

## OPTIMIZATION OF COBALT AND NITROGEN FOR IMPROVING SEED YIELD, PROTEIN CONTENT AND NITROGEN USE EFFICIENCY IN MUNGBEAN

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### ABSTRACT

**Background** Cobalt (Co) is a beneficial element involved in the synthesis of vitamin B<sub>12</sub> in pulses while, nitrogen (N) is essential for pulses because it is the major component of amino acids and proteins. There was a need to evaluate the combined effect of Co and N application on grain yield and quality of mungbean (*Vigna radiata* L.).

**Methodology** Field experiment consisting of four levels of cobalt sulfate (0, 0.04, 0.09, 0.14 kg ha<sup>-1</sup>) and three N levels (0, 12.5, 25.0 kg ha<sup>-1</sup>) was conducted to evaluate their effects on mungbean growth and yield. The experiment was laid out in a randomized complete block design with the factorial arrangement. Mungbean variety NIAB-Mung 2011 was used as a test crop.

**Results** Significantly ( $p \leq 0.05$ ) the maximum number of nodules plant<sup>-1</sup> (12.33), pods plant<sup>-1</sup> (32.83), seeds pod<sup>-1</sup> (12.83), seed yield (1303 kg ha<sup>-1</sup>), protein (25.96%) and Co contents (108.97 ppb) were recorded in the mungbean plants supplied with cobalt sulfate @ 0.14 kg ha<sup>-1</sup> and N 25 kg ha<sup>-1</sup>. Maximum agronomic N use efficiency (34.64%) was recorded with the application of cobalt sulfate @ 0.14 kg ha<sup>-1</sup> and N 12.5 kg ha<sup>-1</sup> while, physiological N use efficiency (18.6-20.6%) was same at both levels of N (12.5 and 25 kg ha<sup>-1</sup>) application with cobalt sulfate levels of 0.04-0.09 kg ha<sup>-1</sup>. Further increase in the level of cobalt sulfate decreased the physiological N use efficiency.

**Conclusion** Integrated application of cobalt sulfate @ 0.14 kg ha<sup>-1</sup> and N 25 kg ha<sup>-1</sup> proved to be the best for improving the grain yield, protein content and N use efficiency of mungbean

### INTRODUCTION

Mungbean (*Vigna radiata* L.) is one of the best pulse crops as a protein supplement in the world. It is also a well-known pulse crop of Pakistan. However, the average yield of mungbean in Pakistan is only 661.5 kg ha<sup>-1</sup>, which is far below than its potential yield (1971 kg ha<sup>-1</sup>) (Government of Pakistan 2013). Yield loss in mungbean may be due to many physiological and management factors that are responsible for plant growth and development. The use of high yielding cultivars and balanced fertilization are important factors to enhance the yield of mungbean. Quddus et al. (2011) reported that nutrient deficiency is a major factor of lower yield of pulses including mungbean. Among plant nutrients, nitrogen (N) is the most important element for all crops including legumes because it is an integral component of amino acids and proteins (Khan et al. 2016). Moreover, the legumes are more susceptible

to micronutrients deficiency which significantly affects their production and quality (Kaisher et al. 2010).

Cobalt (Co) is not classified as an essential element, but it is usually described as beneficial (Young 1983). Cobalt is important for legume crops due to its role in vitamin B<sub>12</sub> synthesis and to fix the atmospheric N (Basu et al. 2005; Gad et al. 2011). Vitamin B<sub>12</sub> is required for the initiation of nodulation. The specific cobalamine dependent enzyme systems in *Rhizobium*, which may account for the influence of Co on nodulation and N-fixation, are methionine synthase, ribonucleotide reductase and methylmalonyl co-enzyme A mutase (Das 2000). The combination of Co and N had a positive effect on chlorophyll content, shoot growth, number of nodules, seed quality and N use efficiency (NUE) (Akbar et al. 2013). Increasing NUE and limiting the N fertilizer use are important challenges to preserve the environment, and

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improve the sustainable and productive agriculture (Daubresse et al. 2010). It has been reported that combined use of ammonium nitrate and Co increased the growth and concentration of N, phosphorus (P), potassium (K), manganese (Mn), zinc (Zn) and copper (Cu) in soybean leaves and seeds as well as oil and protein contents in seeds, and crop yield. However, Co concentration beyond 12 mg kg<sup>-1</sup> had an adverse effect on plant growth (Kandil and El-Maghraby 2016). The addition of Co saved about 75 and 33.3% of inorganic and organic N fertilizer, respectively which helps in mitigation of soil contamination and reduces the input expenditure bear by farmers (Gad 2006). Keeping in view the above-mentioned facts, a field study was conducted to determine the interactive effect of N with Co on growth, yield, nutrient uptake and NUE in mungbean.

