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Enhancement of Grain Yield and Nutritional Composition of faba bean by Foliar Application of Cobalt

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FABA bean is a nutrient-rich winter legume in Egypt that improves soil fertility through biological nitrogen fixation (BNF). Cobalt (Co) can further increase its productivity and boost BNF. This nutrient has been recently listed as a micronutrient for fertilization in the Official Journal of the European Union, Regulation (EU) 2019/1009, but information on its foliar application remains limited. Probably, this foliar application at 35 days after sowing increase plant growth, and also indirectly enhance root nodulation during its peak activity because of its intermediate mobility in plant phloem. However, excess Co may impair plant growth and photosynthesis, though optimal concentrations should be decided to avoid growth inhibition. This study evaluates the effects of foliar application of Co (0, 5, 10, and 15 mg L^{-1}) in two forms ($CoSO_4$ and $CoCl_2$) on faba bean plants under field conditions for two successive years (2022/2023 and 2023/2024). Results revealed that foliar application of cobalt (Co) significantly enhanced morphological (e.g. plant height), physiological (e.g. chlorophyll content), biochemical (e.g. nutrient accumulation in shoots and biological nitrogen fixation), and yield-related traits in faba bean (100 grain weight, total seed yield and their contents of protein and carbohydrate). These applications also recorded significant indirect impacts on nodule biomass and nitrogenase activity within the rhizosphere, with optimal results at 10 mg Co L^{-1}. A higher Co dose (15 mg L^{-1}) further improved straw yield and chlorophyll levels, yet, its had adverse effects on grain yield-related parameters and negatively diminished biological nitrogen fixation. Probably, diluting Co concentrations through increasing the vegetative growth was survival strategy under stress; however, reproductive processes — such as flowering, grain formation, and filling require a balanced supply of nutrients and assimilates. Among the two sources of Co, $CoSO_4$ was more effective than $CoCl_2$ in increasing phosphorus, iron, manganese, and zinc uptake, whereas $CoCl_2$ favoured nitrogen accumulation, though potassium remained unaffected. Generally, seed yield and quality parameters were positively correlated with nutrient accumulation in shoots, chlorophyll content and nodulation. These findings highlight the importance of cobalt role in promoting the physiological performance and nutritional value of faba beans, with 10 mg L^{-1} identified as the optimum level. Future research should focus on molecular and transcriptomic responses of cobalt under field conditions, including nutrient uptake regulations, its mobility within both plants and nodules, considering stress mitigation routes

Keywords: *Vicia faba*; Nutrient uptake; Chlorophyll; Nitrogenase; Co salts.

1. Introduction

Faba bean (*Vicia faba* L.), a member of the Fabaceae family (Bangar and Kajla, 2022), is an important winter legume crop in many parts of the world (Abou-Khater *et al*., 2022), because of its high protein content (20–38%) (Abd El-Aty *et al*., 2023; Essa *et al*., 2023) and balanced essential amino acids (Bangar and Kajla, 2022; Martineau-Côté *et al*., 2022). Additionally, it contains carbohydrates (58.3%), fiber (25%), and bioactive antioxidants such as phenolics and flavonoids (Dhull *et al*., 2022). Moreover, it is known by its health benefits such as cholesterol reduction and anti-inflammatory effects (Martineau-Côté *et al*., 2022). Faba bean remains an affordable staple in Egypt and other Mediterranean regions (Abdelaal and Soliman, 2022; Al-Suwaina *et al*., 2022), where it is used in preparing traditional dishes like falafel and Medamis (Martineau-Côté *et al*., 2022). Despite that, the local production of faba bean in Egypt does not meet domestic demand (Abou-Khater *et al*., 2022), so Egypt imports to meet local consumption (Al-Suwaina *et al*., 2022). Faba beans help to improve soil fertility through biological nitrogen fixation (BNF) (Sabry et al., 2021; Abd El-Aty *et al*., 2023; Essa *et al*., 2023. This process (BNF) reduces the dependence on synthetic fertilizers for both the current and subsequent crops, thereby sustaining farming systems (Jithesh *et al*., 2024; Pu *et al*., 2025). Among grain legumes, faba beans exhibits the highest potential for BNF (Klippenstein *et al*., 2022) mediated by *Rhizobium leguminosarum* (Klippenstein *et al*., 2022).

