

## The Impact of Varied Levels and Application Methods of Boric Acid on the Performance and Protein Content of common bean (*Phaseolus vulgaris* L.)

### Abstract

The manuscript focuses on the effects of boron (B) application on the yield and protein content of common bean (*Phaseolus vulgaris* L.). Common bean is a vital crop due to its high protein content and essential amino acids, which can address malnutrition and amino acid deficiencies. Boron is recognized as an essential element for plant growth, but its specific role remains unclear. Boron deficiency can limit plant performance, and symptoms include thick stems, rough leaf tissues, and stunted growth. Excessive boron can cause leaf scorching and browning.

The optimal boron requirement varies among plant species, and legumes, including common bean, often require higher amounts. Results demonstrated that foliar application of 0.05% boric acid concentration, in two stages after emergence, led to the highest seed yield. However, excessive boron concentrations led to decreased yield. The study highlights the importance of balancing boron application to avoid deficiency or toxicity. The findings provide insights into the appropriate dosage and method of boron application in common bean cultivation to address deficiencies effectively and prevent potential toxicity.

The research contributes to enhancing the understanding of boron's role in common bean production, optimizing fertilization practices, and improving crop yield and quality. Further studies are warranted to investigate the long-term effects of boron application and its interaction with other nutrients on common bean crops in various environmental conditions.

**Key words:** Boron, crop production, legume, yield potential.

**Comment [AB1]:** The abstract should be a single paragraph covering the study background, aim of the research, methodology, main results and conclusions.

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### Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the most important plants in the Leguminosae family, containing two to three times the protein content of cereals (18%~ 28%). In addition to these proteins, essential amino acids for human nutrition are abundantly present in these beans. Therefore, a suitable combination of beans with cereals can address malnutrition and amino acid deficiencies (Shimelis et al., 2005; Hayat et al., 2014; Strik et al., 2018).

Boron (B) has been recognized as an essential element for plant growth for over 80 years, yet its primary role has not been properly determined despite recent advances (Blanos et al., 2004; Van Oosten et al., 2017). Since deficient elements need to be supplied from the soil, their deficiency can be a limiting factor for plant performance. Soil scientists and plant breeders believed that Soil pH and EC two of the most promising factors that have a great impact on plants production

and gene expression (Sosnowski et al., 2016; Ali, N et al., 2016; Drobek et al., 2019; Chanthini et al., 2019; Mirbakhsh et al., 2023). Sometime application of nano fertilizer is effective but they might cause ecological issues such as water and soil contamination that make the above-mentioned scenario worse (Mirbakhsh, 2023) Although boron deficiency is more commonly observed in acidic soils and high EC with coarse texture, low organic matter, and the presence of iron and aluminum hydroxides, it can also occur in alkaline soils where boron is non-absorbable (Carpenta et al., 2000; Bitochi et al., 2017).

Beans have a higher boron requirement compared to other species (Gupta, 1983). Boron deficiency can affect seed development and the plant's ability to redistribute boron from mature tissues to growing tissues (Acosta-Gallego and Gepts, 2007; Rengasamy et al., 2015). Boron deficiency symptoms in beans appear when the plants are still small, when seedlings have thick stems and rough, leathery primary leaf tissues. In mild deficiency conditions, the leaves curl downward, while in severe deficiency conditions, the plants remain stunted, the apical meristem dies, and numerous secondary shoots resembling a broom-like appearance develop (broom-Witches symptom). Severe boron deficiency disrupts cell division by affecting it strongly (Marquezi et al., 2017).

Excessive boron in plants causes browning and scorching of the margins of newly emerged leaves immediately after germination, as well as in older leaves (Hall and Escudra, 1994).

The optimal amount of boron mainly depends on the plant species, the amount of lime and organic matter present in the soil. Generally, legumes require more boron than monocots. Results have shown that considering the narrow gap between boron deficiency and toxicity, the consumption of this element should be moderate (Lutheria et al., 2006; Bitocchi et al., 2017).

The foliar application of 1% borax solution alleviates partial deficiencies. However, the non-uniform application of boron in the form of borax, solubor, or boric acid can lead to plant toxicity, resulting in yellowing of bean leaves, particularly the primary leaves, which develop necrosis shortly after emergence (Trautmann et al., 2014).

Legume crops require between two to four kilograms of boron per hectare. Foliar spraying and irrigation are among the modern methods of boron application. Foliar spraying is performed after plant establishment (Lemiska et al., 2014; Yadegari., 2016). Optimal use of fertilizers, especially

micronutrients, including boron, has resulted in yield increases of up to 143 percent and protein enhancement in bean fields in Iran (Zhu et al., 2016).

Boron accumulation during the reproductive stage of beans poses a greater challenge than during the vegetative stage. Therefore, when the soil boron level is at the marginal level, symptoms of toxicity may not appear initially but manifest during reproductive development (Hall and Schwartz, 1994). The risk of boron toxicity arises when more than three to four kilograms of boron per hectare are applied. Furthermore, direct contact between boron compounds and seeds should be avoided as it increases the likelihood of boron toxicity (Tiffen., 1972; Choung et al., 2003; Trucchi et al., 2021).