## MATERIALS AND METHODS

### Site description

For physico-chemical analysis of experimental soil, composite and representative soil samples to a depth of 0-30 cm were obtained with soil auger before and after the experiment. The soil was analyzed for its different chemical characteristics using the techniques as illustrated by Homer and Pratt (1961). The soil was analyzed for N, P, K and Co. The soil analysis report before sowing and after harvesting of mungbean is presented in Table 1 which indicated that Co was deficient in the soil. Nitrogen and Co doses for the production of mungbean were adjusted on the basis of this analysis. The weather data of crop season were obtained from the meteorological observatory, University of Agriculture Faisalabad situated approximately 150 m away from the experimental site. The weekly mean values of

temperature, relative humidity, rainfall and pan evaporation are given in Figure 1.

### Experimental detail

The experiment was conducted at the Agronomic Research Area, University of Agriculture Faisalabad, Pakistan. The treatments comprised of four cobalt sulfate levels i.e. 0 (control), 0.04, 0.09 and 0.14 kg ha<sup>-1</sup> and three N levels i.e. 0 (control), 12.5 and 25.0 kg ha<sup>-1</sup>. The experiment was laid out in randomized complete block design in a factorial arrangement with three replications using a net plot size of 5 m x 1.8 m. Mungbean variety NIAB-Mung 2011 was used as a test crop. The crop was sown in 30 cm apart rows using a seed rate 30 kg ha<sup>-1</sup> with the help of hand drill and plant to plant distance of 10 cm was maintained by thinning. Nitrogen was applied as basal dose according to treatments. Phosphorus and potassium were applied at 57 and 62 kg ha<sup>-1</sup> in the form of triple superphosphate and muriate of potash, respectively at sowing time. Cobalt sulfate was applied as a foliar spray at anthesis stage of mungbean. In addition to rainfall received during the whole growing period of the crop, a total of three irrigations were applied at different plant growth stages till the physiological maturity of the crop. First irrigation was applied 25 days after crop sowing while subsequent irrigations were applied at flowering and seed formation stages. Hand weeding was done twice during the experimental period (25 and 45 days after sowing) to keep the field free from weeds. At appropriate growth stage, when 90% of the pods were ripened and turned yellowish brown, the crop was harvested manually. The crop was sun dried for four days in the field and then threshing was done manually.

