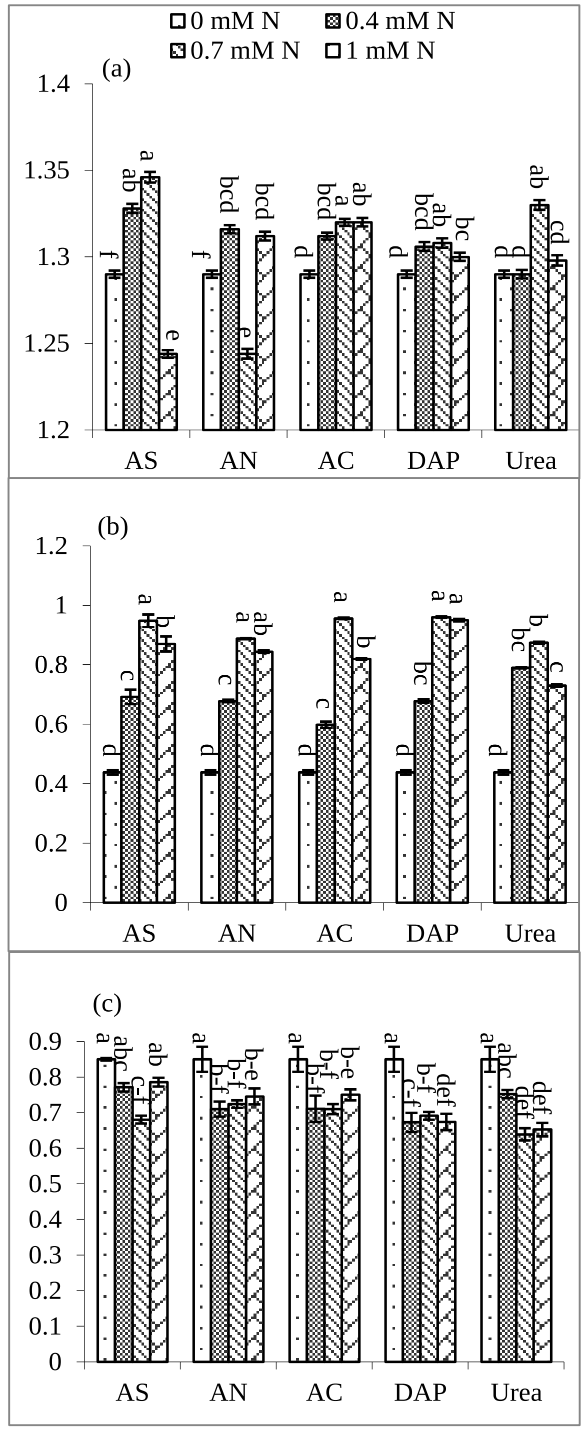

Figure 5a. Effect of various concentration of nitrogen fertilizers (ammonium sulfate (AS), ammonium nitrate (AN), ammonium chloride (AC), diammonium phosphate (DAP) and urea) on chlorophyll *a* (mg g^{-1} fresh weight) (a), chlorophyll *b* (mg g^{-1} fresh weight) (b) and chlorophyll *a/b* ratio (c) of cluster bean (*Cyamopsis tetragonoloba* L.)