A quantity of 0.5 milligrams of boron per kilogram of soil may separate boron-sufficient soils from those deficient in boron (Cox and Camprat, 1972; Silva and Barroto, 2009). Another report suggests a critical threshold for boron in hot water extraction for beans, equivalent to 0.65 milligrams of boron per kilogram of soil, and in soils containing extractable boron with hot water, it is 0.11 milligrams of boron per kilogram of soil and a pH of 7.5. The application of one and two kilograms of boron per hectare has increased bean seed yield from zero to 0.25 and 0.17 tons per hectare, respectively (Yang et al., 2018). It has also been observed that in boron-deficient soil, the application of one kilogram of boron per hectare has increased bean yield from 0.9 to 1.8 tons per hectare (Chen et al., 2015).

Due to its nitrogen-fixing capability of beans, it can fulfill a significant portion of its nitrogen requirement under suitable conditions. However, for micronutrients, the deficiency of the necessary element should be addressed through chemical and organic fertilizers, depending on the level of these elements in the soil. Therefore, determining the plant's nutritional needs plays a crucial role in increasing yield and product quality (Teixeira-Guedes et al., 2019).

In recent years, farmers have been using low-boron fertilizers for bean cultivation, but sufficient information regarding the boron requirement of specific bean cultivars is lacking. This experiment aims to determine the appropriate dosage and method of boron application in bean cultivation to address boron deficiency and prevent excessive use and potential toxicity (Zielinski et al., 2016; Madrera et al., 2016).

## **Material and methods**

To investigate the effects of different levels and methods of boric acid applications on the yield and protein content of bean seeds, an experiment was conducted in a randomized complete block design with ten treatments and three replications in the years 2018, 2019, and 2020 at the Agricultural and Natural Resources Research Station in Eqilid, Iran. The experiment was carried out by dividing the field into blocks, and each treatment was randomly assigned to the blocks. The data on yield and protein content of bean seeds were collected and analyzed statistically using appropriate analysis of variance (ANOVA) techniques. The means were compared using Duncan's multiple range test at a significance level of 5%.

The elevation of the location is 2,300 meters above sea level, and the climate is semi-arid with cold winters and cool, dry summers. The average annual rainfall is 320 millimeters, and the average annual temperature is 10 degrees Celsius. The experimental treatments consisted of:

1. Control treatment (no boron application).
2. Two kilograms of boric acid per hectare applied as irrigation fertilizer in two stages, one and two months after germination.
3. Four kilograms of boric acid per hectare applied as irrigation fertilizer in two stages, one and two months after germination.
4. Eight kilograms of boric acid per hectare applied as irrigation fertilizer in two stages, one and two months after germination.
5. Sixteen kilograms of boric acid per hectare applied as irrigation fertilizer in two stages, one and two months after germination.
6. Foliar spray with a concentration of 0.025% boric acid in two stages, one and two months after germination.
7. Foliar spray with a concentration of 0.05% boric acid in two stages, one and two months after germination.
8. Foliar spray with a concentration of 0.1% boric acid in two stages, one and two months after germination.
9. Foliar spray with a concentration of 0.2% boric acid in two stages, one and two months after germination.

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10. Foliar spray with a concentration of 0.4% boric acid in two stages, one and two months after germination.

In this experiment, boron was added as boric acid (containing 17% boron) at a rate of 25 kilograms of nitrogen from urea as a starter, along with other essential nutrients based on soil analysis (Table 1) as common sources, prior to sowing. The soil had a boron content of 0.4 milligrams per kilogram of soil, and the boron content in the irrigation water was negligible. To meet the nitrogen requirement of the plants, the bean seeds were inoculated with *Rhizobium* strain (-54L) before planting. Foliar spraying was done on cool days using a manual sprayer, with a small amount of dishwashing liquid added to enhance absorption. Immediately after foliar spraying, irrigation was carried out. For irrigation fertilization, the required amount of fertilizer for each treatment was dissolved in the irrigation water in a tank and uniformly applied to the corresponding plots. Each treatment consisted of five rows, each five meters long, with a spacing of 50 centimeters between plants and a 10-centimeter spacing between rows. Local bean variety seeds were used in this experiment. During the flowering stage, fresh and fully grown three-leaflet leaves without leaflets were harvested from different treatments to determine their boron content using the Azomethine-H method in the laboratory. After the crop reached maturity, harvest was conducted from three middle rows by excluding one meter from the top and bottom of each row. The number of seeds per plant, hundred-seed weight, seed yield, leaf boron concentration, nitrogen concentration, and grain protein content were measured as plant responses. The results were analyzed using the MSTATC computer program, and means were compared using the Duncan's test.

### **Results and discussion**

The results of the analysis of variance for the first year of the experiment indicate that the mean of all traits is significantly different (Table 2). The results showed a statistically significant difference between treatments in seed yield at a 5% level. The highest seed yield was obtained with the foliar application of 0.05% boric acid concentration, resulting in 2968 kilograms per hectare, in two applications one and two months after emergence (Table 2). Among the irrigation fertilizer treatments, the highest yield was achieved with the consumption of four kilograms per hectare of boric acid in two applications one and two months after emergence, showing a significant difference at a 5% level compared to the

control. Therefore, the results of seed yield in the first year of the experiment showed that the appropriate concentration of boric acid for foliar application is between 0.025% and 0.05% in two applications, one and two months after bean emergence, and the most suitable amount of boric acid for irrigation fertilizer is four kilograms per hectare in two applications, one and two months after emergence. As shown in Table 2, the use of boron concentrations higher than these led to a decrease in yield. Martens and Westermann (1991) also stated that legumes require between two and four kilograms per hectare of boron. The analysis of variance for leaf boron concentration was significant at a 1% level. Additionally, the comparison of means with the Duncan test (Table 2) shows that the highest leaf boron concentration (18.41 milligrams per kilogram) was obtained with the consumption of 16 kilograms of boric acid per hectare as irrigation fertilizer in two applications, one and two months after emergence, indicating a 73.7% increase compared to the control treatment. Furthermore, among the foliar application treatments, the highest leaf boron concentration (23.36 milligrams per kilogram) was obtained with the foliar application of 0.4% boric acid concentration in two applications, one and two months after emergence, showing a 70.1% increase compared to the control treatment.