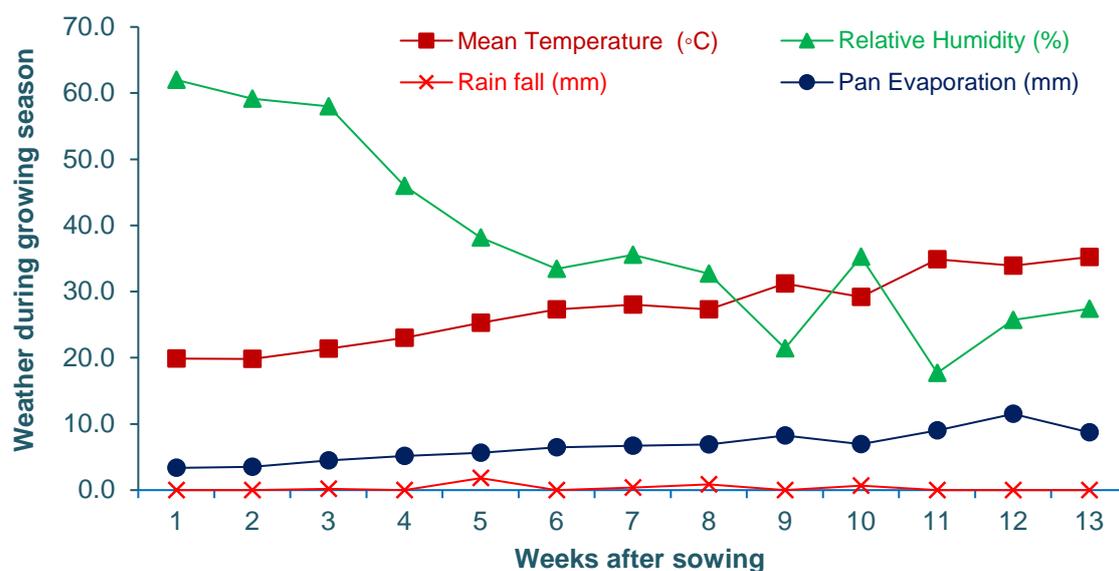


Figure 1 Weather data of mungbean growing season

**Growth and yield related traits**

To record the data of number of nodules plants<sup>-1</sup>, three plants per plot were taken out by digging their roots and then washed with water to clean them. Nodules were counted manually and their average was calculated. Ten randomly selected plants at maturity were used to determine the number of pods per plant. The pods of all ten plants were removed by hands and then counted. Average was taken to get the number of pods per plant. The removed pods of ten randomly selected plants were used for calculating number of seeds per pod. The seeds from pods were removed by hand and seeds were counted using a seed counter. Total number of seeds was divided by total number of pods to get number of seeds per pod. Two samples of 1000-seed from each treatment were taken and their weight was recorded using an electric balance and then the average was computed to get 1000-seed weight. Randomly two samples (1 m<sup>2</sup>) for grain yield were taken from each plot. Samples were sun-dried, threshed manually, and grain yield was recorded. The grain yield m<sup>-2</sup> was converted to kg per hectare.

**Table 1** Physico-chemical analysis of soil before sowing and after harvesting

| Characteristics                            | Soil analysis |              |
|--|---------------|--------------|
|  | Pre-sowing    | Post-harvest |
| Sand (%)                                   | 33.80         | 33.80        |
| Silt (%)                                   | 34.00         | 34.00        |
| Clay (%)                                   | 32.20         | 32.20        |
| Textural class                             | Loam          |              |
| Saturation (%)                             | 37            | 37           |
| SAR (mmol L <sup>-1</sup> ) <sup>1/2</sup> | 6.0           | 6.0          |
| ESP  | 7.0           | 7.0          |
| pH   | 8.0           | 8.0          |
| EC (dS m <sup>-1</sup> )                   | 1.2           | 1.2          |
| Organic matter (%)                         | 0.78          | 0.89         |
| Total nitrogen (%)                         | 0.048         | 0.60         |
| Available P (mg kg <sup>-1</sup> )         | 8.8           | 9.2          |
| Available K (mg kg <sup>-1</sup> )         | 130           | 135          |
| CaCO <sub>3</sub> (%)                      | 5.0           | 4.8          |
| Available Co (%)                           | 0.03          | 0.04         |

**Seed nitrogen and protein content**

One gram of oven dried plant material was taken in a digestion flask, 30 mL of concentrated H<sub>2</sub>SO<sub>4</sub> and 5 g of digestion mixture [K<sub>2</sub>SO<sub>4</sub>: FeSO<sub>4</sub>: CuSO<sub>4</sub> (20: 2: 1)] was added, and then the material was digested in a digestion chamber at 400 °C for 2-3 hours. The digestion mixture was cooled down and dilution was made with distilled water in 250 mL volumetric flask. Distillation was done taking 10 mL of diluted samples in Kjeldahl’s apparatus and N evolved as NH<sub>3</sub> gas was collected in receiver containing 2% H<sub>3</sub>BO<sub>3</sub> solution with mixed indicator and this was titrated against standard 0.1 N H<sub>2</sub>SO<sub>4</sub> till golden yellow color (Barbano et al. 1990) Nitrogen percentage was calculated by following formula:

$$\text{Nitrogen (\%)} = \frac{(V1 - V2)N}{100 w} \times 100 \times 14$$

Where

V1= Sample titration (mL)

V2= Blank titration (mL)

N= Normality of standardized H<sub>2</sub>SO<sub>4</sub>

W= 100 (sample weight) (g)

Then N% was multiplied by 6.25 to get crude protein percentage.

$$\text{Crude protein (\%)} = \text{Nitrogen (\%)} \times 6.25$$

Where 6.25 = Protein conversion factor

**Leaf cobalt content**

A 0.5 g sample of ground plant material was taken in 30-50 mL porcelain crucibles. Porcelain crucibles were placed in a cool muffle furnace and the temperature was increased gradually up to 550°C. Ashing was continued for 5 hours after attaining 550°C then muffle was shut off, the sample was taken out when cooled. The cooled ash was dissolved in 5 mL of 2 N hydrochloric acid and mixed thoroughly by a plastic rod. The digest was diluted, filtered through Whatman No. 42 filter paper and made up to 50 mL volume. The solution was directly aspirated to an Atomic Absorption Spectro-photometer, with air/ acetylene flame for estimating Co concentration in leaves of plants (Champan and Pratt 1961).