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Cobalt (Co) is an essential nutrient for the growth of many lower plants and benefits higher plants (Gowidan et al., 2022), especially those in the Leguminosae family, when being supplied at optimal levels (Hu *et al*., 2021; Kafeel *et al*., 2023). It is a component of several enzymes and proteins in plant metabolism and is also a part of cobalamin (vitamin B_{12}), which plays a critical role in N_2 fixation within bacterial nodules (Hu *et al*., 2021). In this process, N-fixers reduce N_2 to NH_3, through a process mediated by the nitrogenase enzyme complex (Kafeel et al., 2023). Accordingly, soil application of cobalt can directly stimulate rhizobial activity, leading to enhanced nodulation and nitrogenase enzymes (Hu *et al*., 2021; Faiyad and AbdEL-Azeiz, 2024). The present objective is to determine how foliar spraying of cobalt could enhance root nodulation, but information available on its foliar application remains limited. Since Co is needed in small amounts in leaves for plant health (Elsonbaty and Elsherpiny, 2024; Ulukapı *et al*., 2024; Radi *et al*., 2025); then the absorbed Co by foliar application may be redistributed within plants, exhibiting intermediate mobility in phloem (Riesen and Feller, 2005). This nutrient can be translocated from shoots to roots, then transferred to nodules at lower non-toxic concentrations (Hu *et al*., 2021).

In faba beans, nodulation becomes visible approximately 14–20 days after planting, with maximum nodulation and activity between 35 and 50 days. Thus, foliar cobalt application at around 30–35 days might ensure that sprayed cobalt reaches nodules in a suitable time to support efficient nitrogen fixation while also enhancing plant resilience under stress conditions. In this aspect, Gad *et al*. (2025) found that the application of Co at 50 days after sowing may also improve nodulation parameters of faba beans. Besides, foliar application provides rapid uptake of cobalt while avoiding micronutrient antagonism in soil. Recently, Co has been explicitly listed as a micronutrient for fertilization in the Official Journal of the European Union, Regulation (EU) 2019/1009 (2019); yet this does not classify it as a universally essential nutrient for all plants. Nevertheless, relatively high cobalt concentrations can negatively affect plant growth and photosynthetic efficiency (Ali *et al*., 2023); thus, it is important to find out the suitable concentrations of Co for foliar application.

This study examines the impacts of spraying Co on faba bean plants grown under field conditions across two growing seasons, with three Co levels (5, 10, and 15 mg Co L^{-1}) in two forms ($CoCl_2$ and CoSO. It is hypothesized that the effectiveness of Co addition depends on its chemical form ($CoCl_2$ vs. $CoSO_4$) and concentration **(Hypothesis I)**. At optimal levels, cobalt is thought to improve the physiological and biochemical performance of faba beans, thereby promoting plant growth and yield **(Hypothesis II)**. It can also have indirect impacts via increasing nodule biomass and nitrogenase activity in the rhizosphere **(Hypothesis III)**. On the contrary, high cobalt doses could be toxic to both plants while exhibits less toxicity on nitrogen-fixing bacteria if Co primary reached through the phloem downward pathway from shoots to roots owing to the detoxifying mechanisms of cobalt within plant tissues **(Hypothesis IV)**. This research provides new insights into how foliar application of Co influences the growth and productivity of faba beans. Unlike most previous studies, it compares different cobalt forms ($CoSO_4$ and $CoCl_2$), considering their distinct physiological effects. It also determines the optimal dose for Co foliar application through systematic assessment of three doses (5, 10, and 15 mg Co L^{-1}) to maximize benefits while avoiding toxicity. Additionally, this study establishes detailed mechanistic relationships among shoot nutritional status, nodule activity, and seed quality parameters. This work aligns with several US Sustainable Development Goals, particularly (1) **SDG 2 (Zero Hunger)**: by using Co to increase food production, (2) **SDG 13 (Climate Action**: decreasing dependence on chemical synthetic fertilizers, and (3) **SDG 15 (Life on Land)**: by following sustainable practices.