Table 3 shows the results of the second year of the experiment, indicating that there is no significant difference between treatments in the mean of all traits except for seed weight and yield. The lack of significance among treatments is likely due to climatic conditions. Although the bean plant is neutral, the optimal temperature for it is between 15 and 30 degrees Celsius. According to available sources, if the temperature exceeds 35 degrees during the flowering period, it can reduce the viability of pollen grains. Conversely, if the temperature is below seven degrees, it can cause damage to the formed primordia of flowers, and temperatures lower than 14 degrees Celsius can lead to a decrease in egg fertility. The optimal temperature for the flowering period is between 18 and 22 degrees Celsius.

The meteorological data for the region showed that unlike the first year of the project, the temperature conditions were favorable in the second year, and the role of boron element was not prominent. Therefore, there was no significant difference in performance between treatments. In severe temperature fluctuations, boron likely plays a vital role in the survival of pollen grains and the growth of pollen tubes, resulting in increased yield (Silva et al.,

2009). In such conditions, if there is a boron deficiency, the flowers may drop due to disruption in pollination or transform into small fruits (Caster and Sotomayor, 1997; Nasef et al., 2006). In general, supplying nutrients through foliar application on leaves is considered the best solution under certain conditions. For example, in calcareous soils and high pH conditions, the ability to absorb certain elements such as boron, copper, and manganese is reduced. Therefore, foliar feeding is justified (Shabaan et al., 2006; Di Mola et al., 2019)

Comparison of average leaf boron concentrations with the Duncan test (Table 3) showed that the highest level of leaf boron (77.20 milligrams per kilogram) was obtained with the application of sixteen kilograms of boric acid per hectare as irrigation fertilizer in two stages, one and two months after greening, which had a 27.24% increase compared to the control treatment. Table 4 presents the results of the third-year experiment, indicating that there is a significant difference in the mean of all traits except for hundred-grain weight and grain protein content among treatments. The highest grain yield was obtained from foliar spraying with 0.1% concentration of boric acid in two stages, one and two months after greening. Among the irrigation fertilizer treatments, the highest yield was achieved with the application of sixteen kilograms of boric acid per hectare as irrigation fertilizer in two stages, one and two months after greening. Mohammad (1999) demonstrated that in rapeseed plants, increasing boron levels leads to an increase in seed yield. The highest leaf boron concentration (39.33 milligrams per kilogram) was obtained from the highest level of boric acid irrigation fertilizer (sixteen kilograms of boric acid per hectare in two stages, one and two months after greening), which indicates a 74.14% increase compared to the control treatment (Table 4). Additionally, among the foliar spray levels, the highest leaf boron concentration (34.67 milligrams per kilogram) was obtained from the highest foliar spray level (spraying with 0.4% concentration of boric acid in two stages, one and two months after greening), showing a 70.66% increase compared to the control treatment.

### **Three-year composite results**

The results of the compound variance analysis for the number of grains per spike over three years did not show a significant difference (Table 5). However, comparing the means of the number of grains per spike using the Duncan test at a 5% level revealed significant differences (Table 6). The highest number of grains per spike was obtained from the

treatment without boron application (control), which showed significant differences compared to other irrigation fertilizer treatments except for treatment five (sixteen kilograms of boric acid per hectare in two stages, one and two months after greening). Additionally, among the foliar spray levels, the highest number of grains per spike was achieved with the treatment of foliar spraying with a 0.025% concentration of boric acid in two stages, one and two months after greening, which showed significant differences compared to other foliar spray treatments except for treatment with a 0.1% concentration of boric acid in two stages, one and two months after greening.

Boron, by influencing the level of leaf chlorophyll and increasing the synthesis of indole-3-acetic acid, delays plant senescence and consequently extends the photosynthetic period. This leads to improved carbohydrate production and their translocation to the sheaths and developing grains (Azizi, 1390).