**Plant nitrogen use efficiency**

Agronomic and physiological NUEs were calculated by the following formulae:

$$\text{Agronomic NUE} = \frac{\text{Grain yield F} - \text{Grain yield C}}{\text{Fertilizer nutrients applied}} \text{ kg kg}^{-1}$$

Where

Grain yield F = Grain yield with N fertilizer application

Grain yield C = Grain yield of control plot (without N fertilizer application)

$$\text{Physiological NUE} = \frac{\text{Grain yield F} - \text{Grain yield C}}{\text{N uptake F} - \text{N uptake C}} \text{ kg kg}^{-1}$$

Where

Grain yield F = Grain yield with N fertilizer application

Grain yield C = Grain yield of control plot (without N fertilizer application)

N uptake F = Total N uptake with N fertilizer application

N uptake C = Total N uptake without N fertilizer application

**Statistical analysis**

All the data collected were analyzed statistically using Fisher’s analysis of variance technique and least significant difference (LSD) test was used to compare differences among treatments means at 0.05 probability level (Steel et al. 1997).

**RESULTS AND DISCUSSION**

**Number of nodules plant<sup>-1</sup>**

A higher number of nodules (11.83-12.33 plant<sup>-1</sup>) was recorded in mungbean plants treated with higher dose of N (25 kg ha<sup>-1</sup>) and nodules formation was

increased with the increasing level of cobalt sulfate (Table 2). While, the lowest number of nodules was found in control (no application of fertilizer). Increase in number of nodules by Co application might be due to increased nitrogenase and leghaemoglobin contents that promoted nodulation in mungbean. Cobalt application reduced the ethylene production and inhibitory effect of nitrate on nodulation that increased the nitrogen fixation in mungbean (Jain and Nainawatee 2000). These results are supported by the findings of Gad (2012) who stated that cobalt sulfate significantly increased the number of nodules, nodule weight and its efficiency due to enhanced nitrogenase activity, especially with both 100 and 75% rich N fertilizers in groundnut crop. Moreover, significantly ( $p \leq 0.05$ ) higher number and dry weight of nodules were observed when Co was applied at the rate of 0.21 kg ha<sup>-1</sup>. Similar research findings were obtained by Gad (2006), Mohamed et al. (2011) and Gad et al. (2011).

#### Number of pods plant<sup>-1</sup>

Number of pods plant<sup>-1</sup> is one of major yield contributing parameters which contribute towards final grain yield in legumes. The effect of different levels of cobalt sulfate and N on the number of pods plant<sup>-1</sup> of mungbean are shown by the data presented in Table 2. The results of the current study showed that interaction between Co and N was found significant for number of pods plant<sup>-1</sup>. The application of cobalt sulfate and N at the rate of 0.14 kg ha<sup>-1</sup> and 25 kg ha<sup>-1</sup>, respectively gave the maximum number of pods plant<sup>-1</sup> (32.83) and it was statistically at par with that observed with the application of 0.09 kg ha<sup>-1</sup> cobalt sulfate and 25 kg ha<sup>-1</sup> N (31.47). The minimum number of pods plant<sup>-1</sup> (18.53) was recorded in control plot (no application of cobalt sulfate and N). Gad (2006) reported that Co application at 8 ppm increased the number of pods plant<sup>-1</sup> and also increased the uptake efficiency of inorganic N fertilizers (NH<sub>4</sub>NO<sub>3</sub> and urea). The

combination of Co, molybdenum (Mo) and N had an opposite effect on chlorophyll and number of pods plant<sup>-1</sup> of groundnut crop (Mohamed et al. 2011).

#### Number of seeds pod<sup>-1</sup>

It is a key factor towards the yield of leguminous crops. The results concerning to the effect of different levels of cobalt sulfate and N on the number of seeds pod<sup>-1</sup> of mungbean are presented in Table 2 which showed that this parameter was significantly influenced by cobalt sulfate and N interaction. The number of seeds pod<sup>-1</sup> was increased with increasing level of cobalt sulfate and N. The highest number of seeds pod<sup>-1</sup> (12.83) was observed in plots where cobalt sulfate and N were applied at the rate of 0.14 kg ha<sup>-1</sup> and 25 kg ha<sup>-1</sup>, respectively. The lowest number of seeds pod<sup>-1</sup> (7.96) was recorded in the plot where cobalt sulfate was applied at the rate of 0.04 kg ha<sup>-1</sup> and no N was applied. According to Hanan et al. (2012), the combined application of cobalt sulfate and N increased the number of seeds and thus yield of cowpea. Similar research findings were obtained by Pattanyak et al. (2000) on mungbean, Junior et al. (2008) on common bean and Gad et al. (2011) on tomato. The reason was that Co works as a cofactor of cobalamine (Vitamin B<sub>12</sub>) which functions as a coenzyme in N<sub>2</sub> fixation and nodule growth (Licht et al. 1996; Jordan and Reichard 1998) and thus enhances the efficiency of N and other primary nutrients, leading ultimately to increase seeds per pod of mungbean.

