



Role of Soil Addition of Cobalt in Enhancing productivity and Symbiotic Relationships in Faba Bean

Mahmoud Elshony^{1,2}, Ihab M. Farid¹, Nader Habashy² and Mohamed H.H. Abbas^{1*}



¹ Soils and Water Department, Faculty of Agric., Benha University, Egypt

² Soil, Water and Environment Res. Inst, Agric. Res. Center (ARC), Giza, Egypt

FABA bean is a protein-rich crop, yet its productivity is often limited by nitrogen deficiency. The current study evaluated the effects of cobalt, applied to soil as sulfate (CoSO_4) or chloride (CoCl_2) at different concentrations, on growth, yield, and biological nitrogen fixation (BNF) in faba bean. A two-season field experiment was conducted in a randomized complete block design with 7 treatments: three doses of Co (5.0, 10.0, and 15.0 mg L^{-1}) from each source were applied directly to the soil around plants, in addition to the reference control treatment (0 mg Co L^{-1}). Soil application of cobalt significantly, regardless of the salt type, enhanced BNF by increasing number and biomass of bacterial nodules, and also increased nitrogenase enzyme activity at doses up to 10 mg Co L^{-1} . These improvements subsequently elevated available N-content in soil and N-accumulation in plant shoots and seeds, resulting in concurrent increases in yield and yield components. However, higher Co doses (15 mg Co L^{-1}) negatively affected nodulation, despite increasing nitrogen and cobalt concentrations in shoots, and chlorophyll content. This dose did not improve straw or grain yield beyond those achieved with 10 mg L^{-1} , except for grain yield under CoCl_2 . Overall, CoSO_4 outperformed CoCl_2 in most measured parameters, except straw yield. Strong positive correlations were found between cobalt concentrations in shoots and each of chlorophyll content, nodule weights and nitrogenase activity, supporting cobalt's essential role in the legume-rhizobia symbiosis. Also, all growth and yield parameters, excluding number of branches per plant, showed significant correlations with nitrogen content in plant tissues and also with chlorophyll concentration. Future studies should focus on the long-term effects of different cobalt forms on soil microbes and biological nitrogen fixation, considering timing and dose of application and how cobalt interacts with other soil amendments. Such studies may effectively help in establishing sustainable fertilization programs to increase crop yields while preserving healthy soils.

Keywords: Biological nitrogen fixation; Soil available N; Nitrogenase activity; Faba bean; Cobalt sources.

1. Introduction

Faba bean (*Vicia faba* L.), also known as broad beans, horse beans, or field beans (Vishnupriya *et al.*, 2024), is an affordable and valuable source of proteins (Abou-Khater *et al.*, 2022) in over 66 countries around the world (Long *et al.*, 2022; Zehring *et al.*, 2022). It is considered a low-fat diet (Labba *et al.*, 2021), rich in antioxidants and bioactive elements (Dhull *et al.*, 2022). It also contains polyphenols that help in reducing cardiovascular and neurological risk (Poonia *et al.*, 2022). Approximately 32% of global faba bean production occurs in the Mediterranean and East African area (Sabry *et al.*, 2021; Abou-Khater *et al.*, 2022). In Egypt, faba bean is a staple of the national diet (Hegab *et al.*, 2014). Its production was estimated at approximately 200,000 tons in 2024, obtained from 52.08 hectares (EAS, 2024). Nevertheless, this production fails to meet the local demand (Attia and El-Sayed, 2022) and often yields relatively low total revenues (Kandil, 2022).

Enhancing biological N fixation (BNF) is a promising strategy to cut down input costs, and also guarantees not only to enhance faba bean yield but also increases residual soil nitrogen availability for subsequent crops (Jithesh *et al.*, 2024). Various additives, such as cobalt were found to be effective in enhancing biological N fixation (Moro *et al.*, 2021; Barbosa *et al.*, 2023). In particular, Co is a beneficial element for many higher plants (Hu *et al.*, 2021; Gowidan *et al.*, 2022), which can increase plant uptake of macro- and micronutrients from soil (Banerjee and Roychoudhury, 2021b), besides improving plant tolerance towards biotic (pathogens and herbivores) and abiotic stresses (drought and salinity) (Naz *et al.*, 2023). It is also a component of several enzymes and co-enzymes in plants, including antioxidant enzymes (Hareem *et al.*, 2024) and nitrogenase in nitrogen-fixing bacteria (Li *et al.*, 2024). Many studies highlighted the role of cobalt in boosting plant

*Corresponding author e-mail: mohamed.abbas@fagr.bu.edu.eg

Received: 02/08/2025; Accepted: 12/09/2025

DOI: 10.21608/EJSS.2025.410062.2297

©2025 National Information and Documentation Center (NIDOC)

productivity (Abdalla *et al.*, 2024; Elshamly and Nassar, 2025; Gad *et al.*, 2025), yet this study uniquely connects the form and dose of cobalt with the physiological and biochemical responses of plants and also with the biological nitrogen fixation in faba bean

The current study investigates the impacts of using cobalt as a soil additive - either in the form of sulphates or chlorides- on faba bean growth, yield components, biological nitrogen fixation, and associated physiological traits. Probably, cobalt sulphate is more effective than cobalt chloride in promoting plant growth and increasing biological nitrogen fixation, because of its lower toxicity in addition to its incorporation in sulphate assimilated pathways of proteins, allowing plants to absorb and use Co more easily (**hypothesis I**). Fenerally, cobalt application increases nodulation and nitrogenase activity, which boosts nitrogen levels in plants and soil (**hypothesis II**). Additionally, cobalt helps to prevent chlorophyll breakdown under stress, supporting better photosynthesis and yield (**hypothesis III**). However, high cobalt doses ($> 10 \text{ mg L}^{-1}$), particularly in chloride forms, can be toxic, minimizing plant growth (**hypothesis IV**). The current research aims align with the United Nations Sustainable Development Goals, particularly **SDG 2** (Zero Hunger) and **SDG 12** (Responsible Consumption and Production), via endorsing sustainable agriculture, improving soil fertility through biological nitrogen fixation, and increasing crop through efficient nitrogen use.

2. Materials and Methods

2.1 Materials of study

This study was conducted for two consecutive winter seasons (2022/2023 and 2023/2024) at the Agricultural Research Station in El-Giza Governorate, Egypt ($30^{\circ}48'52''$ N latitude, $31^{\circ}81'25''$ E longitude). Before cropping, soil samples (0-30 cm) were collected from the farm, air dried, crushed and analyzed for their physical and chemical characteristics as outlines by Dane and Topp (2020) and Sparks *et al.* (2020), respectively. These characteristics are presented in Table 1.

Table 1. Selected physical and chemical properties of the experimental soil.

Soil characteristics	Value	Soil characteristics	2022	2023
Particle size distribution (%)		Chemical properties		
Clay	46.21	pH (1:2.5 soil water suspension)	7.69	7.53
Silt	29.18	$\text{CaCO}_3 \text{ (g kg}^{-1}\text{)}$	15.20	16.20
Sand	24.61	Organic carbon (g kg^{-1})	12.04	9.40
Textural class	Clay	EC (dS m^{-1} , paste extract)	1.25	1.32
Physical properties		Available nutrients (mg kg^{-1})		
Bulk density (Mg m^{-3})	1.23	N	24.18	22.16
Wilting point moisture (%)	16.28	P	9.33	8.445
Field capacity moisture (%)	33.12	K	258.66	273.6
Available water (%)	16.84	Co	0.069	0.13
		Fe	6.32	7.31
		Mn	0.98	1.05
		Zn	0.56	0.64

Seeds of *Vicia faba* L. (cv. Giza 716) were kindly obtained from the Field Crops Research Institute, Agricultural Research Center (ARC), Giza, Egypt. Thirty minutes before sowing, seeds were inoculated with *Rhizobium leguminosarum* (sourced from Cairo Mercin) using a 10% sugar solution as adhesive material. Two cobalt sources—cobalt sulfate ($\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$ >99% purity, Merck, Germany) and cobalt chloride ($\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, >98% purity, Merck, Germany)—were used to prepare four different cobalt concentrations: 0.0, 5.0, 10.0, and 15.0 mg L^{-1} . Previous research indicates that cobalt effects are dose-dependent, i.e. low concentrations up to $10.0 \text{ mg Co L}^{-1}$ may enhance plant growth (Hu *et al.*, 2021); whereas higher levels can induce toxicity (Hu *et al.*, 2021; Faiyad and AbdEl-Azeiz, 2024).