2. Materials and Methods

2.1. Materials of study

A field experiment was conducted at the El-Giza Agricultural Research Station, El-Giza Governorate, Egypt (Latitude: 30°48′52″ N; Longitude: 31°81′25″ E) for two successive winter years (2022/2023 and 2023/2024). Prior to crop cultivation, the soil was sampled (0-30 cm), air dried, crushed and analysed for their physical and chemical properties as suggested by Dane and Topp (2020) and Sparks *et al*. (2020), respectively, and the obtained results are presented in Table 1.

Table 1. Physical and chemical characteristics of the experimental soil .

Soil characteristics	Value	Soil characteristics	2022	2023
Particle size distribution%:		*Soil chemical properties:*		
Clay	46.21	pH (1:2.5 soil water suspension)	7.69	7.53
Silt	29.18	$CaCO_3$ (g kg^{-1})	15.2	16.20
Sand	24.61	Organic carbon (g kg^{-1})	12.04	9.40
Textural class *	Clay	EC (dS m^{-1}; soil paste extract)	1.25	1.32
Soil physical properties:				
Bulk density Mg m^{-3}	1.23	Soil moisture at wilting point %	16.28	
Soil moisture at field capacity %	33.12	Available Water %	16.84	

Available Nutrients, mg kg^{-1}	N	P	K	Co	Fe	Mn	Zn
2022	24.18	9.33	258.66	0.069	6.32	0.98	0.56
2023	22.16	8.45	273.6	0.13	7.31	1.05	0.64

Seeds of faba bean (Vicia faba L., var. Giza 716) were obtained from the Field Crops Research Institute, Agricultural Research Center (ARC), Giza, Egypt. *Rhizobium leguminosarum* was obtained from Cairo Mercin. This inoculant was then with seeds using a 10% sugar solution as an adhesive material, air-dried in shade for about 30 minutes before sowing. Two sources of cobalt were used in this study, i.e. CoSO4 $CoSO_4$.$7H_2O$ >99% purity, Merck, Germany) and CoCl2 ($CoCl_2$.$6H_2O$, >98% purity, Merck, Germany). These salts were used to prepare four Co concentrations: 0.0, 5.0, 10.0, and 15.0 mg L^{-1}.

2.2. Experimental procedures

A field experiment was conducted for two consecutive winter seasons (2022/2023 and 2023/2024), comprising foliar application of Co at three rates (5.0, 10.0, and 15.0 mg L^{-1}) from two sources—cobalt sulfate and cobalt chloride. All plots were 3.0 × 3.5 meters (approximately 10.5 m^2) arranged in split-split design with three replicates. The source of Co was assigned to main plots while the dose was placed in the subplots. Seed sowing was carried out in all plots in October 2022 and 2023. Three weeks after seedling emergence, plants were thinned to maintain two plants per hill. All experimental plots received the recommended doses of N, P and K, i.e.50 kg ha^{-1} ammonium sulfate (20.6% N), split into three equal applications during the growing season, 46 kg P ha^{-1} as single superphosphate (8.5% P) during land preparation and 80 kg K ha^{-1} as potassium sulfate (40% K) at the flowering stage. Cobalt treatments were sprayed twice: at 45 and 60 days after sowing.

At the flowering stage, plant height and number of branches per plant were measured in 5 plants selected randomly from the two inner ridges of each plot. Total chlorophyll content was also estimated in the fourth upper leaf using the DMSO extraction method (Nayek et al., 2014) and a UV/VIS spectrophotometer (JENWAY 6705, UK). At the maturity stage (pods turned into yellow or brown), yield and yield components such as 100-seed fresh weight, total seed yield, and straw biomass were recorded. Seed biochemical traits, including total protein and carbohydrate, were also considered following AOAC (2005).