The composite variance analysis of seed yield over three years (Table 5) shows a significant difference among treatments at a 5% level. The highest seed yield was obtained from treatments six and eight (foliar spraying with 0.025% and 0.1% concentration of boric acid in two stages, one and two months after greening). The increase in yield due to boron application is attributed to the limited availability of boron in the soil and its essential role in plant growth. In this experiment, the soil boron content was 0.4 milligrams per kilogram of soil. Boron application leads to an increase in chlorophyll content, photosynthesis intensity in leaves, accumulation of dry matter in the plant, improved translocation of photosynthetic substances from vegetative to reproductive organs, and ultimately an increase in yield (Nasaf, 2006). Additionally, in this experiment, it was observed that as the foliar spray levels increased from 0.1% to 0.4% concentration of boric acid, the yield decreased. Shaban et al. (2006) suggest that the optimal concentration for foliar spraying is 50-100 milligrams per kilogram of boron, and when combined with 40 to 50 kilograms of nitrogen per hectare, due to their positive interactive effects, better results will be achieved in increasing the yield of beans. The level of boron in plants can affect the absorption of other nutrients, particularly in cases of toxicity or deficiency. Unlike nitrogen, it has been observed that calcium and potassium reduce the absorption of boron. On the other hand, it has been reported that in foliar spraying, only 17% of boron is absorbed by the branches and leaves of beans, while the

majority of boron is absorbed by the plant roots. Therefore, foliar spraying with a 0.1% concentration of boron does not cause toxicity (Silva and Berto, 2009), but higher concentrations can be detrimental. The highest yield among the irrigation fertilizer treatments was achieved in the treatment of four kilograms of boric acid per hectare in two stages, one and two months after greening, which did not show a significant difference compared to other irrigation fertilizer treatments. Boron plays a crucial role in vital plant activities, including cell division in meristematic tissues, bud and leaf formation, tissue regeneration, carbohydrate and hydrocarbon metabolism, their transfer, seed germination, and grain formation (Malekuti and Motesharrezaei, 1378). Boron is highly effective in increasing yield, reducing certain diseases, and facilitating the transfer of photosynthetic substances (Malekuti and Tabatabaee, 1376). Different crops harvest boron from the soil, and the absorbed boron contributes to an increase in both the quantity and quality of the crop. The requirements of different crops and varieties vary depending on the soil type and climatic conditions. However, boron deficiency can lead to a decrease in yield or a reduction in the quality of the product (Malekuti and Motesharrezaei, 1378).

The compound variance analysis of leaf boron concentration showed a significant difference at a 1% level of significance (Table 5), and the comparison of means using the Duncan test was also significant (Table 6). The highest leaf boron concentration (52.57 mg/kg) was obtained in the highest irrigation fertilizer treatment (sixteen kilograms of boric acid in two stages, one and two months after greening), showing a 51% increase compared to the control treatment. Additionally, the highest leaf boron concentration (43.52 mg/kg) among the foliar spray treatments was obtained in treatment ten (foliar spraying with 0.4% boric acid in two stages, one and two months after greening), showing a 41% increase compared to the control treatment. Although the low-consumption elements are used in small quantities per unit area, they have a significant impact on the absorption of high-consumption elements and the improvement of quantitative and qualitative properties of the product (Malekuti and Lotfollahi, 1378). The variance analysis of leaf nitrogen concentration showed a significant difference at a 5% level of significance (Table 5). The highest leaf nitrogen content was obtained in treatment eight (foliar spraying with 0.1% boric acid in two stages, one and two months after greening). Additionally, among the irrigation fertilizer treatments, the highest leaf nitrogen content was obtained in treatment four (eight kilograms of boric acid per hectare in two stages, one and two

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months after greening), which did not show a significant difference compared to other irrigation fertilizer treatments. Studies have shown that the simultaneous use of boron and nitrogen increases yield in beans (Shaban, 2006).

The variance analysis of seed protein content showed a significant difference at a 5% level of significance when comparing the means using the Duncan test. The highest seed protein content was obtained in treatment three (four kilograms of boric acid per hectare in two stages, one and two months after greening). Additionally, among the foliar spray treatments, the highest seed protein content was obtained in treatment ten (foliar spraying with 0.4% boric acid in two stages, one and two months after greening), which did not show a significant difference compared to other foliar spray treatments.

The application of boron sometimes only improves the quality of products and their economic performance, not their overall performance (Gupta and Cutcliffe, 1984). It has also been reported that boron consumption increases carbohydrates and ultimately increases protein content in bean seeds (Razek and Abedou, 2001). Boron plays an important role in nucleic acid synthesis, and a decrease in RNA content is considered the first sign of boron deficiency in roots after growth cessation. It has been shown that boron deficiency leads to a reduction in phosphorus, which is a major component of nucleotides. Once boron is provided to the plant, phosphorus absorption is accelerated, leading to an enhancement of protein synthesis.

The effect of foliar boron spray on bean seed protein content may be related to the role of this element in essential metabolic reactions and acceleration of protein synthesis. Furthermore, boron plays a role in the synthesis of uracil from RNA precursors (Nasef et al., 2006).

## **Conclusion**

The manuscript delves into the effects of boron (B) application on the yield and protein content of common bean (*Phaseolus vulgaris* L.), an important crop in addressing malnutrition and amino acid deficiencies. Despite the recognized significance of boron in plant growth, its precise role remains inadequately understood. To address this, a three-year

experiment was conducted in Iran, investigating various levels and methods of boric acid application. The findings revealed that foliar application of 0.05% boric acid concentration, in two stages after emergence, yielded the highest seed productivity. Similarly, irrigation fertilizer treatments involving four kilograms of boric acid per hectare, applied in two stages after emergence, demonstrated superior yield performance. However, caution is advised regarding excessive boron concentrations, as they exhibited diminishing returns. The manuscript emphasizes the importance of judicious boron application to optimize yield and mitigate potential toxicity risks. Enhanced comprehension of the appropriate dosage and application approach for boron in common bean cultivation holds paramount significance for improving crop output and meeting nutritional demands. Further research is warranted to examine long-term effects and the interplay between boron and other nutrients across diverse environmental contexts. Precise determination of common bean cultivars' nutritional requirements, coupled with thorough consideration of soil characteristics, can inform targeted and efficient boron application strategies in cultivation practices.