2.2. Field Investigation

A field experiment was conducted following a randomized complete block design (RCBD), in which the following 7 treatments were considered: 3 doses of Co applied to soils (5.0, 10.0, and 15.0 mg L⁻¹) from two sources—cobalt sulfate and cobalt chloride in addition to the reference control treatment (0 mg Co L⁻¹). Each plot was 3.0 × 3.5 meters (approximately 10.5 m²), and all treatments were replicated three times. Seeds were sown on the 15th of October 2022 and 2023, and thinned at three weeks after emergence to two plants per hill. All plots received the recommended doses of NPK, i.e. 50 kg of N in the form of ammonium sulfate (20.6% N) ha⁻¹ applied as a starter dose to stimulate the activities of N fixers; 247.1 kg ha⁻¹ single superphosphate (15% P₂O₅) during land preparation; and 123.6 kg ha⁻¹ potassium sulfate (48% K₂O) at flowering. Soil applications of Co were carried out twice, i.e. at 45 and 60 days after planting (DAP).

2.3. Plant Measurements

Plant height and number of branches per plant were recorded at the flowering stage from five randomly selected plants per every experimental plot. Also, total chlorophyll was determined at this growth stage using the DMSO extraction method (Nayek et al., 2014) and a UV-VIS spectrophotometer (JENWAY 6705, UK). At physiological maturity (pods turned into yellow or brown), yield parameters including 100-seed weight, total seed yield, and straw biomass were measured.

2.4. Plant and Soil Analyses

Samples of the collected plant materials were cleaned from dirt, washed with deionized water, oven-dried at 70°C, ground, and digested using a 1:1 mixture of sulfuric acid–hydrogen peroxide (Jones et al., 1991). Total nitrogen was determined via the micro-Kjeldahl method, while phosphorus was determined colorimetrically after being reduced with ascorbic acid (Sparks et al., 2020) then determined with a Spectrophotometer model SM1600 UV-VIS. Potassium was measured using flame photometry (JENWAY PFP7). Micronutrients (Fe, Mn, Zn, Cu) were determined in grains using Atomic Absorption Spectrophotometer (Perkin-Elmer 372). Carbohydrates were determined in grains using the procedure of Dubois et al. (1956), and crude protein content was calculated by multiplying total nitrogen by 6.25 (Deyoe and Shellenberger, 1965).

Available nitrogen (N) in the soil was extracted using 2N potassium chloride (KCl). Then reduced to ammonium (NH₄⁺) using Devarda's alloy, then the available ammonium content was determined by micro Kjeldahl. Nodulation was evaluated at 50 DAP as follows: numbers and weights of active nodules were quantified per plant, while nitrogenase activity was assessed using the acetylene reduction assay (Hardy et al., 1968).

2.5. Statistical Analyses

Data were analyzed using SPSS software (version 18). Treatment means were compared using Tukey's HSD test at the 0.05 significance level. All graphical presentations were generated using Sigma Plot version 10.

3. Results

3.1. Activity of N-fixing bacteria

Application of Co, irrespective of its form (CoCl₂ or CoSO₄), significantly increased the number of nodules per plant, their weights and nitrogenase activity enzyme in soil up to an application dose of 10 mg Co L⁻¹ (Fig 1). Higher dose of Co (15 mg Co L⁻¹) led to significant reductions in these parameters, with an exception of nodule weights per plant which did not vary significantly between the higher application dose of Co (15 mg Co L⁻¹) in the form of CoCl₂ and the lower dose of this salt, i.e. 10 mg Co L⁻¹. Notably these abovementioned parameters were noteworthy higher for the application of CoSO₄ versus CoCl₂.

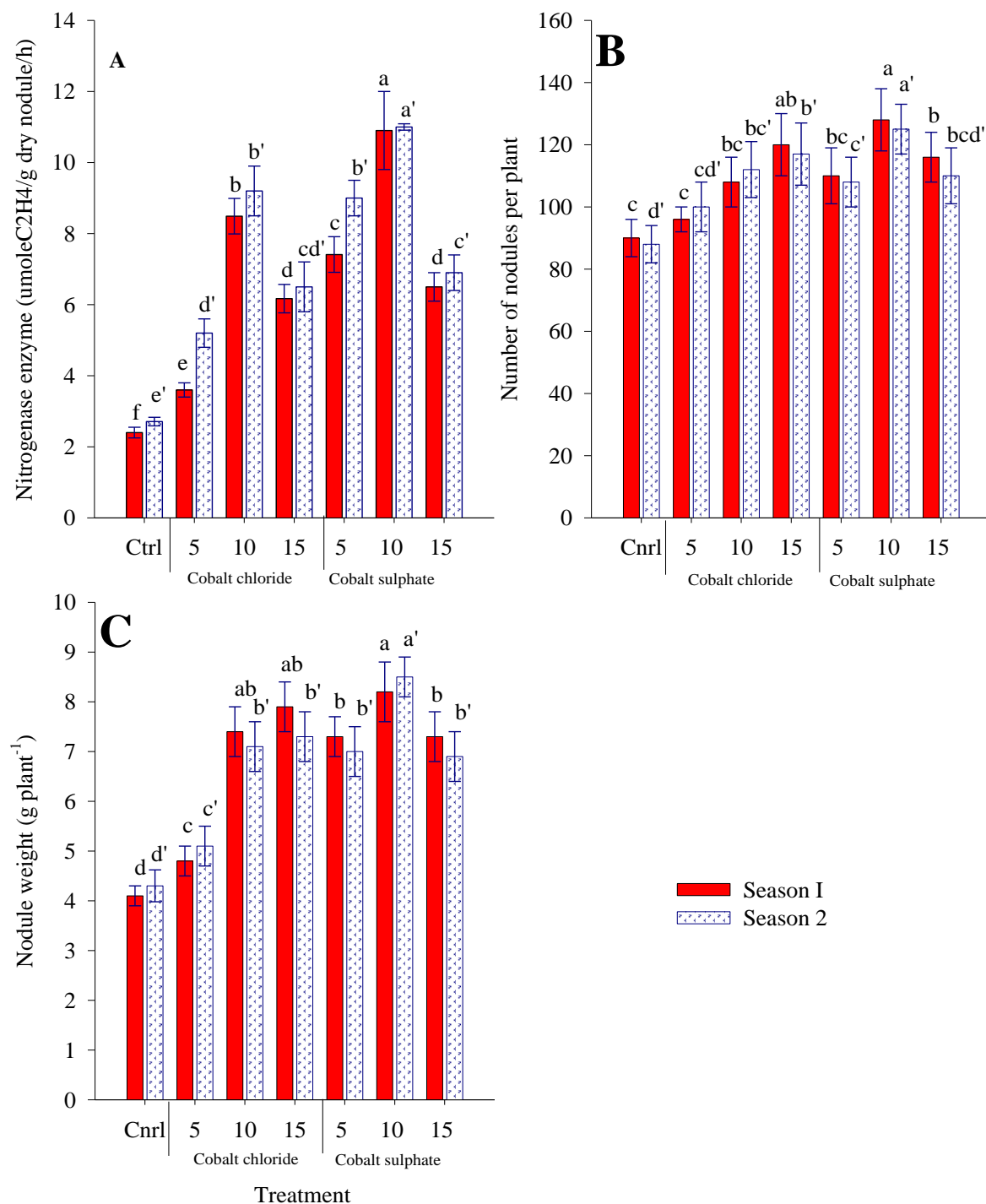


Fig. 1. Effects of cobalt chloride and sulphate on bacterial nodulation and nitrogenase activity in soil (mean \pm standard deviation). Similar letters indicate no significant variations among treatments.