#### 1000-seed weight

The data pertaining to the effect of different levels of cobalt sulfate and N application on 1000-seed weight of mungbean are presented in Table 2 which showed the significant effect of treatments on the parameter under discussion. The maximum 1000-seed weight (70.87 g) was recorded with the application of cobalt sulfate and N at the rate of 0.14

**Table 2** Interactive effect of cobalt sulfate and N levels on yield parameters of mungbean (*Vigna radiata* L.)

| Treatments                     | Number of nodules plant <sup>-1</sup> | Number of pods plant <sup>-1</sup> | Number of seeds pod <sup>-1</sup> | 1000-seed weight (g) | Seed yield (kg ha <sup>-1</sup> ) |
|--------------------------------|---------------------------------------|------------------------------------|-----------------------------------|----------------------|-----------------------------------|
| N <sub>0</sub> CS <sub>0</sub> | 5.10f                                 | 18.53i                             | 6.77h                             | 47.73f               | 678.30k                           |
| N <sub>0</sub> CS <sub>1</sub> | 5.97f                                 | 20.27h                             | 7.96g                             | 52.57ef              | 712.30j                           |
| N <sub>0</sub> CS <sub>2</sub> | 8.37e                                 | 21.50gh                            | 8.43fg                            | 57.70de              | 719.30j                           |
| N <sub>0</sub> CS <sub>3</sub> | 10.10c                                | 22.57fg                            | 9.00ef                            | 64.26bc              | 790.30h                           |
| N <sub>1</sub> CS <sub>0</sub> | 8.63de                                | 23.33f                             | 9.97cde                           | 62.50cd              | 742.30i                           |
| N <sub>1</sub> CS <sub>1</sub> | 9.67cde                               | 24.93e                             | 9.83cde                           | 65.33abc             | 888.00g                           |
| N <sub>1</sub> CS <sub>2</sub> | 9.87cd                                | 26.70cd                            | 10.10cd                           | 66.80abc             | 1044.0e                           |
| N <sub>1</sub> CS <sub>3</sub> | 10.57bc                               | 25.57de                            | 10.83bc                           | 70.87a               | 1111.30d                          |
| N <sub>2</sub> CS <sub>0</sub> | 9.53cde                               | 27.93bc                            | 9.57de                            | 67.37abc             | 980.3f                            |
| N <sub>2</sub> CS <sub>1</sub> | 11.83ab                               | 28.97bc                            | 10.73bc                           | 69.80ab              | 1177.00c                          |
| N <sub>2</sub> CS <sub>2</sub> | 11.83ab                               | 31.47a                             | 11.63b                            | 69.96ab              | 1234.00b                          |
| N <sub>2</sub> CS <sub>3</sub> | 12.33a                                | 32.83a                             | 12.83a                            | 66.30abc             | 1303.30a                          |
| LSD value                      | 1.375                                 | 1.383                              | 1.002                             | 5.738                | 8.547                             |

Means not sharing the same letters differ significantly at 5% probability level. N = Nitrogen levels (kg ha<sup>-1</sup>), CS = Cobalt sulfate levels (kg ha<sup>-1</sup>). N<sub>0</sub>= N at 0.00 kg ha<sup>-1</sup>, N<sub>1</sub>= N at 12.5 kg ha<sup>-1</sup>, N<sub>2</sub>= N at 25.0 kg ha<sup>-1</sup>, CS<sub>0</sub>= Cobalt sulfate at 0.00 kg ha<sup>-1</sup>, CS<sub>1</sub>= Cobalt sulfate at 0.04 kg ha<sup>-1</sup>, CS<sub>2</sub>= Cobalt sulfate at 0.09 kg ha<sup>-1</sup>, CS<sub>3</sub>= Cobalt sulfate at 0.14 kg ha<sup>-1</sup>.

kg ha<sup>-1</sup> and 12.5 kg ha<sup>-1</sup>, respectively followed by application of cobalt sulfate and N at the rate of 0.09 kg ha<sup>-1</sup> + 25 kg ha<sup>-1</sup>. Barbo et al. (2009) reported that application of Co and Mo resulted in significant increase in seed weight in different cultivars of soybean. The enhanced seed weight by Co application might be the result of improved efficiency of macro and micronutrients uptake by the plants and thus vegetative and reproductive growth. Cobalt also reduced the titrate-able acidity which increased the seed weight (Gad 2005).

