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# The Impact of Boron Molybdenum and Nickel on Leghaemoglobin, nitrate reductase & urease activity and quality of blackgram (*Vigna mungo* L. Hepper)

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## Abstract

A field experiment was conducted on the Influence of Boron Molybdenum and Nickel on Biochemical traits and quality of blackgram (*Vigna mungo* L. Hepper) at Agricultural college farm, Bapatla during *Rabi* 2017-18 in randomized block design with eight treatments of micronutrient application *viz*., control ($T_1$), B ($T_2$), Mo ($T_3$), Ni ($T_4$), B+Mo ($T_5$), B+Ni ($T_6$), Mo+Ni ($T_7$) and B+Mo+Ni ($T_8$) replicated thrice. The results revealed that leghaemoglobin content in nodules, activity of urease in leaves and nodules and protein content in seed increased with B, Mo and Ni application individually and in combination. Activity of NR in leaves increased with $T_2$ to $T_8$ except Ni ($T_4$) application alone.

***Keywords:*** Blackgram; micronutrients; leghaemoglobin; nitrate reductase; urease and protein

## Background

Pulses are one of the important groups of food crops in Indian agriculture after cereals and oilseeds, which are excellent and indispensable source of protein, essential amino acids, fatty acids, fibers, minerals and vitamins. This group of crops improves soil health by enriching nitrogen status and ensures long term fertility for sustainability of the cropping systems. Black gram (*Vigna mungo* L.Hepper), an important legume crop, responds to micronutrients. Among various constraints leading to low productivity of pulses, inadequate and imbalanced plant nutrition assumes great significance. Boron, Molybdenum and Nickel have a marked effect on plants. To increase or to sustain the productivity of black gram, there is a need for application of micronutrients (B, Mo and Ni).

Boron helps in the nodulation, nitrogen fixation, protein synthesis. It is associated with meristematic activity, auxin, cell well, maintaining correct water relations within the plant and sugar translocation, Patra, P. K and Bhattacharya, C. (2009). Molybdenum is an essential component of the enzyme nitrate reductase, which catalyzes the conversion of $NO_3$ to $NO_2$ and optimum nodulation. It is also a structural component of enzyme nitrogenase which is actively involved in atmospheric $N_2$ fixation by root nodule bacteria of leguminous crops. Molybdenum acts in enzyme system which brings about oxidation-reduction reactions, especially the reduction of nitrate to ammonia prior to amino acids and protein synthesis in the cells of plant besides activator of dehydrogenases and co-factor in the synthesis of ascorbic acid. Singh et al. (2014) Nickel is an essential micronutrient for plant growth as a constituent of several metallo-enzymes such as urease, Ni deficiency in plants reduces urease activity, disturbs nitrogen assimilation, (Welch, 1991). In blackgram, the concentration of Mo in leaves and seed as well as activity of nitrate reductase was found increased with an increase in Mo supply. (Gopal et al., 2015). Naz et al. (2018) reported that application of B, Mo and Ni alone and individually helps in the increase of NRA, Urease activity and leghaemoglobin content of the plants.

## Materials and Methods

A field experiment was conducted to the impact of Boron Molybdenum and Nickel on Leghaemoglobin, nitrate reductase & urease activity and quality of blackgram (*Vigna mungo* L. Hepper) at Agricultural College Farm, Bapatla during *Rabi* season of 2017-18. The experiment consisted of 8 treatments viz., $T_1$- no micronutrient application (control), $T_2$- Borax @ 2.5 kg $ha^{-1}$, $T_3$- Ammonium molybdate @ 1.5 kg $ha^{-1}$, $T_4$- Nickel chloride @1 kg $ha_{-1}$, $T_5$- Borax @ 2.5 kg and Ammonium molybdate @ 1.5 kg $ha^{-1}$, $T_6$- Borax @ 2.5 kg and Nickel chloride @1 kg $ha^{-1}$, $T_7$- Ammonium molybdate @ 1.5 kg and Nickel chloride @1 kg $ha^{-1}$, T8- Borax @ 2.5 kg, Ammonium molybdate @ 1.5 kg and Nickel chloride @1 kg $ha^{-1}$ and applied as basal. It was laid in randomized block design and replicated thrice. The soil was neutral in reaction, low in salinity and nitrogen, medium in available phosphorus and organic carbon and very high in potassium. The standard packages of cultural practices were followed throughout crop growth period. The data on leghaemoglobin content of nodules was estimated as per the method of Appleby and Bergersen (1980) and it was calculated and expressed as μg. $g^{-1}$ FWT. To estimate nitrate reductase activity in the leaf, the method suggested by Hageman and Flesher (1960) was followed. The nitrite formed was estimated by the method described by Nicholas et al. (1976) by measuring the absorbance of the pink colour at 540 nm using spectrophotometer. For the estimation of urease enzyme we followed sodium phenate solution method. For the estimation of total proteins of seeds were determined by the method of Lowry's (1951). The data were analyzed as per the statistical procedures given by Panse and Sukhatme (1985).