3.2. Available N, and its accumulations in faba bean parts

Significant increases occurred in nitrogen available content in soil as well as N- contents in both shoots and roots due to the application of Co, either in the form of CoCl₂ or CoSO₄ (Fig 2). Increasing the rate of applied Co up to 10 mg Co L⁻¹ led to concurrent significant increases in N concentrations in both shoots and seeds, with superiority for CoSO₄ applications on straw. The higher dose of CoCl₂ lessened considerably available N content in soil, probably because of Cl toxicity on plants. This, in turn, led to significant reductions in N content in shoots, while it did not significantly affect N content in seeds. On the contrary, the higher dose of CoSO₄ raised significantly available N content in soil and also raised N concentrations within faba bean shoots, but not in grains. These results were confirmed in the two successive seasons of study.

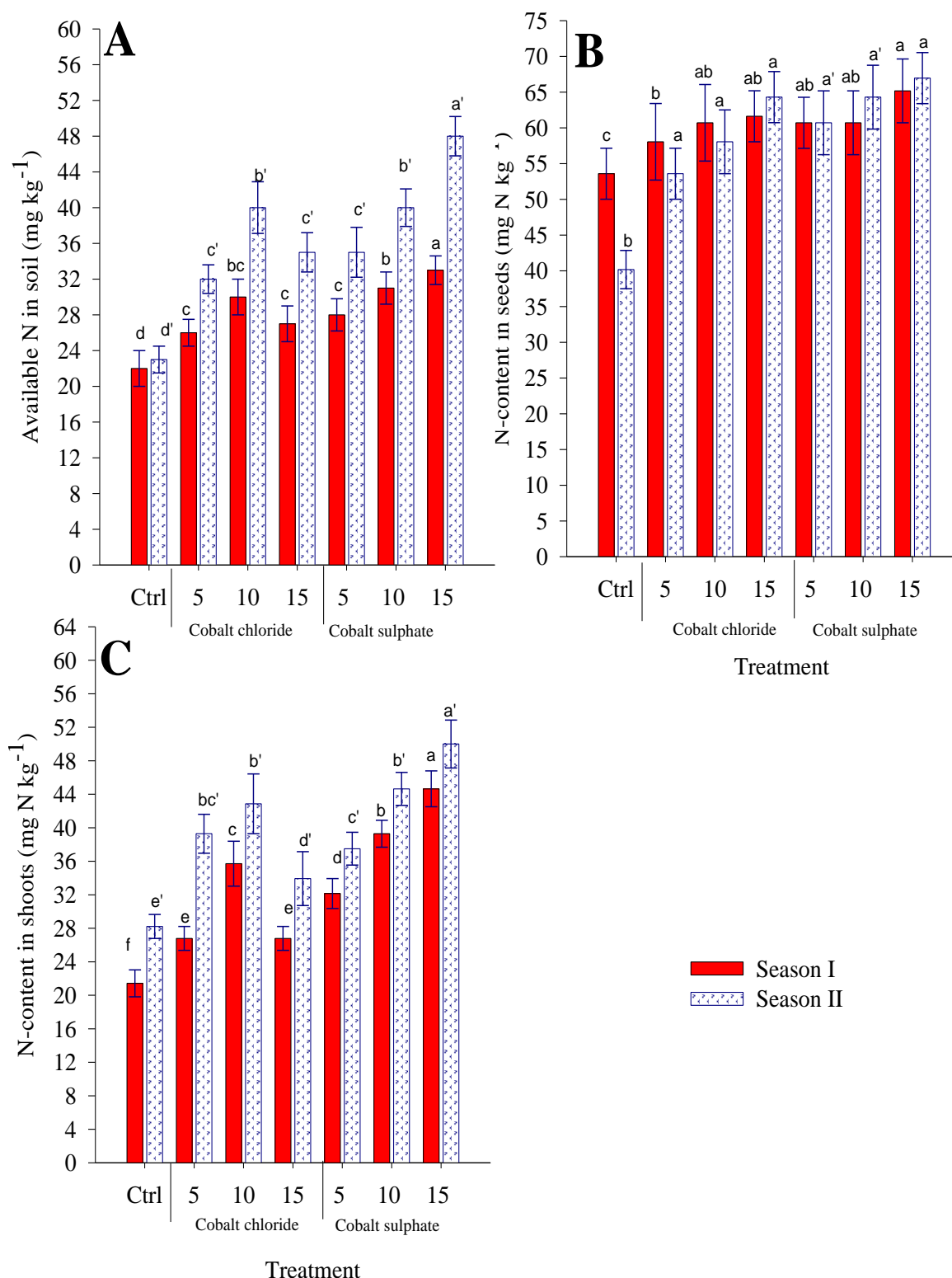


Fig. 2. Effects of cobalt chloride and sulphate on available N and its accumulation in plant tissues (mean \pm standard deviation). Similar letters indicate no significant variations among treatments.

3.3. Cobalt concentrations in plant tissues and chlorophyll content

Application of Co raised significantly its content within plant tissues (Fig 3). Such increases were more pronounced with increasing the rate of applied Co, and in particular, CoSO_4 applications recorded significantly higher increases in Co concentrations versus CoCl_2 . Remarkably, Co levels increased substantially for the

application of 15 mg Co L⁻¹ (CoCl₂ or CoSO₄) versus the application of 10 mg Co L⁻¹. In other words, the dilution effect might be remarkable upon Co additions at lower doses (up to 10 mg Co L⁻¹), while at the higher one (15 mg L⁻¹) high accumulation of Co occurred without equivalent increases in plant growth. Likewise, application of Co raised significantly chlorophyll content in plant shoots, with superiority of CoSO₄ application versus CoCl₂. These results were confirmed in two successive seasons of study, especially with increasing the rate of application of Co.