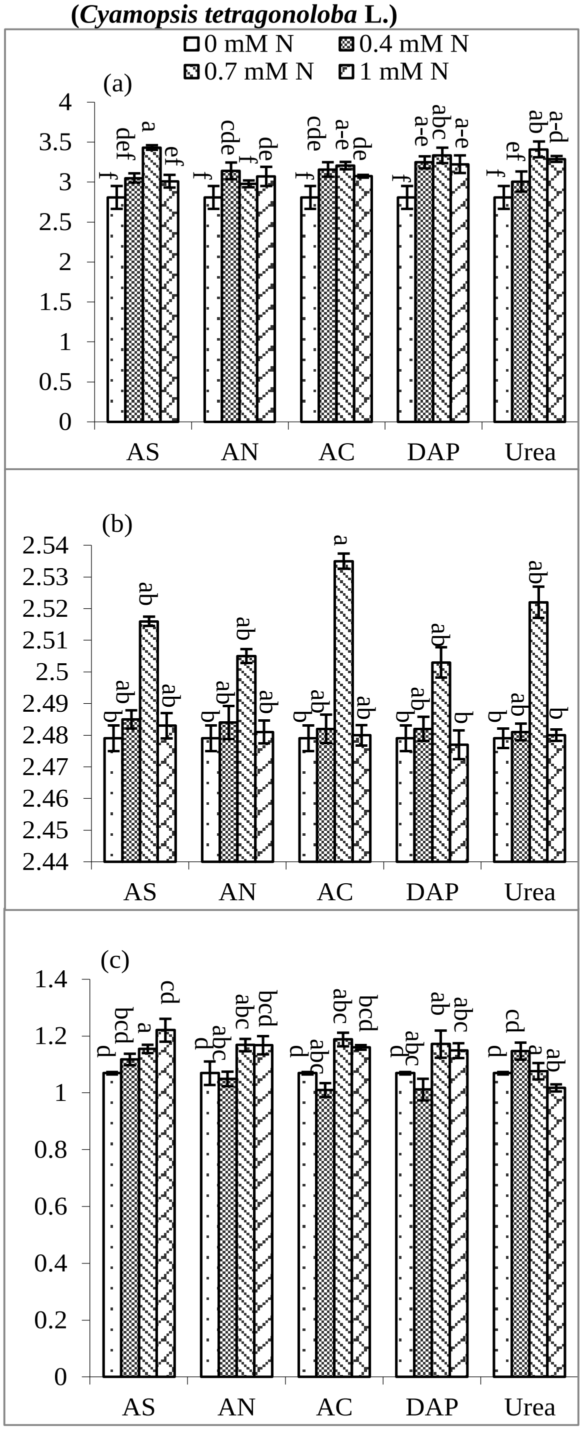

Figure 5b. Effect of various concentration of nitrogen fertilizers (ammonium sulfate (AS), ammonium nitrate (AN), ammonium chloride (AC), diammonium phosphate (DAP) and urea) on total chlorophyll (mg g^{-1} fresh weight) (a), carotenoid contents (mg g^{-1} fresh weight) (b), total chlorophyll/carotenoid ratio (c) of cluster bean (*Cyamopsis tetragonoloba* L.)

But in case of *E*, the application of AC at 0.4 mM and 1 mM levels showed approximately 10% decrease as compared to control. The C_i was highly improved by DAP at 1 mM in comparison to other applied nitrogen fertilizers. Since, the application of different fertilizer treatments showed significant effect for *A*/*E* which increased consistently up to 0.7 mM but gradually decreased at 1 mM level. Among all fertilizer treatments, the application of AS gave the highest increase and urea gave the least increase in (*A*/*E*) at 0.7 mM level. Non-significant differences were observed by AN, AC and DAP at this level as compared to control (Fig. 6).

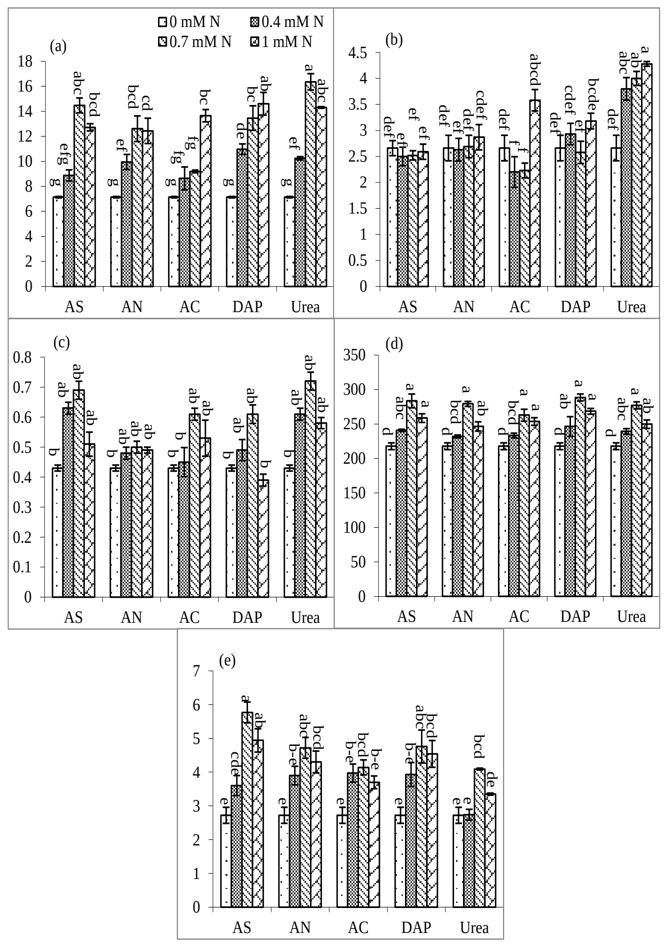

Figure 6. Effect of various concentration of nitrogen fertilizers (ammonium sulfate (AS), ammonium nitrate (AN), ammonium chloride (AC),