## Results and Discussion

### *Leghaemoglobin Content (μg $g^{-1}$ FWT)*

Leghaemoglobin content in nodule was significantly influenced and increased by micronutrient application (Table 1). At 40 DAE, it varied from 9.3 μg to 18.0 μg, maximum leghaemoglobin (LB) content was noted in B+Mo+Ni application and minimum in control which was on par with Ni application. The next higher LB content was noted in B+Mo application which showed parity with Mo+Ni application. The next higher values were recorded in combined application of B+Ni followed by Mo+Ni application which is on parity with B+Mo application. The significantly highest value among all the treatments recorded by B+Mo+Ni application. At 60 DAE also, the LB content in micronutrient treatments increased over control, varied from 5.3 μg to 8.4 μg. The lower LB content was recorded in control and higher in $T_8$ treatment. After control the next lower value was found in $T_4$ (Ni) treatment, followed by $T_2$ (B) and T3 in the order. Combined application showed higher increase in LB than alone application. After $T_8$, the $T_5$ (B+Mo) and $T_7$ (Mo+Ni) showed increased LB content followed by $T_6$ (B+Ni).

| ***Treatments*** | ***Leghaemoglobin content (μg $g^{-1}$ FWT)*** | | ***Protein content (mg $g^{-1}$)*** |
|---|---|---|---|
| | ***40 DAE*** | ***60 DAE*** | ***In seeds*** |
| T1: Control | 9.3 | 5.3 | 203.3 |
| T2: Borax @ 2.5 Kg $ha^{-1}$ | 10.8 | 6.2 | 236.2 |
| T3: Ammonium molybdate @ 1.5Kg $ha^{-1}$ | 11.5 | 6.5 | 229.8 |
| T4: Ni $Cl_2$. $6H_2O$ @ 1.0 Kg $ha^{-1}$ | 9.9 | 5.9 | 223.0 |
| T5: Borax @ 2.5&Ammonium molybdate @ 1.5 Kg $ha^{-1}$ | 15.3 | 7.7 | 256.2 |
| T6: Borax @ 2.5 & Ni $Cl_2$. $6H_2O$ @ 1.0 Kg $ha^{-1}$ | 13.4 | 7.3 | 247.6 |
| T7: Ammonium molybdate @ 1.5 & Ni $Cl_2$. $6H_2O$ @ 1.0 Kg $ha^{-1}$ | 14.5 | 7.6 | 245.2 |
| T8: Borax @ 2.5 & Ammonium molybdate @ 1.5 & Ni $Cl_2$.$6H_2O$ @ 1.0 Kg $ha^{-1}$. | 18.0 | 8.4 | 270.1 |
| SE(m)± | 0.3 | 0.1 | 2.9 |
| CD(0.05) | 0.9 | 0.2 | 8.7 |
| CV (%) | 3.8 | 1.6 | 2.1 |

***Table 1:*** Effect of micronutrients (B, Mo & Ni) on Leghaemoglobin content (μg $g^{-1}$ FWT)and protein content (mg $g^{-1}$) in Blackgram.

The impact of these three micronutrients in enhancing LB content in nodule was more effective at 40 DAE. The treatments $T_2$ to $T_4$ increased the LB content by 16.0, 23.6 and 6.4 percent respectively. In combination treatments $T_5$ to $T_8$ LB content increased by 64.5, 44.1, 55.9 and 93.5 percent respectively. Compared to B and Ni, Mo application alone and in combination resulted in greater increase in LB content. The similar positive effect of Mo and Ni on LB content in pea and chickea was reported by Naz et al (2018).

### *Nitrate Reductase activity*

The data presented in Table 2 on nitrate reductase activity (NRA) in leaves of blackgram revealed that application of B, Mo and Ni application significantly influenced and increased the NRA. At 20 DAE, B+Ni application resulted in NRA on par with control. NRA was found maximum in B+Mo+Ni treated plants, which showed parity with other combination treatments. Mo application increased the NRA compared to control, but inferior to that found in combination treatments. At 40 DAE, NRA was found maximum in plants treated with B+Mo+Ni application followed by B+Mo, which showed parity with Mo+Ni and B+Ni application. It was minimum in control and Ni application alone. B and Mo application showed NRA on par with each other, superior to control and inferior to that in combination treatments. At 60 DAE, the influence of $T_2$ to $T_4$ on NRA was on par with control and combination treatments. The combination treatments significantly influenced the NRA over control and all were on par with each other.