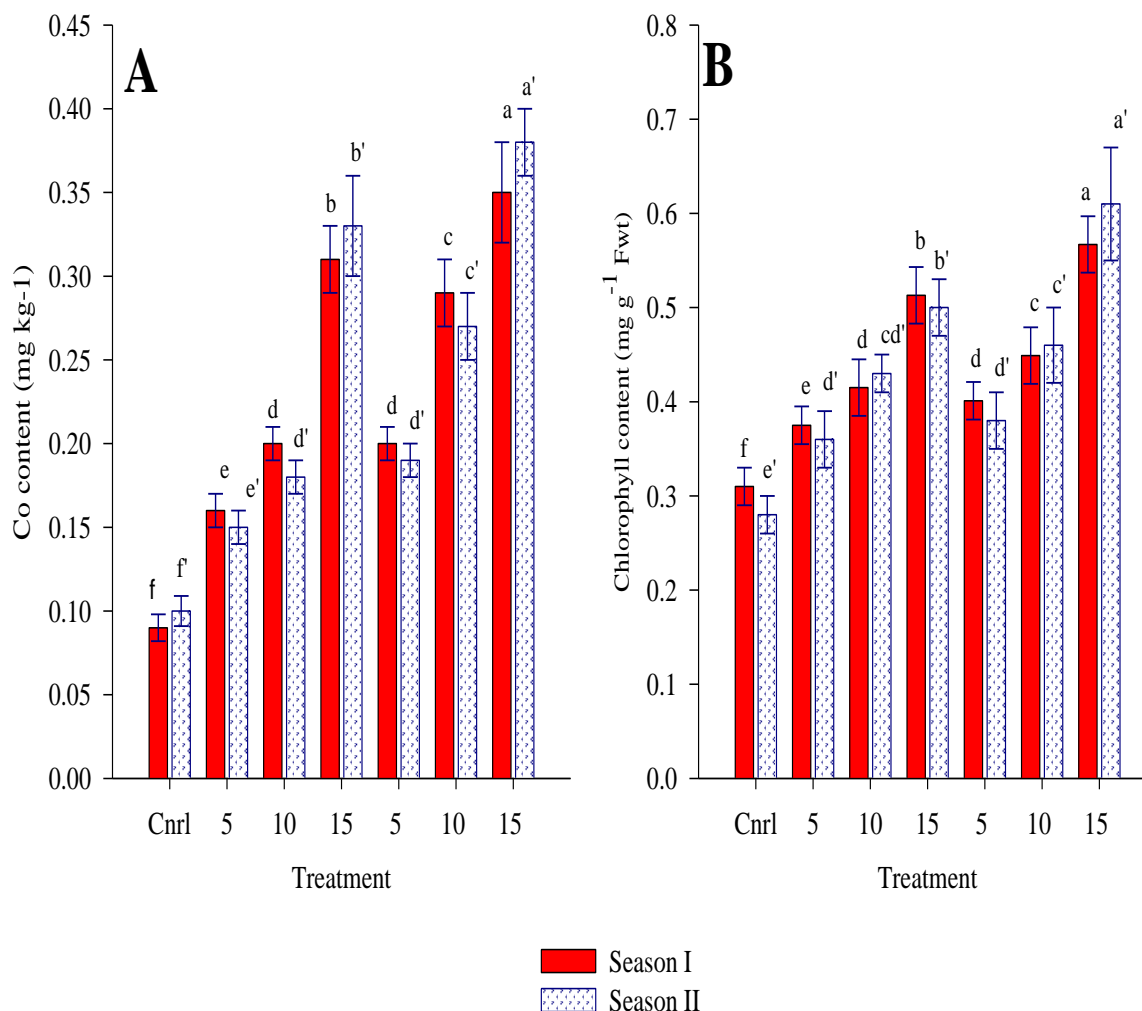


Fig. 3. Effects of cobalt chloride and sulphate on chlorophyll and cobalt accumulation in bean shoots. Similar letters indicate no significant variations among treatments.

3.4. Faba bean growth parameter and yield components

Application of cobalt boosted significantly grains and straw yields of faba bean as well as the seed components named 100 grain weight and no of pods per plant, though these applications did not significantly affect either the number of branches per plant or even plant height (Fig 4). Probably, Co was more involved in initiating and developing seeds rather than enhancing plant vegetative growth. The effects of CoSO₄ on straw and seed yields were considerably different from those of CoCl₂. For example, increasing the dose of applied CoCl₂ resulted in concurrent significant increases in straw and grain yields. On the other hand, only significant increases occurred in straw and grain yields due to application of CoSO₄ at 5 mg Co/L; nevertheless, the increases in Co doses seemed to be almost ineffective. Overall, CoSO₄ impacted seed yields, while CoCl₂ augmented more the vegetative growth.

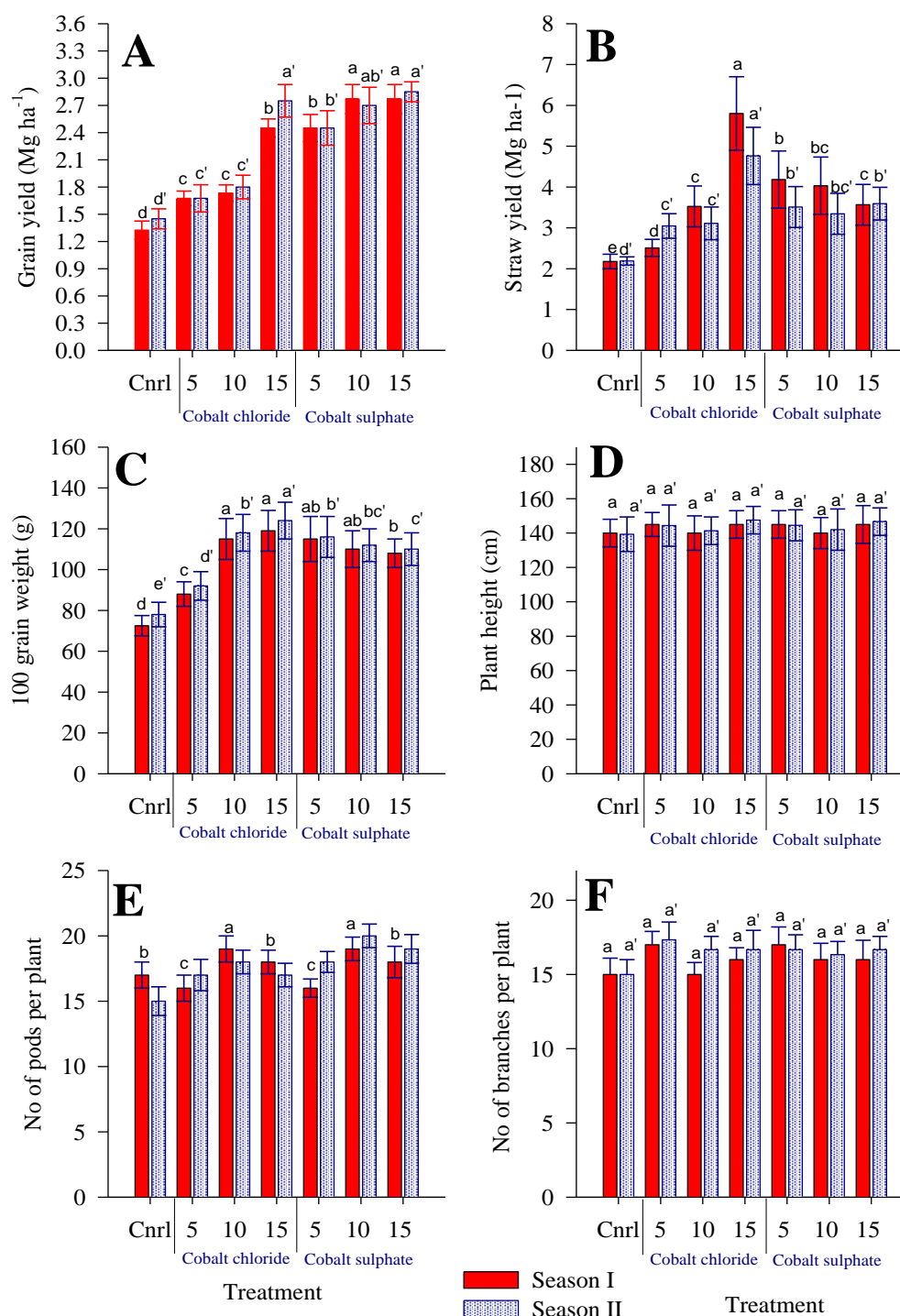


Fig. 4. Effects of cobalt chloride and sulphate on the growth and yield of faba bean (mean± standard deviation). Similar letters indicate no significant variations among treatments.