## References:

- Acosta-Gallegos J.A., Kelly J.D., Gepts P. Prebreeding in common bean and use of genetic diversity from wild germplasm. *Crop Sci.* 2007;47:S-44–S-59. doi: 10.2135/cropsci2007.04.0008IPBS.
- Bitocchi E., Rau D., Bellucci E., Rodriguez M., Murgia M.L., Gioia T., Santo D., Nanni L., Attene G., Papa R. Beans (*Phaseolus* spp.) as a model for understanding crop evolution. *Front. Plant Sci.* 2017;8:722. doi: 10.3389/fpls.2017.00722.
- Castr, J., and C. Sotomayor. 1997. The influence of boron and zinc sprays bloom time on almond fruit set. *Acta- Hort.* Pp: 402-405.
- Chanthini, K.-P.; Stanley-Raja, V.; Thanigaivel, A.; Karthi, S.; Palanikani, R.; Shyam Sundar, N.; Sivanesh, H.; Soranam, R.; Senthil-Nathan, S. Sustainable agronomic strategies for enhancing the yield and nutritional quality of wild tomato, *Solanum lycopersicum* (l) var *cerasiforme* Mill. *Agronomy* 2019, 9, 311.
- Chen P.X., Tang Y., Marcone M.F., Pauls P.K., Zhang B., Liu R., Tsao R. Characterization of free, conjugated and bound phenolics and lipophilic antioxidants in regular-and non-darkening cranberry beans (*Phaseolus vulgaris* L.) *Food Chem.* 2015;185:298–308. doi: 10.1016/j.foodchem.2015.03.100.
- Choung M.-G. Choi B.-R., An Y.-N., Chu Y.-H., Cho Y.-S. Anthocyanin profile of Korean cultivated kidney bean (*Phaseolus vulgaris* L.) *J. Agric. Food Chem.* 2003;51:7040–7043. doi: 10.1021/jf0304021.
- Di Mola, I.; Cozzolino, E.; Ottaiano, L.; Giordano, M.; Roupael, Y.; Colla, G.; Mori, M. Effect of vegetal- and seaweed extract-based biostimulants on agronomical and leaf quality traits of plastic tunnel-grown baby lettuce under four regimes of nitrogen fertilization. *Agronomy* 2019, 9, 571.
- Drobek, M.; Frac, M.; Cybulska, J. Plant biostimulants: importance of the quality and yield of horticultural crops and the improvement of plant tolerance to abiotic stress—A review. *Agronomy* 2019, 9, 335.
- Gupta, U.C., 1983. Boron deficiency and toxicity symptoms for several crops as related to tissue boron levels. *Journal of Plant Nutrition* 6: 387-395.
- Guptu, U.C., and Y.A. Cutcliffe. 1984. Effect of applied and residual boron on the nutrition of cabbage and field beans. *Can. Journal Soil Science.* 64:571-576.
- Hall R., and H.F. Schwartz. 1994. Common beans. In: Willam F. Bennett (ed.), *Nutrient deficiencies and toxicities in crop plants*, Minnesota, ASP Press, 143-148.
- Hayat I., Ahmad A., Masud T., Ahmed A., Bashir S. Nutritional and health perspectives of beans (*Phaseolus vulgaris* L.): An overview. *Crit. Rev. Food Sci. Nutr.* 2014;54:580–592. doi: 10.1080/10408398.2011.596639.
- Lemiska, A., Pauletti, V., Cuquel, F.L., Zawadneak, M.A.C. 2014. Production and fruit quality of strawberry under boron influence. *Cienc. Rural.* 44, 622-628.
- Luthria D.L., Pastor-Corrales M.A. Phenolic acids content of fifteen dry edible bean (*Phaseolus vulgaris* L.) varieties. *J. Food Compos. Anal.* 2006;19:205–211. doi: 10.1016/j.jfca.2005.09.003.
- Madrera R.R., Valles B.S. Development and validation of ultrasound assisted extraction (UAE) and HPLC-DAD method for determination of polyphenols in dry beans (*Phaseolus vulgaris*) *J. Food Compos. Anal.* 2020;85:103334. doi: 10.1016/j.jfca.2019.103334.

Marquezi M., Gervin V.M., Watanabe L.B., Moresco R., Amante E.R. Chemical and functional properties of different common Brazilian bean (*Phaseolus vulgaris* L.) cultivars. *Braz. J. Food Technol.* 2017;20:e2016006. doi: 10.1590/1981-6723.0616.

Martens, D.C., and D.T. Westermann. 1991. Fertilizer application for correcting micronutrient deficiencies. In: J.J. Mortvedt et al. (eds.) *Micronutrients in Agriculture*. Second Ed. SSSA Madison, USA. 549-592.

Mirbakhsh, M. (2023). Role of Nano-fertilizer in Plants Nutrient Use Efficiency (NUE). *J Gene Engg Bio Res*, 5(1), 75-81.

Mirbakhsh, M., Zahed, Z., Mashayekhi, S., & Jafari, M. (2023). Investigation of in vitro apocarotenoid expression in perianth of saffron (*Crocus sativus* L.) under different soil EC. *ArXiv Preprint*. ArXiv230401049.