To summarise, during vegetative phase (20 DAE) among individual applications ($T_2$ to $T_4$) only Mo showed the impact (4.9 %) on NRA. All combination treatments resulted in greater increase in NRA (6.8, 5.8, 6.1 and 9.9 %, respectively in $T_5$, $T_6$, $T_7$ & $T_8$). At flowering stage (40 DAE), both $T_2$ and $T_3$ showed the impact on NRA (5.6 and 6.7 %, respectively). Among combination treatments $T_8$ and $T_5$ showed greater impact (17.1 & 13.1 %, respectively) than $T_7$ and $T_6$ (11.9 & 10.4 %, respectively). At pod maturity stage (60 DAE), increase in NRA over control was noted only in combination treatments (3.9, 4.9, 7.1 & 7.8 % in $T_6$, $T_7$, $T_5$ & $T_8$ respectively). Increased nitrate reductase activity in micronutrient treated plants might be due to the enhancement of nitrogen uptake by plant, more availability of nitrate in the metabolic pool, modulation in hormones level and enhancement in the availability of trace elements and also the growth promoting substances (cytokinins) released by the microbes. These findings were positively supported by findings of Gupta et al (2015) and Anbuselvi et al (2011).

### *Urease activity (In leaves and Nodules)*

The effect of micronutrients (Table 2) on urease activity in leaves and nodules in blackgram was found significant and increased values were observed. In leaves at 20 DAE, urease activity in different treatments varied from 8.9 to 20.9 mg. The significantly highest value was observed in B+Mo+Ni treated plants, followed by B+Mo and Mo+Ni which were on par with each other. The next in the order was observed in B+Ni treated plants. Among individual applications, Ni treated plants showed greater increase, followed Mo and then boron treated plants. At 60 DAE also, the significantly highest value was observed in B+Mo+Ni treated plants. It was followed by other three combinations, which were on par with each other. B application alone showed the impact on par with control, which recorded the lowest value. Mo and Ni application alone increased the urease activity to an extent greater than control and lesser than combination treatments and two were found on par.

Urease activity in nodules under different treatments varied from 44.2 to 58.5 mg. The lowest was observed in control, which showed parity with B application. The highest activity was observed in B+Mo+Ni treated plants. It was followed by other three combinations, which were on par with each other Mo and Ni application alone increased the urease activity in nodules to an extent greater than $T_1$ and $T_2$ and lesser than $T_5$ to $T_8$ and two were found on par.

| *Treatments* | *Nitrate reductase activity* (µM $NO_{2-}$ $g^{-1}$ $hr^{-1}$) | | | *Urease activity (mg/dl)* | | |
|---|---|---|---|---|---|---|
| | | | | *In Leaves* | | *In nodules* |
| | *20 DAE* | *40 DAE* | *60 DAE* | *20 DAE* | *60 DAE* | *40 DAE* |
| T1: Control | 102.3 | 110.3 | 96.9 | 8.9 | 22.6 | 44.2 |
| T2: Borax @ 2.5 Kg $ha^{-1}$ | 106.2 | 116.5 | 99.6 | 11.6 | 23.5 | 46.0 |
| T3: Ammonium molybdate @ 1.5Kg $ha^{-1}$ | 107.3 | 117.7 | 101.1 | 12.7 | 24.5 | 47.1 |
| T4: Ni $Cl_{2.}$ $6H_2O$ @1.0 Kg $ha^{-1}$ | 105.7 | 113.3 | 98.8 | 14.2 | 25.2 | 48.3 |
| T5: Borax @ 2.5&Ammonium molybdate @ 1.5 Kg $ha^{-1}$ | 109.3 | 124.7 | 103.8 | 18.0 | 28.4 | 55.4 |
| T6: Borax @ 2.5 & Ni $Cl_{2.}$ $6H_2O$ @1.0 Kg $ha^{1}$ | 108.2 | 121.8 | 100.7 | 16.4 | 27.6 | 53.1 |
| T7: Ammonium molybdate @ 1.5 & Ni $Cl_{2.}$ $6H_2O$ @1.0 Kg $ha^{-1}$ | 108.5 | 123.4 | 101.6 | 18.0 | 27.6 | 54.8 |
| T8: Borax @ 2.5 & Ammonium molybdate @ 1.5 & Ni $Cl_{2.}6H_2O$ @1.0 Kg $ha^{-1}$. | 112.4 | 129.2 | 104.5 | 20.9 | 33.3 | 58.5 |
| SE(m)± | 1.5 | 1.6 | 2.1 | 0.3 | 0.4 | 0.8 |
| CD(0.05) | 4.6 | 4.8 | 6.4 | 0.8 | 1.2 | 2.4 |
| CV (%) | 2.4 | 2.3 | 3.6 | 3.0 | 2.7 | 2.6 |

***Table 2:*** Effect of micronutrients (B, Mo & Ni) on Nitrate reductase (µM $NO_2$- $g^{-1}$ $hr^{-1}$) and urease activity (mg/dl) in Blackgram.