3.5. Correlations among Co accumulation in shoots, biological nitrogen fixation in soil, and growth performance in faba bean plants

Seed yield and its attributes (100 seed weight and no of pods per pant) were significantly correlated with Co content in shoots. Likewise, plant chlorophyll and soil N-related biological activities (nodule numbers, weights and nitrogenase activity) were both positively and significantly correlated with Co content in plants. Furthermore, chlorophyll content in plants was correlated significantly with all growth parameters and yield attributes as well as with the soil biochemical markers under investigations (nodule numbers, weights and nitrogenase activity).

Table 2. Correlations Among Cobalt accumulation in plant shoots, their contents in photosynthetic pigment, Nitrogen Fixation, and Agronomic Traits of faba beans.

	Nitro	Seed yield	Nodule weight	Av N	Straw yield	Nodule no	N shoot	100 Grain weight	N grain	Plant height	Pod no	No branches	Co-shoot	chlorophyll
nitrogenase														
Seed yield	0.625**													
Nod weight	0.882**	0.808**												
Available N	0.566**	0.520**	0.599**											
Straw yield	0.420**	0.690**	0.739**	0.520**										
Number of nodules per plant	0.828**	0.333*	0.951**	0.604**	0.724**									
N shoot	0.358*	0.597**	0.561**	0.488**	0.118	0.356*								
100- grain weight	0.763**	0.727**	0.322*	0.349*	0.788**	0.827**	0.519**							
N grain	0.529**	0.751**	0.725**	0.611**	0.687**	0.762**	0.380*	0.758**						
Plant height	0.045	0.370*	0.219	0.323*	0.302	0.325*	0.239	0.393*	0.551**					
No of pods per plant	0.539**	0.346*	0.604**	0.425**	0.274	0.653**	0.449**	0.490**	0.378*	0.282				
No branches	0.223	0.343*	0.227	0.427**	0.220	0.338*	0.374*	0.405**	0.523**	0.813**	0.082			
Co-shoot	0.468**	0.903**	0.776**	0.343**	0.503**	0.554**	0.370*	0.638**	0.649**	0.203*	0.314*	0.218		
Chlorophyll	0.423**	0.815**	0.353*	0.488**	0.623**	0.708**	0.648**	0.672**	0.768**	0.442**	0.511**	0.317*	0.799**	

*P<0.05

**P<0.01

Nitro: nitrogenase;

Probably, increasing the biological activities of N-fixers increased plant assimilation and photosynthesis, though in our cases we assume that this relation is two way, For example N-fixers improved the nutritional status of the plant as a mechanism to increase plants metabolites that reach nodules from the host plant. This in turn increased the symbiotic relation between plants and N-fixers. Overall, the increases that took place in shoot and root biomasses were the consequences of increasing N available content and its accumulation in plants as a result of inspiring the biological activities in soil

4. Discussion

4.1. Bacterial nodulation and nitrogenase activity in soil

Soil Co application enhanced significantly biological nitrogen fixation (BNF) via increasing the number and weights of bacterial nodules as well as the activities of the nitrogenase enzyme in soil. These effects were noticeable within the two seasons of study as Co is a structural component of vitamin B₁₂ (cobalamin) (Warren *et al.*, 2002), needed by *Rhizobia* metabolism to enhance symbiotic efficiency (Li *et al.*, 2024). Additionally, cobalt stabilized FeMo-cofactor in nitrogenase, which is responsible of breaking the strong triple bond of N₂ (Dos Santos *et al.*, 2012). Another indirect benefit is cobalt's role in reducing oxidative stress in nodules via supporting bacterial antioxidant enzymes like superoxide dismutase (SOD), which protects from reactive oxygen species (ROS) produced during photosynthesis (Ma *et al.*, 2021). The impacts of Co soil applications were the highest in case of addition of 10 mg Co L⁻¹, while decreased at higher doses (15 mg Cd/L), likely because cobalt toxicity inhibited microbial proliferation (Zaborowska *et al.*, 2016) or probably due to competition with essential micronutrients like Zn or Mn for uptake (Chatterjee and Chatterjee, 2000). Overall, cobalt sulphate stimulated better bacterial nodulation than cobalt chloride, within the two seasons of study. This difference is mostly attributed to the less stressful anion (SO₄²⁻ vs. Cl⁻) on *Rhizobia* viability, as well as sulfur's additional benefit in supporting amino acid synthesis as mentioned above. It is worth mentioning that the contents of Co within faba bean shoots were significantly correlated with nodule weight per plant ($r^2=0.776$) and also with the nitrogenase enzyme activity ($r^2=0.468$). This provides strong evidence of cobalt's crucial involvement in legume–rhizobia symbiosis (Hu *et al.*, 2021).

4.2. Cobalt accumulation in bean shoots and plant biochemical marker (chlorophyll)

Cobalt concentrations significantly increased in plant shoots as the dose of Co increased. Likewise, chlorophyll content was notably enhanced by cobalt application, especially at higher doses. This beneficial nutrient (Co) stimulates nitrate reductase activity, which supports nitrogen assimilation and is also considered critical for chlorophyll biosynthesis (Ali *et al.*, 2010). Additionally, it modulates reactive oxygen species (ROS) levels (Tourky *et al.*, 2023), which can degrade chlorophyll, if not controlled, (Pospíšil, 2016). Chlorophyll content was generally much higher in plants treated with CoSO_4 compared to those treated with CoCl_2 as the latter exhibited less Co content in plant tissues than the former. Mostly, sulfate-based salts are generally less phytotoxic than chloride-based salts (Fort *et al.*, 2014; Hütsch *et al.*, 2018), resulting in more efficient uptake and translocation of the metal (Kabata-Pendias, 2010). In contrast, chloride ions may cause mild root toxicity that hampers overall nutrient absorption (Geilfus, 2018). A strong positive correlation ($r^2 = 0.799$) was detected between cobalt concentrations in shoots and plant chlorophyll content, indicating cobalt's critical role in maintaining photosynthetic efficiency (Hong *et al.*, 2019). This contradicts the findings by Pérez-Espinosa *et al.* (2002), who reported that cobalt application induced a significant decrease in chlorophyll and carotenoid contents in tomato plants (Perez-Espinosa *et al.*, 2002), probably because they used high levels which induced toxicity (Patra *et al.*, 2019).

4.3. Available N, and its accumulations in faba bean straw and grains

Application of cobalt significantly raised available nitrogen content in soil and increased its accumulation in plant shoots and seeds within the two seasons of study. In this regard, CoSO_4 showed a superior effect on nitrogen availability and accumulation in plant tissues than CoCl_2 , likely because of the higher toxicity of chloride ions (Cl^-) relative to sulfate ions (SO_4^{2-}), as mentioned above. Increasing cobalt application rate from 5 to 10 mg/L resulted in significant increases in concentrations of available N in soil and also Co content in shoots. However, further increases in the dose of applied Co from 10 to 15 mg/L led to contrasting effects between the two cobalt sources. For example, available soil N and shoot N content declined at high doses of CoCl_2 , while for CoSO_4 , these two parameters continued to increase, indicating that CoSO_4 is safer and more plant-compatible.