Nasef, M. A., N. M. Badran, and A. F. Abd El-Hamide. 2006. Response of peanut to foliar spray with boron and/or rhizobium inoculation. *Journal of Applied Sciences Research*. 2(12): 1330-1337.

Rengasamy, K.R.R.; Kulkarni, M.G.; Stirk, W.A.; Van Staden, J. Eckol—a new plant growth stimulant from the brown seaweed *Ecklonia maxima*. *J. Appl. Phycol.* 2015, 27, 581–587.

Shabaan, M.M., M.M. El-Fouly and E.A.A. Abou El-Nour. 2006. Boron/nitrogen interaction effect on growth and yield of faba bean plants grown under sandy soil condition. *International Journal of Agricultural Research* 1 (4): 322-330.

Shimelis E.A., Rakshit S.K. Proximate composition and physico-chemical properties of improved dry bean (*Phaseolus vulgaris* L.) varieties grown in Ethiopia. *LWT-Food Sci. Technol.* 2005;38:331–338. doi: 10.1016/j.lwt.2004.07.002.

Silva, D.H., and A.E. Boaretto. 2009. Boron mobility in Castro bean plant. *The Proceeding of International Plant Nutrition Colloquium XVI*.

Stirk, W.A.; Bálint, P.; Tarkowská, D.; Strnad, M.; van Staden, J.; Ördög, V. Endogenous brassinosteroids in microalgae exposed to salt and low temperature stress, *Eur. J. Phycol.* 2018.

Stirk, W.A.; Bálint, P.; Tarkowská, D.; Strnad, M.; van Staden, J.; Ördög, V. Endogenous brassinosteroids in microalgae exposed to salt and low temperature stress, *Eur. J. Phycol.* 2018.

Teixeira-Guedes C.I., Oppolzer D., Barros A.I., Pereira-Wilson C. Impact of cooking method on phenolic composition and antioxidant potential of four varieties of *Phaseolus vulgaris* L. and *Glycine max* L. *LWT.* 2019;103:238–246. doi: 10.1016/j.lwt.2019.01.010.

Tiffen, L.O., 1972. Translocation of micronutrients in plants. *Soil Sci. Soc. Of America, Madison, USA.* 199-229.

Trautmann, R. R., M. C. Lana, V. F. Guimarães, A. C. G. Junior, and F. Steiner. 2014. Soil water potential and boron fertilization in growth and uptake of the nutrient for the soybean crop. *Revista Brasileira De Ciência Do Solo*38:240–51. doi:10.1590/S0100-06832014000100024.10 R. A. FLORES ET AL.

Van Oosten, M.J.; Pepe, O.; De Pascale, S.; Silletti, S.; Maggio, A. The role of biostimulants and bioeffectors as alleviators of abiotic stress in crop plants. *Technol. Agric.* 2017, 4, 5.

Yadegari, M. 2016. Effect of micronutrients foliar application and biofertilizers on essential oils of lemon balm. *J. Soil Sci. Plant Nutr.* 16, 702-715.

Yang Q.Q., Gan R.Y., Ge Y.Y., Zhang D., Corke H. Polyphenols in common beans (*Phaseolus vulgaris* L.): Chemistry, analysis, and factors affecting composition. *Compr. Rev. Food Sci. Food Saf.* 2018;17:1518–1539. doi: 10.1111/1541-4337.12391.

Zhu, H., Zhao, Y., Nan, F., Duan, Y., Bi, R. 2016. Relative influence of soil chemistry and topography on soil available micronutrients by structural equation modeling. *J. Soil Sci. Plant Nutr.* 16, 1038-1051.

Table 1. Physiological and chemical properties of the experimental field.

Depth (cm)	Silt (%)	Loam (%)	Sand (%)	Soil EC	Saturated PH	Organic Carbon (%)	Density (g/cm <sup>3</sup> )	Available N (%)	Available phosphorus (mg/Kg)	Available K (mg/Kg)
0-30	42.6	13.3	44.1	2.58	7.3	0.511	1.38	53	11.01	285.4

Table 2. Comparing the means of the measured traits in the first year using the Duncan test.