From the above results, it can be suggested that application of B, Mo and Ni influences and the urease activity in leaves and nodules of blackgram. Boron application showed the impact on urease activity of leaves only at vegetative stage. The combination treatments had greater influence (1.8, 2.0, 2.0 and 2.3 folds increase in $T_6$, $T_5$, $T_7$ and $T_8$ respectively) than individual applications (1.3, 1.4 and 1.6 folds increase in $T_2$, $T_3$ and $T_4$ respectively). At 60 DAE, $T_8$ increased the urease activity by 1.5 folds, $T_5$ by 1.3 folds, $T_6$ and $T_7$ by 1.2 folds and $T_3$ and $T_4$ by 1.1 folds. Urease activity in nodules increased by 1.3 times in treatments $T_8$ and $T_5$, 1.2 times in $T_6$ and $T_7$ and 1.1 times in $T_3$ and $T_4$. This indicate that among individual application, Ni showed greater effect on urease activity in leaves, while in nodules both Ni and Mo showed the similar impact. Olivera et al. (2013) reported that Ni applied as $NiCl_2$ increased the leaf urease activity in lettuce. Gheibi et al. (2009) similarly reported that Ni applied as Ni So4 in maize increased the leaf urease activity.

### *Protein content of seeds (mg g-1)*

The data presented in Table 1 on total soluble protein in seeds of blackgram plants treated with B, Mo and Ni indicated that there was a significant difference among the treatments. The protein content in seeds of blackgram under different treatments varied from 203.3 to 270.1 mg $g^{-1}$. The significantly highest protein content was recorded in B+Mo+Ni application, while the lowest was observed in control. The next high protein content of the seed was noted by B+Mo application, which showed parity with B+Ni application, which in turn was found on par with Mo+Ni application. Protein content in grain was relatively less in individual application of micronutrients. Among these ($T_2$ to $T_4$), B application resulted in protein content on par with Mo application, which in turn was found on par with nickel application. Besides this all treatments were found superior to control.

From the above results, it can be stated that protein content in seed of blackgram could be enhanced through application of B, Mo and Ni. Compared to the application of these nutrients individually, the application in combination exhibited greater impact. The increase in protein content was 1.1 folds in $T_3$ and $T_4$; 1.2 folds in $T_2$, $T_6$ & $T_7$ and 1.3 folds in $T_5$ and $T_8$. This suggested that compared to Mo and Ni application alone, showed greater impact on protein content of seed and then compared to B+Ni and Mo+Ni application, B+Mo+Ni and B+Mo showed greater impact in increasing protein content. Increase in protein content might be due to the involvement of boron in the synthesis of protein similar to that reported by Anbuselvi et al. (2011) and Awomi et al, (2012).

## Conclusions

From the present study it can be informed that, the treatments $T_2$ to $T_4$ increased the LB content by 16.0, 23.6 and 6.4 and $T_5$ to $T_8$ increased it by 64.5, 44.1, 55.9 and 93.5 per cent respectively. Nitrate reductase activity at all stages of crop growth was found high in combination treatments compared to individual treatments (5.6 & 6.7 % in $T_2$ & $T_3$). Among combination treatments $T_8$ and $T_5$ showed greater impact (17.1 & 13.1 %, respectively) than $T_7$ and $T_6$ (11.9 & 10.4 %, respectively). Ni treated plants showed greater increase in leaf urease acivity (1.6 folds) followed by Mo (1.4 folds) than B treated ones (1.3 folds) which exhibited the impact only at vegetative stage. The combination treatments $T_5$ to $T_8$ had greater influence (2.0, 1.8, 2.0 & 2.3 folds, respectively) on nodule urease activity than individual applications. Protein content of seed increased to a greater extent in $T_8$ and $T_5$ (1.3 folds) compared to others (1.2 in $T_2$, $T_6$ & $T_7$ and 1 .1 folds in $T_3$ & $T_4$) individual application of B, Mo and Ni had less increase in leghaemoglobin content, nitrate reductse, urease activity and Protein content of seeds when compared to same micronutrients applied as combination.

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