These findings suggest that plants treated with CoSO_4 could use more cobalt without experiencing toxicity, because cobalt is a beneficial plant nutrient, which regulates various developmental and metabolic aspects of plants (Banerjee and Bhattacharya, 2021a). In addition, the counter ion (sulfate) is essential in protein synthesis and chlorophyll formation (Koprova and Rennenberg, 2004; Marschner, 2012). On the contrary, Cl ions, though being essential at small amounts, higher concentrations can be toxic, affecting osmotic balance and membrane integrity of plants (Geilfus, 2018). On the other hand, variations in nitrogen content in grains were minimal among treatments. Nevertheless, all cobalt treatments—regardless of source or dose—raised grain N content. This elevated N appears to have been efficiently utilized in enhancing seed productivity. This nitrogen is therefore needed for seed productivity rather than being accumulated in plants in high concentrations. Regarding the N cycle in soil, it was found that increases in N contents in both shoots and grains were positively and significantly correlated with N available content in soil and also with the activity of nitrogenase enzyme. These results confirm the dual pathways of nitrogen acquisition in legumes—through biological nitrogen fixation (BNF), and better root development (Hu *et al.*, 2021) to increase mineral uptake from soil (Ul Hassan *et al.*, 2023). Moreover, all growth and yield parameters—except number of branches per plant—showed significant correlations with both nitrogen content in plant tissues and chlorophyll content. These physiological factors serve as integrated indicators for plant growth and demonstrate cobalt's multifaceted role in plant physiology through preserving chlorophyll from degradation under stressful conditions (Akeel and Jahan, 2020; Inayat *et al.*, 2024) as well as its role in symbiotic enhancement (Hu *et al.*, 2021).

4.4. Faba bean growth parameters and yield components

Cobalt application significantly enhanced both grain and straw yields as well as 100-grain weight ($P < 0.05$). These results are consistent with the previous reports that observed significant increases in faba bean yield due to Co supplementation (Sherif *et al.*, 2017; Gad *et al.*, 2022; Elsonbaty *et al.*, 2024; Gad and El-Habbak, 2024), even under salt stress conditions (Baddour *et al.*, 2024; Faiyad and AbdEL-Azeiz, 2024). This is because cobalt is an essential element of several enzymes and co-enzymes that affect the growth and metabolism of plants (Hu *et al.*, 2025). The highest improvements in shoot and grain yields were achieved herein for the application of 10 mg Co L⁻¹, while higher doses had no further impacts on plant yield (except for the grains of plants treated with CoCl_2). Thus, 10 mg L⁻¹ is the optimal concentration threshold beyond which additional Co provides diminishing returns. Again, the foliar application of CoSO_4 exhibited superior efficiency in boosting grain yield

and 100 grain weight versus CoCl_2 . This might be attributed to the role of sulfur in the biosynthesis of sulfur-containing amino acids (cystine, methionine) which are crucial for the storage of proteins in grains (Kopriva *et al.*, 2015). Contrastingly, CoSO_4 application exhibited much lower straw yield than that of CoCl_2 . As chloride ions mediate certain physiological responses, such as osmotic adjustment and ethylene production (Fatma *et al.*, 2022); yet it is toxic at high levels. Nevertheless, this efficiency was limited, as the grain yield recorded for CoCl_2 were still lower than that of CoSO_4 . The application of cobalt recorded minimal or no significant impacts on either plant height, number of nodules per plant, or the number of branches per plant.

5. Conclusion

Soil application of cobalt, especially at a rate of 10 mg L^{-1} , enhanced significantly biological nitrogen fixation, as well as the growth, yield and yield components of faba bean. Higher Co dose (15 mg L^{-1}) exhibited acute toxicity to BNF as it decreased considerably number and activity of nitrogen fixing bacteria, while exhibited slight toxicity on plants as their straw and grain yields remained largely unaffected as this higher application rate except in grain plants sprayed with CoCl_2 . Overall, plant growth and yield of faba bean were significantly correlated with both nitrogenase activity in soil and Co level in shoots. These results confirm the dual roles of Co in legumes, which are: (1) increasing biological nitrogen fixation (BNF), and (2) benefiting plants themselves. Markedly, CoSO_4 exhibited superior effects than CoCl_2 on enhancing faba bean growth and productivity. Thus, it is important to choose the appropriate form and dose of cobalt to achieve the goals of developmental sustainability. Future studies should focus on the long-term effects of different cobalt forms on soil microbes and biological nitrogen fixation, considering timing and dose of application, and how cobalt interacts with other soil amendments. Such studies may effectively help in establishing sustainable fertilization programs to increase crop yields while preserving healthy soils.

Declarations

Ethics approval and consent to participate

Consent for publication: The article contains no such material that may be unlawful, defamatory, or which would, if published, in any way whatsoever, violate the terms and conditions as laid down in the agreement.

Availability of data and material: Not applicable.

Competing interests: The authors declare that they have no conflict of interest in the publication.

Funding: Not applicable.

Authors' contributions: All authors contributed equally in writing the original draft, editing and finalizing the manuscript. All authors read and agree for the submission of manuscript to the journal.

Acknowledgments: Authors gratefully acknowledge Prod Dr. Hassan H. Abbas (Faculty of Agriculture, Benha University) for his valuable guidance and constructive suggestions throughout this study. His support and expertise greatly enriched the quality of this work. They also extend their sincere thanks to the editor and anonymous reviewers of the Egyptian Journal of Soil Science (EJSS) for their careful reading and insightful comments that improved the quality of this manuscript.

References

- Abd El-Aty, M., EL-Hity, M., Abo Sen, T., Eid, M., Sheteiwy, M., and Abd EL-Rahaman, I. (2025). Improvement the yield and its components as response to selection in early generations of two crosses of Faba bean.. *Egyptian Journal of Agronomy*, 47(3), 405–417. <https://doi.org/10.21608/agro.2025.361265.1633>
- Abdalla, K., Mousa, S., Ibrahim, M., and Metwally, A. (2024). Impacts of Cobalt, Selenium and Silicon Biofortification on the Growth, Productivity and Nutritional Value of Lettuce. *Egyptian Journal of Horticulture*, 51(1), 71–86. <https://doi.org/10.21608/ejoh.2023.231176.1261>
- Abou-Khater, L., Maalouf, F., Rubiales, D. (2022). Status of Faba Bean (*Vicia faba* L.) in the Mediterranean and East African Countries. In: Jha, U.C., Nayyar, H., Agrawal, S.K., Siddique, K.H.M. (eds) Developing Climate Resilient Grain and Forage Legumes. Springer, Singapore. https://doi.org/10.1007/978-981-16-9848-4_14
- Akeel, A., Jahan, A. (2020). Role of Cobalt in Plants: Its Stress and Alleviation. In: Naeem, M., Ansari, A., Gill, S. (eds) Contaminants in Agriculture. Springer, Cham. https://doi.org/10.1007/978-3-030-41552-5_17
- Ali, B., Hayat, S., Hayat, Q., and Ahmad, A. (2010). Cobalt stress affects nitrogen metabolism, photosynthesis and antioxidant system in chickpea (*Cicer arietinum* L.). *Journal of Plant Interactions*, 5(3), 223–231. <https://doi.org/10.1080/17429140903370584>
- Attia, R., and El-Sayed, M. (2022). An economic study of gap bridging proposal of faba beans in Egypt. *Egyptian Journal of Agricultural Research*, 100(4), 692–700. <https://doi.org/10.21608/ejar.2022.153503.1257>