**Seed yield**

Nitrogen and cobalt sulfate application had significant interactive effects on seed yield of mungbean. Maximum mungbean seed yield (1303.3 kg ha<sup>-1</sup>) was recorded in the plots treated with cobalt sulfate at the rate of 0.14 kg ha<sup>-1</sup> and N at the rate of 25 kg ha<sup>-1</sup> (Table 2). The minimum yield was observed in control plots. The results of the current study are supported by the findings of Gad (2006) that cobalt sulfate applied at the rate 8 ppm along with N fertilizer increased the number of pod-bearing branches, number of nodules and seed yield of pea plants. Kandil (2007) studied the influence of different levels of Co viz. 0, 5, 15 and 20 ppm on faba bean plants and revealed that greatest increase in growth, yield and nutrients uptake occurred by using 20 ppm Co. Junior et al. (2008) evaluated the efficiency of N-fixing inoculum associated with Co and Mo spray on the common bean grain yield and grain nutrients, and showed that Co and Mo spray at higher doses increased grain yield. Awomi et al. (2012) reported that P, Co and Mo fertilization is needed for proper growth, yield and quality of legumes.

**Seed nitrogen content**

The analyzed data showed that interaction of cobalt sulfate and N was non-significant while the individual effect of cobalt sulfate on N content (%) of mungbean was significant. The results showed that the highest level of cobalt sulfate and N gained the highest seed N content of mungbean (3.59 and 3.61%, respectively) (Table 3). These results are in harmony with those obtained by Kandil et al. (2013) who reported that Co is an essential element for the growth of rhizobium, the specific bacteria involved in legume nodulation and fixation of atmospheric N into amino acids in legumes. Cobalt enhanced the N<sub>2</sub> fixation process in legumes and consequently increased plant N content (Gad 2006). Cobalt is an essential element for legumes due to bacteria that live in root nodules and synthesize vitamin B<sub>12</sub> which is required for the microorganisms fixing N in nodules and nitrogenase activity in mungbean (Basu et al. 2005).

**Seed protein content**

The results of the current study revealed that interaction of cobalt sulfate and N levels remained

significant ( $p \leq 0.05$ ) regarding protein contents of mungbean. The result revealed that increased level of cobalt sulfate and N increased the protein content of seed (Table 4). The maximum protein content (25.96%) was recorded with the highest level of cobalt sulfate (0.14 kg ha<sup>-1</sup>) and N (25 kg ha<sup>-1</sup>). Minimum protein content (14.88%) was recorded with control (no application of cobalt sulfate and N). Basu et al. (2005) reported that Co and N application improved protein content in groundnut. Another study on groundnut revealed that Co addition in plant media increased protein, total soluble solids, total carbohydrates and total soluble sugars in groundnut seeds (Gad 2012). According to Sarada and Polasa (1992), and Gad (2012), Co is an essential element for the growth of rhizobium that fixes atmospheric N into amino acids and N markedly increased seed proteins in legumes. Finally, Co is an essential element for legumes due to its requirement for the microorganisms fixing N in nodules and nitrogenase activity in mungbean. Therefore, Co application increased the grain protein content of mungbean in current study.

**Leaf cobalt content**

In our study, application of cobalt sulfate and N at the rate of 0.14 kg ha<sup>-1</sup> and 25 kg ha<sup>-1</sup>, respectively showed maximum Co content (108.97 ppb) in mungbean leaves and it was statistically at par with that observed with 0.14 kg ha<sup>-1</sup> and 0.0 kg ha<sup>-1</sup> of cobalt sulfate and N, respectively. While, the minimum Co contents (11.63 ppb) were recorded in control (no application of cobalt sulfate and N) treatment. The results obtained in the current study are in good agreement with those obtained by Gad et al. (2011). From these results, it could be concluded that increased Co levels in plant media increased Co content in mungbean plants compared with control.