diammonium phosphate (DAP) and urea) on photosynthetic rate (*A*) (µmol m^{-2} s^{-1}) (a), transpiration rate (*E*) (mmol m^{-2} s^{-1}) (b), stomatal conductance (g_s) (mmol m^{-2} s^{-1}) (c), internal CO_2 concentration (C_i) (µmol mol^{-1}) (d) and photosynthesis/transpiration (*A*/*E*) ratio (mmol CO_2/mmol H_2O) (e) of cluster bean (*Cyamopsis tetragonoloba* L.)

DISCUSSION

Nitrogen is very necessary for proper growth and development of plants to meet adequate yield. However, due to various natural and anthropogenic activities, the imbalance in nitrogen contents in plants has been reported (Liu *et al*., 2014) that ultimately lead to loss of plant productivity. Present study has shown that proper application of various nitrogen fertilizers can not only improve various physio-chemical attributes of cluster bean but also improve plant yield. Results revealed that application of various fertilizer treatments had a significant effect on growth and yield attributes of cluster bean. Soil pH significantly decreased in almost all fertilizers applied here in this study. AS, AN, AC and DAP are some inorganic ammonium fertilizers, which may be applied to soils of high pH and low nutrient availability thus lowering soil pH (Table 1). This causes an increase in available nutrient content and ultimately leads to increased growth and yield of plants (Liu *et al*., 2014).

In this study, application of nitrogen fertilizers improved the cluster bean growth at 0.7 mM level. These results showed that plant height, leaf area, plant fresh and dry biomass and nodule fresh and dry weights increased consistently up to 0.7 mM after which there was a slight decrease at 1 mM level. However, the increase in nodule fresh and dry weights was non-significant at all levels (Meyer *et al*., 2019), which indicated partial positive effects of nitrogen content on plant growth at the highest level. The fertilizer application significantly affected leaf area in all fertilizer treatments, however, DAP exhibited the highest increase in leaf area. This may be due to the reason that the application of DAP, supplied phosphates as additional nutrients which may have been effective in increasing leaf area. The literature survey revealed almost similar reports on the effects of various nitrogen fertilizers on soil characteristics and plant grown under various fertilizer treatments (Lee *et al.*, 2012; Fiebig and Dodd, 2016; Meyer *et al*., 2019). The increase in these morphological parameters appeared to more encouraging for inorganic fertilizers as compared to that of conventional organic nitrogen source, i.e. urea (Chatervedi, 2005).

Different yield parameters such as number of pod and yield $plant^{-1}$ also increased significantly by various nitrogen fertilizer treatments. The highest yield was obtained at the highest level of DAP (1 mM), which may be due to the higher supply of nitrogen and phosphorous, and greater availability of micronutrients (Khurana *et al*., 2004; Ali *et al*., 2017). In comparison, all other fertilizers showed reduction at highest level i.e. 1 mM level. The inhibitory effects of excessive nitrogen at 1 mM level as observed in case of all other fertilizer treatments were not seen in DAP even at its highest level of application. This could be due to the reason that the inhibitory effects of nitrogen at this level in DAP was balanced by the additional supply of phosphorus. Similar findings were obtained for various N sources as urea, $(NH_4)_2SO_4$, NH_4NO_3 etc. by various research workers on different crops (Bationo, 2000).

The results for chlorophyll *a*, *b* and total chlorophyll indicated an increase in chlorophyll contents with increasing fertilizer level. This increase was greater up to 0.7 mM after while lesser increase was noted at 1 mM level. This increase may be due to increase in plant nitrogen content. N is an important element in the tetrapyrol structure of chlorophyll (Taiz and Zeiger, 2015). Moreover, pigment decomposition may have been delayed and photosynthetic activity extended at higher N rate (Bassi *et al*., 2018). These results are consistent with the morphological as well as yield parameters and agreed to the work of Lee *et al.* (2002). In comparison, carotenoid contents were not affected by fertilizer application. This may be due to the ability of plant to maintain carotenoid content according to their genetically inherited makeup (Karczmarczyk *et al.,* 1993).