- Baddour, A., El-Sherpiny, M., Sakara, H. (2021). Effect of Rhizobium inoculant, Nitrogen Starter and Cobalt on Stimulation of Nodulation, N Fixation and Performance of Faba Bean (*Vicia faba* L.) Grown under Salinity Stress. *Journal of Soil Sciences and Agricultural Engineering*, 12(2), 61-69. <https://doi.org/10.21608/jssae.2021.153322>
- Banerjee, A., and Roychoudhury, A. (2021a). Beneficial aspects of cobalt uptake in plants exposed to abiotic stresses. In *Frontiers in plant-soil interaction: Molecular insights into plant adaptation* (Chapter 18, pp. 523–529). Elsevier. <https://doi.org/10.1016/B978-0-323-90943-3.00012-2>
- Banerjee, P., Bhattacharya, P. (2021b) Investigating Cobalt in Soil-plant-animal-human system: Dynamics, Impact and Management. *J Soil Sci Plant Nutr* **21**, 2339–2354. <https://doi.org/10.1007/s42729-021-00525-w>
- Barbosa, H. M., Alvarez, R. C. F., de Lima, S. F., Cordeiro, M. A. S., Zanella, M. S., and Bernardo, V. F. (2023). *Bradyrhizobium* and *Azospirillum* co-inoculation associated with cobalt and molybdenum application in the soybean crop. *Ciência Rural*, 53(7). <https://doi.org/10.1590/0103-8478cr20210871>
- Chatterjee, J., and Chatterjee, C. (2000). Phytotoxicity of cobalt, chromium and copper in cauliflower. *Environmental Pollution*, 109(1), 69–74. [https://doi.org/10.1016/S0269-7491\(99\)00238-9](https://doi.org/10.1016/S0269-7491(99)00238-9)
- Dane, J.H., and Topp, G.C. (2020). *Methods of soil analysis: Part 4—Physical methods* (3rd ed.). Soil Science Society of America.
- Dhull, S. B., Kidwai, M. K., Noor, R., Chawla, P., and Rose, P. K. (2022). A review of nutritional profile and processing of faba bean (*Vicia faba* L.). *Legume Science*, 4(3), e129. <https://doi.org/10.1002/leg3.129>
- Dos Santos, P.C., Fang, Z., Mason, S.W. Setubal, J. C., and Dixon, R. (2012) Distribution of nitrogen fixation and nitrogenase-like sequences amongst microbial genomes. *BMC Genomics* **13**, 162. <https://doi.org/10.1186/1471-2164-13-162>
- EAS (2024), Bulletin of The Agricultural Statistics Part (1) winter crops (2024). Economic Affairs Sector (EAS) of the Ministry of Agriculture and Land Reclamation, Arab republic of Egypt.
- Elshamly, A.M.S., and Nassar, S.M.A. (2024)Impacts of cobalt and zinc on improving peanuts nutrient uptake, yield and irrigation water use efficiency under different irrigation levels. *Sci Rep* **14**, 7188 . <https://doi.org/10.1038/s41598-024-56898-2>
- Elsonbaty, A., and elsherpiny, M. (2024). Improving the Yield and Quality of Faba Bean Grown in Alkaline Soils Using Agricultural Gypsum, Organic Fertilizers and Cobalt. *Egyptian Journal of Soil Science*, 64(3), 1285-1303. <https://doi.org/10.21608/ejss.2024.292907.1778>
- Faiyad, R., and AbdEL-Azeiz, E. (2024). Mitigation The Deleterious Effect of Salinity on Faba Bean by Cobalt and Bio-stimulants. *Egyptian Journal of Soil Science*, 64(1), 181-192. <https://doi.org/10.21608/ejss.2023.238554.1667>
- Fatma, M., Asgher, M., Iqbal, N., Rasheed, F., Sehar, Z., Sofu, A., and Khan, N. A. (2022). Ethylene signaling under stressful environments: Analyzing collaborative knowledge. *Plants*, 11(17), 2211. <https://doi.org/10.3390/plants11172211>
- Fort, D. J., Mathis, M. B., Walker, R., Tuominen, L. K., Hansel, M., Hall, S., Richards, R., Grattan, S. R., and Anderson, K. (2014). Toxicity of sulfate and chloride to early life stages of wild rice (*Zizania palustris*). *Environmental Toxicology and Chemistry*, 33(12), 2802–2809. <https://doi.org/10.1002/etc.2744>
- Gad, N., and El-Habbak, A. (2024). Can Cobalt and/or Nitrogen Sources be an Optimum Solution for Improving Characteristics of Broad Bean Plants?. *Journal of Sustainable Agricultural Sciences*, 50(1), 1-11. <https://doi.org/10.21608/jsas.2024.266293.1447>
- Gad, N., El-Mettwally, I. M., and Solieman, N. Y. (2022). Response of faba bean (*Vicia faba* L.) productivity and economic evaluation to cobalt. *Journal of Positive School Psychology*, 6(2), 6261–6271.
- Gad, N., Elrahman, E. A. A., Ali, M. E. F., Abou-Shlell, M. K., Teiba, I. I., Almutairi, M. H., and Yousef, A. F. (2025). Role and importance of cobalt in faba bean through rationalization of its nitrogen fertilization. *BMC Plant Biology*, 25, 872. <https://doi.org/10.1186/s12870-025-06596-6>
- Gad, N., Fekry Ali, M. E., and Abou-Hussein, S. D. (2017). Improvement of faba bean (*Vicia faba* L.) productivity by using cobalt and different levels of compost under new reclaimed lands. *Middle East Journal of Applied Sciences*, 7(3), 493–500.

- Geilfus, C.-M. (2018). Chloride: From nutrient to toxicant. *Plant and Cell Physiology*, 59(5), 877–886. <https://doi.org/10.1093/pcp/pcy071>
- Gowidan, M. K., Gad, N., Abbas, M. H., and Farid, I. M. (2022). Improving sunflower (*Helianthus annuus*) growth and productivity using cobalt application. *Annals of Agricultural Science, Moshtohor*, 60(4), 9321–1246. <https://doi.org/10.21608/assjm.2022.285137>
- Hareem, M., Danish, S., Obaid, S. A., Ansari, M. J., and Datta, R. (2024). Mitigation of drought stress in chili plants (*Capsicum annuum* L.) using mango fruit waste biochar, fulvic acid and cobalt. *Scientific Reports*, 14, 14270. <https://doi.org/10.1038/s41598-024-65082-5>
- Hegab, A. S. A., Fayed, M. T. B., Hamada, M. M. A., and Abdrabbo, M. A. A. (2014). Productivity and irrigation requirements of faba-bean in North Delta of Egypt in relation to planting dates. *Annals of Agricultural Sciences*, 59(2), 185–193. <https://doi.org/10.1016/j.aos.2014.11.004>
- Hong, D. D., Anh, H. T. L., Tam, L. T., Show, P. L., and Leong, H. Y. (2019). Effects of nanoscale zerovalent cobalt on growth and photosynthetic parameters of soybean *Glycine max* (L.) Merr. DT26 at different stages. *BMC Energy*, 1(6). <https://doi.org/10.1186/s42500-019-0007-4>
- Hu X, Wei X, Ling J and Chen J (2021) Cobalt: An Essential Micronutrient for Plant Growth? *Front. Plant Sci.* 12:768523. <https://doi.org/10.3389/fpls.2021.768523>
- Hütsch, B. W., Keipp, K., Glaser, A. K., and Schubert, S. (2018). Potato plants (*Solanum tuberosum* L.) are chloride-sensitive: Is this dogma valid? *Journal of the Science of Food and Agriculture*, 98(8), 3161–3168. <https://doi.org/10.1002/jsfa.8819>
- Inayat H, Mehmood H, Danish S, Alharbi SA, Ansari MJ, Datta R. (2024) Impact of cobalt and proline foliar application for alleviation of salinity stress in radish. *BMC Plant Biol* 24(1):287. <https://doi.org/10.1186/s12870-024-04998-6>
- Jithesh, T., James, E. K., Iannetta, P. P. M., Howard, B., Dickin, E., and Monaghan, J. M. (2024). Recent progress and potential future directions to enhance biological nitrogen fixation in faba bean (*Vicia faba* L.). *Plant-Environment Interactions*, 5, e10145. <https://doi.org/10.1002/pei3.10145>
- Kabata-Pendias, A. (2010). *Trace elements in soils and plants* (4th ed.). CRC Press. <https://doi.org/10.1201/b10158>
- Kandil, S. (2022). Production and marketing of faba bean crop in Egypt. *Alexandria Science Exchange Journal*, 43(1), 93–104. <https://doi.org/10.21608/asejaiqsae.2022.217523>
- Kopriva, S., and Rennenberg, H. (2004). Control of sulphate assimilation and glutathione synthesis: Interaction with N and C metabolism. *Journal of Experimental Botany*, 55(404), 1831–1842. <https://doi.org/10.1093/jxb/erh203>
- Kopriva, S., Calderwood, A., Weckopp, S. C., and Koprivova, A. (2015). Plant sulfur and big data. *Plant Science*, 241, 1–10. <https://doi.org/10.1016/j.plantsci.2015.09.014>
- Labba, I.-C. M., Frøkiær, H., and Sandberg, A.-S. (2021). Nutritional and antinutritional composition of fava bean (*Vicia faba* L., var. minor) cultivars. *Food Research International*, 140, 110038. <https://doi.org/10.1016/j.foodres.2020.110038>
- Li, Y., Liu, Q., Zhang, D.-X., Zhang, Z.-Y., Xu, A., Jiang, Y.-L., and Chen, Z.-C. (2024). Metal nutrition and transport in the process of symbiotic nitrogen fixation. *Plant Communications*, 5(4), 100829. <https://doi.org/10.1016/j.xplc.2024.100829>
- Long J, Wu W, Sun S, Shao Y, Duan C, Guo Y and Zhu Z (2022) *Berkeleyomyces rouxiae* is a causal agent of root rot complex on faba bean (*Vicia faba* L.). *Front. Plant Sci.* 13:989517. <https://doi.org/10.3389/fpls.2022.989517>
- Ma, J., Song, Z., Yang, J., Wang, Y., and Han, H. (2021). Cobalt ferrite nanozyme for efficient symbiotic nitrogen fixation via regulating reactive oxygen metabolism. *Environmental Science: Nano*, 8(1), 188–203. <https://doi.org/10.1039/D0EN00935K>
- Marschner, P. (2012). *Marschner's mineral nutrition of higher plants* (3rd ed.). Academic Press.
- Moro, L., Franz, M. F., Ecco, M., Melgarejo Arrúa, M. A., and Ribas, M. A. (2021). Response of soybean crop with different combinations of seed treatment and application of nitrogen, cobalt, and molybdenum topdressing. *Revista Facultad Nacional de Agronomía Medellín*, 74(3), 9667–9674. <https://doi.org/10.15446/rfnam.v74n3.92760>