**Table 3** Effect of cobalt sulfate and N levels on seed N content of mungbean (*Vigna radiata* L.)

| Treatments                      | N content (%) |
|---------------------------------|---------------|
| Factor A: Cobalt sulfate levels |               |
| CS <sub>0</sub>                 | 2.82d         |
| CS <sub>1</sub>                 | 3.07c         |
| CS <sub>2</sub>                 | 3.29b         |
| CS <sub>3</sub>                 | 3.59a         |
| LSD value ( $p \leq 0.05$ )     | 0.110         |
| Factor B: Nitrogen levels (N)   |               |
| N <sub>0</sub>                  | 2.73c         |
| N <sub>1</sub>                  | 3.25b         |
| N <sub>2</sub>                  | 3.61a         |
| LSD value ( $p \leq 0.05$ )     | 0.096         |

Means not sharing the same letters differ significantly at 5% probability level. N = Nitrogen levels (kg ha<sup>-1</sup>), CS = Cobalt sulfate levels (kg ha<sup>-1</sup>). N<sub>0</sub> = N at 0.00 kg ha<sup>-1</sup>, N<sub>1</sub> = N at 12.5 kg ha<sup>-1</sup>, N<sub>2</sub> = N at 25.0 kg ha<sup>-1</sup>, CS<sub>0</sub> = Cobalt sulfate at 0.00 kg ha<sup>-1</sup>, CS<sub>1</sub> = Cobalt sulfate 0.04 kg ha<sup>-1</sup>, CS<sub>2</sub> = Cobalt sulfate at 0.09 kg ha<sup>-1</sup>, CS<sub>3</sub> = Cobalt sulfate at 0.14 kg ha<sup>-1</sup>.

**Nitrogen use efficiency**

Agronomic NUE (ANUE) is used to describe the capability of yield increase per kilogram N applied. The maximum ANUE (34.64 %) was recorded with the medium level of N (12.5 kg ha<sup>-1</sup>) in combination with the highest level of cobalt sulfate (0.14 kg ha<sup>-1</sup>). However, a further increase in N application rate in combination with all the three cobalt sulfate levels reduced ANUE (Table 4). A significant increase in physiological NUE (PNUE), the plant N uptake per unit of applied N occurred by Co application in response to increasing N level from 0 to 12.5 kg ha<sup>-1</sup>. However, further increase in N application rate could not improve PNUE (Table 4). Even, the highest N dose (25 kg ha<sup>-1</sup>) along with maximum cobalt sulfate level resulted in reduced PNUE. Maximum PNUE (20.63%) was observed with the application of cobalt sulfate at 0.04 kg ha<sup>-1</sup> and N 25 kg ha<sup>-1</sup>. Both N and Co increased the mass of nodulation and N fixation that increased the NUE. These findings have also been supported by Gad et al. (2011), Akbar et al. (2013), Gad (2013), and

Kandil (2013). A previous study showed that Co treatments increased all the tested growth parameters, nutrient uptake and biochemical attributes in canola seeds as compared to control plants (Gad et al. 2011).

**CONCLUSION**

Application of cobalt sulfate at the rate of 0.14 kg ha<sup>-1</sup> and N at 25 kg ha<sup>-1</sup> is the best combination for obtaining higher grain yield of mungbean along with good protein content and increased NUE under the agro-climatic conditions of Faisalabad, Pakistan. This treatment also showed maximum benefit cost ratio of 2.03.

**REFERENCES**

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**Table 4** Effect of cobalt sulfate and N levels on protein contents, cobalt concentration and NUE of mungbean (*Vigna radiata* L.)