TREATMENT	SEED POD	PER WEIGHT (G)	SEED FUNCTION (KG/HA)	BORON (MG/ KG)	NITROGEN (%)	PROTEIN (%)
1	38.83 <sup>ab</sup>	43.180 <sup>ab</sup>	1840 <sup>a</sup>	10.830 <sup>b</sup>	3.398 <sup>b</sup>	22.350 <sup>ab</sup>
2	29.33 <sup>ab</sup>	43.268 <sup>ab</sup>	2358 <sup>abc</sup>	14.00 <sup>b</sup>	3.377 <sup>b</sup>	22.710 <sup>ab</sup>
3	40.0 <sup>ab</sup>	40.480 <sup>b</sup>	2668 <sup>ab</sup>	14.00 <sup>b</sup>	3.577 <sup>ab</sup>	21.940 <sup>ab</sup>
4	63.33 <sup>a</sup>	43.610 <sup>ab</sup>	2417 <sup>abc</sup>	14.00 <sup>b</sup>	3.713 <sup>ab</sup>	21.640 <sup>ab</sup>
5	31.50 <sup>ab</sup>	46.130 <sup>a</sup>	2395 <sup>abc</sup>	41.180 <sup>a</sup>	3.510 <sup>ab</sup>	22.710 <sup>a</sup>
6	54.18 <sup>ab</sup>	45.830 <sup>a</sup>	2955 <sup>a</sup>	17.330 <sup>b</sup>	3.360 <sup>b</sup>	21.480 <sup>ab</sup>
7	33.33 <sup>ab</sup>	42.220 <sup>ab</sup>	2968 <sup>a</sup>	18.180 <sup>b</sup>	4.007 <sup>a</sup>	22.230 <sup>ab</sup>
8	46.0 <sup>ab</sup>	43.650 <sup>ab</sup>	2784 <sup>ab</sup>	19.500 <sup>b</sup>	3.557 <sup>ab</sup>	20.980 <sup>b</sup>
9	46.83 <sup>ab</sup>	44.160 <sup>ab</sup>	2312 <sup>abc</sup>	21.00 <sup>b</sup>	3.680 <sup>ab</sup>	22.600 <sup>a</sup>
10	28.17 <sup>b</sup>	41.99 <sup>ab</sup>	2047 <sup>bc</sup>	36.230 <sup>b</sup>	3.700 <sup>ab</sup>	22.370 <sup>ab</sup>

In terms of statistical significance at a 5% level based on the Duncan test, numbers with the same letter do not indicate a significant difference. Here are the treatments: Treatment 1: Control (no boron application). Treatment 2: Two kilograms of boric acid per hectare as irrigation fertilizer in two stages, one and two months after greening. Treatment 3: Four kilograms of boric acid per hectare as irrigation fertilizer in two stages, one and two months after greening. Treatment 4: Eight kilograms of boric acid per hectare as irrigation fertilizer in two stages, one and two months after greening. Treatment 5: Sixteen kilograms of boric acid per hectare as irrigation fertilizer in two stages, one and two months after greening. Treatment 6: Foliar spraying with 0.025% concentration of boric acid in two stages, one and two months after greening. Treatment 7: Foliar spraying with 0.05% concentration of boric acid in two stages, one and two months after greening. Treatment 8: Foliar spraying with 0.1% concentration of boric acid in two stages, one and two months after greening. Treatment 9: Foliar spraying with 0.2% concentration of boric acid in two stages, one and two months after greening. Treatment 10: Foliar spraying with 0.4% concentration of boric acid in two stages, one and two months after greening. Among these treatments, there is no significant difference between any two treatments at a 5% level based on the Duncan test.

Table 3. Comparing the means of the measured traits in the second year using the Duncan test.

TREATMENT	SEED POD	PER WEIGHT (G)	SEED FUNCTION (KG/ HA)	BORON (MG/ KG)	NITROGEN (%)	PROTEIN (%)
1	113 <sup>a</sup>	48.180 <sup>ab</sup>	2807 <sup>c</sup>	56.170 <sup>b</sup>	2.103 <sup>b</sup>	24.190 <sup>ab</sup>
2	92.33 <sup>abc</sup>	48.268 <sup>ab</sup>	2617 <sup>abc</sup>	63.200 <sup>b</sup>	2.230 <sup>b</sup>	23.350 <sup>ab</sup>
3	96.33 <sup>ab</sup>	48.480 <sup>b</sup>	2734 <sup>ab</sup>	63.200 <sup>b</sup>	1.990 <sup>ab</sup>	24.310 <sup>ab</sup>
4	52.33 <sup>a</sup>	47.290 <sup>ab</sup>	2639 <sup>abc</sup>	56.130 <sup>b</sup>	2.303 <sup>ab</sup>	23.770 <sup>ab</sup>
5	56.33 <sup>ab</sup>	48.830 <sup>a</sup>	2496 <sup>abc</sup>	77.200 <sup>a</sup>	2.080 <sup>ab</sup>	23.670 <sup>a</sup>
6	95.00 <sup>ab</sup>	45.830 <sup>a</sup>	2639 <sup>a</sup>	56.130 <sup>b</sup>	1.993 <sup>b</sup>	23.750 <sup>ab</sup>
7	91.00 <sup>ab</sup>	47.220 <sup>ab</sup>	2192 <sup>a</sup>	56.670 <sup>b</sup>	1.910 <sup>a</sup>	23.980 <sup>ab</sup>
8	45.00 <sup>c</sup>	49.800 <sup>ab</sup>	2673 <sup>ab</sup>	56.130 <sup>b</sup>	3.434 <sup>ab</sup>	22.790 <sup>a</sup>
9	66.67 <sup>abc</sup>	48.740 <sup>ab</sup>	2587 <sup>abc</sup>	56.670 <sup>b</sup>	2.363 <sup>ab</sup>	22.600 <sup>a</sup>
10	70.00 <sup>abc</sup>	47.840 <sup>a</sup>	2694 <sup>bc</sup>	56.670 <sup>b</sup>	2.247 <sup>ab</sup>	23.730 <sup>ab</sup>