- Naz, M., Raza, M. A., Bodlah, M. A., Bouzroud, S., Ghani, M. I., Riaz, M., Shah, T., Zubair, A., Bodlah, I., and Fan, X. (2023). Beneficial elements in plant life under a changing environment. In S. Pandey, D. K. Tripathi, V. P. Singh, S. Sharma, and D. K. Chauhan (Eds.), *Beneficial chemical elements of plants* (Chapter 1). Wiley. <https://doi.org/10.1002/9781119691419.ch1>
- Patra, K. K., Oberoi, D., Joshi, R. K., Prasad, R., and Pandey, D. D. (2019). Changes of Photosynthetic Parameters in *Jatropha curcas* L. Leaves under Cobalt Stress. *INTERNATIONAL JOURNAL OF PLANT AND ENVIRONMENT*, 5(04), 278–283. <https://doi.org/10.18811/ijpen.v5i04.8>
- Pérez-Espinosa, A., Moreno-Caselles, J., Moral, R., Pérez-Murcia, M. D., and Gómez, I. (2002). Effect of cobalt on chlorophyll and carotenoid contents in tomato plants. *Journal of Plant Nutrition*, 25(9), 1933–1940. <https://doi.org/10.1081/PLN-120013285>
- Poonia, A., Vikranta, U., Chaudhary, N., Dangi, P. (2022). Current and Potential Health Claims of Faba Beans (*Vicia Faba*, L.) and Its Components. In: Punia Bangar, S., Bala Dhull, S. (eds) Faba Bean: Chemistry, Properties and Functionality. Springer, Cham. https://doi.org/10.1007/978-3-031-14587-2_13
- Pospíšil P (2016) Production of Reactive Oxygen Species by Photosystem II as a Response to Light and Temperature Stress. *Front. Plant Sci.* 7:1950. <https://doi.org/10.3389/fpls.2016.01950>
- Sabry, A. M., El-Ghozoli, M. A., Ali, I. M. E., and Rashed, H. S. A. (2021). Effects of different biogas manures and their extracts on dry matter yield and nutrient uptake by faba bean (*Vicia faba* L.) grown under sandy soil conditions. *Annals of Agricultural Science, Moshtohor*, 59(1), 123–136. <https://doi.org/10.21608/assjm.2021.182716>
- Sherif, A. E. A., ElKhalawy, S. M. A., and Hegab, E. A. (2017). Impact of nitrogen and cobalt rates on faba bean crop grown on clayey soil. *Journal of Soil Science and Agricultural Engineering, Mansoura University*, 8(9), 459–465. <https://doi.org/10.21608/jssae.2017.37580>
- Sparks, D. L., Page, A. L., Helmke, P. A., Loeppert, R. H., Soltanpour, P. N., Tabatabai, M. A., Johnston, C. T., and Sumner, M. E. (Eds.). (2020). *Methods of soil analysis: Part 3—Chemical methods* (3rd ed.). Soil Science Society of America.
- Tourky, S. M. N., Shukry, W. M., Hossain, M. A., Siddiqui, M. H., Pessarakli, M., and Elghareeb, E. M. (2023). Cobalt enhanced the drought-stress tolerance of rice (*Oryza sativa* L.) by mitigating the oxidative damage and enhancing yield attributes. *South African Journal of Botany*, 159, 191–207. <https://doi.org/10.1016/j.sajb.2023.05.035>
- Ulhassan, Z., Shah, A.M., Khan, A.R., Azhar, W., Hamid, Y. and Zhou, W. (2023). Mechanisms of Cobalt Uptake, Transport, and Beneficial Aspects in Plants. In *Beneficial Chemical Elements of Plants* (eds S. Pandey, D.K. Tripathi, V.P. Singh, S. Sharma and D.K. Chauhan). <https://doi.org/10.1002/9781119691419.ch7>
- Vishnupriya, S., Roshini, D., Bhavaniramy, S., Karthiayani, and Ramar, V. (2024). Faba bean starch: Structure, functionality, and applications. In *Non-Conventional Starch Sources* (pp. 409–438). Elsevier. <https://doi.org/10.1016/B978-0-443-18981-4.00014-8>
- Warren, M. J., Raux, E., Schubert, H. L., and Escalante-Semerena, J. C. (2002). The biosynthesis of adenosylcobalamin (vitamin B12). *Natural Product Reports*, 19(4), 390–412. <https://doi.org/10.1039/B108967F>
- Zaborowska M, Kucharski J, Wyszowska J (2016) Biological activity of soil contaminated with cobalt, tin, and molybdenum. *Environ Monit Assess.* 188(7):398. <https://doi.org/10.1007/s10661-016-5399-8>.
- Zehring, J., Walter, S., Quendt, U., Zocher, K., and Rohn, S. (2022). Phytic Acid Content of Faba Beans (*Vicia faba*)—Annual and Varietal Effects, and Influence of Organic Cultivation Practices. *Agronomy*, 12(4), 889. <https://doi.org/10.3390/agronomy12040889>