Application of nitrogen fertilizers resulted in higher N content in leaves showing a positive correlation between leaf nitrogen content and photosynthesis as reported in many C_3 as well as C_4 species (Evans, 1989; Bassi *et al.,* 2018). Nitrogen impact on photosynthesis has also been reported to be associated with sub-stomatal conductance (g_s) and internal CO_2 concentration (*Ci*) (Guo *et al*., 2007). In present study, the application of various inorganic ammonium fertilizers and urea had no significant effect on g_s and *Ci* of cluster bean plants (Grassi *et al*., 2002). The absence of any observable effect of N on g_s is compatible to the findings and hypothesis formulated by Gassi *et al*. (2002) that N is not correlated to sub-stomatal conductance. The application of inorganic ammonium fertilizers showed low water loss through *E* as compared to urea. This seemed because of non-stomatal limitations on photosynthesis. However, the observed decrease in *E* did not result in low WUE in any of the inorganic N fertilizers because of high photosynthetic rate. Although, net CO_2 assimilation rate (*A*) and water use efficiency (WUE) increased up to 0.7 mM, after which a slight decrease was noted. This decrease at the highest level (1 mM) may be due to excessive N stress (Meuser *et al.,* 1990; Taub, 2010; Jin *et al.,* 2015).

Nitrate reductase is a key enzyme in the nitrogen metabolism of plants. Many reports shows that nitrate reductase activity decreased in degraded soils e.g. soils with high pH, salinity and waterlogging etc. (Meloni *et al.,* 2004; Ren *et al.,* 2017).

Application of inorganic nitrogen fertilizers also increased nitrate reductase activity (Venkatesan and Ganapathy, 2004). In this study, the nitrate reductase activity (NRA) increased with the increase in fertilizer application up to 0.7 mM for all fertilizers studied. This may be either due to stimulation of NRA synthesis or increase in availability of its substrate by both or all sources (Vouillot *et al*., 1996). Although, at 1 mM NRA slightly reduced, however, it remained higher than control indicating partial inhibitory effect of excessive N. The results obtained for growth and yield parameters indicated a positive correlation with nitrate reductase activity as consistent with the work of Zheng *et al*. (1995) and Moinuddin *et al*. (1996).

This study showed that application of various nitrogen fertilizers improved nitrogen contents in shoot, root and leaves of cluster bean. These results affirm the findings for nitrate reductase activity, as in treated plants, N content also increased with increase in fertilizer level as compared to control (Chamizo-Ampudia *et al.,* 2017). Increase in N content may be either due to increase in nitrate reductase synthesis or due to higher availability of substrate (Navarro *et al.,* 2003). As N is a necessary element to synthesize the basic building blocks of plant body and enzymes, higher N content increased plant growth as compared to N deficiency (Haque *et al*., 2001; Achakzai, 2006).

Conclusion: The application of nitrogen fertilizers had promising effect on physiological, nutritional and biochemical attributes of cluster bean. All studied nitrogen fertilizers at 0.7 and1 mM concentration, improved growth related attributes in cluster bean. Medium supplementation with DAP at 0.7 mM highly improved yield related parameters. Application of AS at 0.7 mM improved nitrogen contents while NRA was improved by AN (0.7 mM). Chlorophyll contents were improved by applying AS and AC at 0.7 mM level. By applying urea at 0.7 mM, A, E and g_s were highly improved, while C_i and A/E ratio was improved by DAP and AS respectively. Overall, all studied nitrogen fertilizers proved very effective in improving various studied physiological, biochemical and reproductive attributes of cluster bean. In short, AS at 0.7 mM, improved growth, nitrogen contents, chlorophyll contents Ci and A/E ratio; urea at 10 mM, improved A, E, g_s and DAP at 0.7 mM, improved all studied yield related attributes. Hence, it is recommended that AS and urea at 0.7 mM and DAP at 1mM proved very helpful in improving growth, physiological and reproductive growth of cluster bean.

Acknowledgement: This article has been extracted from M.Phil. thesis of the first author submitted to University of Agriculture, Faisalabad, Pakistan

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[Received 06 Oct 2020; Accepted 17 May. 2021; Published (online) 25 Jun 2021]