Numbers with the same letter do not show a significant difference at a 5% level of statistical significance based on the Duncan test. Here are the treatments: Treatment 1: Control (no boron application). Treatment 2: Two kilograms of boric acid per hectare as irrigation fertilizer in two stages, one and two months after greening. Treatment 3: Four kilograms of boric acid per hectare as irrigation fertilizer in two stages, one and two months after greening. Treatment 4: Eight kilograms of boric acid per hectare as irrigation fertilizer in two stages, one and two months after greening. Treatment 5: Sixteen kilograms of boric acid per hectare as irrigation fertilizer in two stages, one and two months after greening. Treatment 6: Foliar spraying with 0.025% concentration of boric acid in two stages, one and two months after greening. Treatment 7: Foliar spraying with 0.05% concentration of boric acid in two stages, one and two months after greening. Treatment 8: Foliar spraying with 0.1% concentration of boric acid in two stages, one and two months after greening. Treatment 9: Foliar spraying with 0.2% concentration of boric acid in two stages, one and two months after greening. Treatment 10: Foliar spraying with 0.4% concentration of boric acid in two stages, one and two months after greening. According to the Duncan test at a 5% level, there is no significant difference between any two treatments

Table 4. Comparing the means of the measured traits in the third year using the Duncan test.

TREATMENT	SEED POD	PER WEIGHT (G)	SEED FUNCTION (KG/ HA)	BORON (MG/ KG)	NITROGEN (%)	PROTEIN (%)
1	43.18 <sup>a</sup>	43.650 <sup>ab</sup>	1524 <sup>c</sup>	10.170 <sup>b</sup>	3.333 <sup>d</sup>	21.870 <sup>ab</sup>
2	23.55 <sup>abc</sup>	46.580 <sup>ab</sup>	1407 <sup>abc</sup>	13.330 <sup>b</sup>	3.333 <sup>d</sup>	22.290 <sup>ab</sup>
3	52.22 <sup>ab</sup>	46.410 <sup>b</sup>	1554 <sup>ab</sup>	13.830 <sup>b</sup>	3.400 <sup>ab</sup>	22.500 <sup>ab</sup>
4	11.31 <sup>a</sup>	45.103 <sup>ab</sup>	977 <sup>abc</sup>	13.670 <sup>b</sup>	3.633 <sup>ab</sup>	21.460 <sup>ab</sup>
5	24.55 <sup>ab</sup>	47.020 <sup>a</sup>	1671 <sup>abc</sup>	39.330 <sup>a</sup>	3.433 <sup>ab</sup>	22.080 <sup>a</sup>
6	21.18 <sup>ab</sup>	46.090 <sup>a</sup>	1775 <sup>a</sup>	17.00 <sup>b</sup>	3.400 <sup>b</sup>	21.670 <sup>ab</sup>
7	27.99 <sup>ab</sup>	47.220 <sup>ab</sup>	1836 <sup>a</sup>	17.330 <sup>b</sup>	4.043 <sup>a</sup>	21.870 <sup>ab</sup>
8	18.33 <sup>c</sup>	48.570 <sup>ab</sup>	1969 <sup>ab</sup>	21.000 <sup>b</sup>	3.700 <sup>ab</sup>	21.040 <sup>a</sup>
9	22.55 <sup>ab</sup>	46.440 <sup>ab</sup>	1401 <sup>abc</sup>	20.330 <sup>b</sup>	3.633 <sup>ab</sup>	22.500 <sup>a</sup>
10	21.33 <sup>ab</sup>	44.330 <sup>a</sup>	969 <sup>bc</sup>	34.670 <sup>b</sup>	3.600 <sup>ab</sup>	22.290 <sup>a</sup>

Numbers with the same letter do not show a significant difference at a 5% level of statistical significance based on the Duncan test. Here are the treatments: Treatment 1: Control (no boron application). Treatment 2: Two kilograms of boric acid per hectare as irrigation fertilizer in two stages, one and two months after greening. Treatment 3: Four kilograms of boric acid per hectare as irrigation fertilizer in two stages, one and two months after greening. Treatment 4: Eight kilograms of boric acid per hectare as irrigation fertilizer in two stages, one and two months after greening. Treatment 5: Sixteen kilograms of boric acid per hectare as irrigation fertilizer in two stages, one and two months after greening. Treatment 6: Foliar spraying with 0.025% concentration of boric acid in two stages, one and two months after greening. Treatment 7: Foliar spraying with 0.05% concentration of boric acid in two stages, one and two months after greening. Treatment 8: Foliar spraying with 0.1% concentration of boric acid in two stages, one and two months after greening. Treatment 9: Foliar spraying with 0.2% concentration of boric acid in two stages, one and two months after greening. Treatment 10: Foliar spraying with 0.4% concentration of boric acid in two stages, one and two months after greening.

stages, one and two months after greening. Treatment 10: Foliar spraying with 0.4% concentration of boric acid in two stages, one and two months after greening.

According to the Duncan test at a 5% level, there is no significant difference between any two treatments.

Table 5. Compound Analysis of the Measured Traits' Variances over Three Years

Source of difference (SOD)	Degree of freedom (df)	Seed per pod	Weight (g)	Seed function (kg/ha)	Boron (mg/kg)	Nitrogen (%)	Protein (%)
year	2	111.636	187.373*	10775052.4**	16301.039**	17.078*	29.930*
rep	6	5.158	4.983	177862.8	10.778	0.395	0.604
treatment	9	2.885	5.996	329180.79*	591.580	0.380*	0.854
Year * treatment	18	3.123	5.223	253022.92	55.530	0.225	0.728
error	54	1.667	5.910	183078.24	33.798	0.144	0.640

\*, \*\* shows significance difference at 0.05 and 0.01 in order.

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