| Treatments                     | Seed protein content (%) | Leaf Co content (ppb) | Agronomic NUE (kg kg <sup>-1</sup> ) | Physiological NUE (kg kg <sup>-1</sup> ) |
|--------------------------------|--------------------------|-----------------------|--------------------------------------|--|
| N <sub>0</sub> CS <sub>0</sub> | 14.88h                   | 11.63i                | 0.00i                                | 0.00d                                    |
| N <sub>0</sub> CS <sub>1</sub> | 16.92g                   | 64.70g                | 0.00i                                | 0.00d                                    |
| N <sub>0</sub> CS <sub>2</sub> | 17.79fg                  | 89.21d                | 0.00i                                | 0.00d                                    |
| N <sub>0</sub> CS <sub>3</sub> | 18.67ef                  | 106.89ab              | 0.00i                                | 0.00d                                    |
| N <sub>1</sub> CS <sub>0</sub> | 18.09fg                  | 13.06i                | 5.12h                                | 13.58c                                   |
| N <sub>1</sub> CS <sub>1</sub> | 19.55de                  | 68.51f                | 16.77g                               | 20.32a                                   |
| N <sub>1</sub> CS <sub>2</sub> | 20.71cd                  | 92.23c                | 29.25b                               | 20.06a                                   |
| N <sub>1</sub> CS <sub>3</sub> | 22.75b                   | 105.13b               | 34.64a                               | 18.58ab                                  |
| N <sub>2</sub> CS <sub>0</sub> | 19.84cde                 | 12.60i                | 24.16d                               | 19.39a                                   |
| N <sub>2</sub> CS <sub>1</sub> | 21.00c                   | 58.51h                | 19.95f                               | 20.63a                                   |
| N <sub>2</sub> CS <sub>2</sub> | 23.33b                   | 82.98e                | 22.23e                               | 18.59ab                                  |
| N <sub>2</sub> CS <sub>3</sub> | 25.96a                   | 108.97a               | 25.00c                               | 16.72b                                   |
| LSD value                      | 1.194                    | 2.876                 | 0.439                                | 2.952                                    |

Means not sharing the same letters differ significantly at 5% probability level. ppb = parts per billion, N = Nitrogen levels (kg ha<sup>-1</sup>), CS = Cobalt sulfate levels (kg ha<sup>-1</sup>); N<sub>0</sub> = N at 0.00 kg ha<sup>-1</sup>, N<sub>1</sub> = N at 12.5 kg ha<sup>-1</sup>, N<sub>2</sub> = N at 25.0 kg ha<sup>-1</sup>, CS<sub>0</sub> = Cobalt sulfate at 0.00 kg ha<sup>-1</sup>, CS<sub>1</sub> = Cobalt sulfate at 0.04 kg ha<sup>-1</sup>, CS<sub>2</sub> = Cobalt sulfate at 0.09 kg ha<sup>-1</sup>, CS<sub>3</sub> = Cobalt sulfate at 0.14 kg ha<sup>-1</sup>.

**Table 5** Effect of cobalt sulfate and nitrogen levels on benefit cost ratio (BCR) of mungbean (*Vigna radiata* L.)

| Treatments | Total expenditure (Rs. ha <sup>-1</sup> ) | Total Income (Rs. ha <sup>-1</sup> ) | Net income (Rs. ha <sup>-1</sup> ) | BCR  |
|------------|---|--------------------------------------|------------------------------------|------|
| N0CS0      | 53599.0                                   | 61050.0                              | 7451.0                             | 1.13 |
| N0CS1      | 54515.4                                   | 64110.0                              | 9594.6                             | 1.18 |
| N0CS2      | 54945.5                                   | 64740.0                              | 9794.5                             | 1.18 |
| N0CS3      | 55019.8                                   | 71130.0                              | 16110.2                            | 1.29 |
| N1CS0      | 54625.0                                   | 66810.0                              | 12185.0                            | 1.22 |
| N1CS1      | 55541.4                                   | 79920.0                              | 24378.6                            | 1.44 |
| N1CS2      | 55971.0                                   | 93960.0                              | 37989.0                            | 1.68 |
| N1CS3      | 56539.8                                   | 100020.0                             | 43480.2                            | 1.77 |
| N2CS0      | 55670.0                                   | 88230.0                              | 32560.0                            | 1.58 |
| N2CS1      | 56586.4                                   | 105930.0                             | 49343.6                            | 1.87 |
| N2CS2      | 57016.5                                   | 111060.0                             | 54043.5                            | 1.95 |
| N2CS3      | 57584.8                                   | 117300.0                             | 59715.2                            | 2.03 |

N = Nitrogen levels (kg ha<sup>-1</sup>), CS = Cobalt sulfate levels (kg ha<sup>-1</sup>); N<sub>0</sub> = N at 0.00 kg ha<sup>-1</sup>, N<sub>1</sub> = N at 12.5 kg ha<sup>-1</sup>, N<sub>2</sub> = N at 25.0 kg ha<sup>-1</sup>, CS<sub>0</sub> = Cobalt sulfate at 0.00 kg ha<sup>-1</sup>, CS<sub>1</sub> = Cobalt sulfate at 0.04 kg ha<sup>-1</sup>, CS<sub>2</sub> = Cobalt sulfate at 0.09 kg ha<sup>-1</sup>, CS<sub>3</sub> = Cobalt sulfate at 0.14 kg ha<sup>-1</sup>.

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