2.3. Plant and soil analyses

Plant samples were washed with tap water, rinsed with deionized water, dried at 70°C, ground, and digested using H_2SO_4 –H_2O_2 (Parkinson and Allen, 1975). Total nitrogen was measured in these plant digests using the micro-Kjeldahl method (Page et al., 1982), while P was measured by UV/Vis spectrophotometer (JENWAY 6705, UK) and potassium by flame photometer (JENWAY PFP7). Micronutrients (Fe, Mn, Zn, Cu) were analysed via Atomic Absorption Spectrophotometer (Perkin-Elmer 372). Carbohydrates were measured colorimetrically according to Dubois *et al*. (1956), and crude protein was calculated by multiplying total N by 6.25 (Deyoe and Shellenberger, 1965). Nodulation was assessed at 50 days post-sowing, in which total and active biomass were recorded and nitrogenase activity was evaluated via the acetylene reduction assay (Hardy *et al*., 1968) and expressed as µmol C_2H_4 g^{-1} h^{-1}.

2.4. Statistical Analyses

Data analysis was conducted using SPSS statistical software (SPSS 18), followed by Tukey's test at the 0.05 significance level to compare means. Graphs were created with SigmaPlot 10.

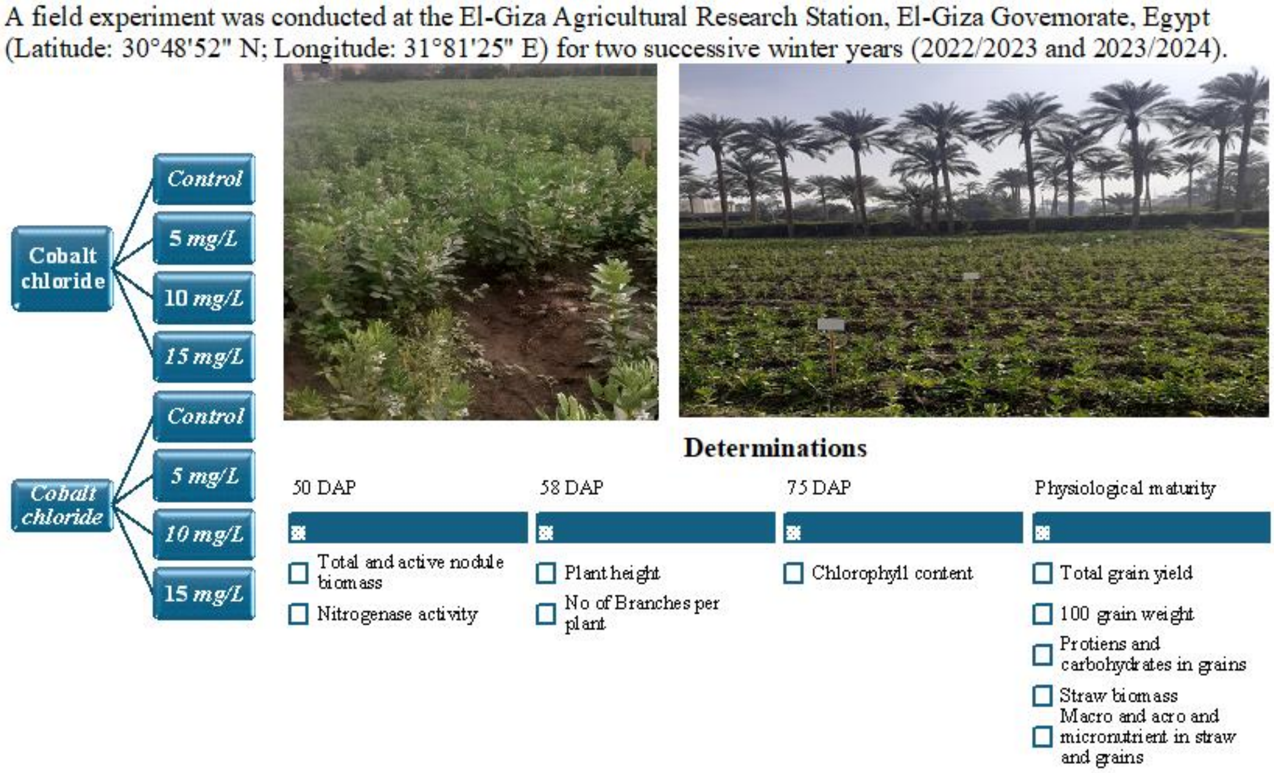


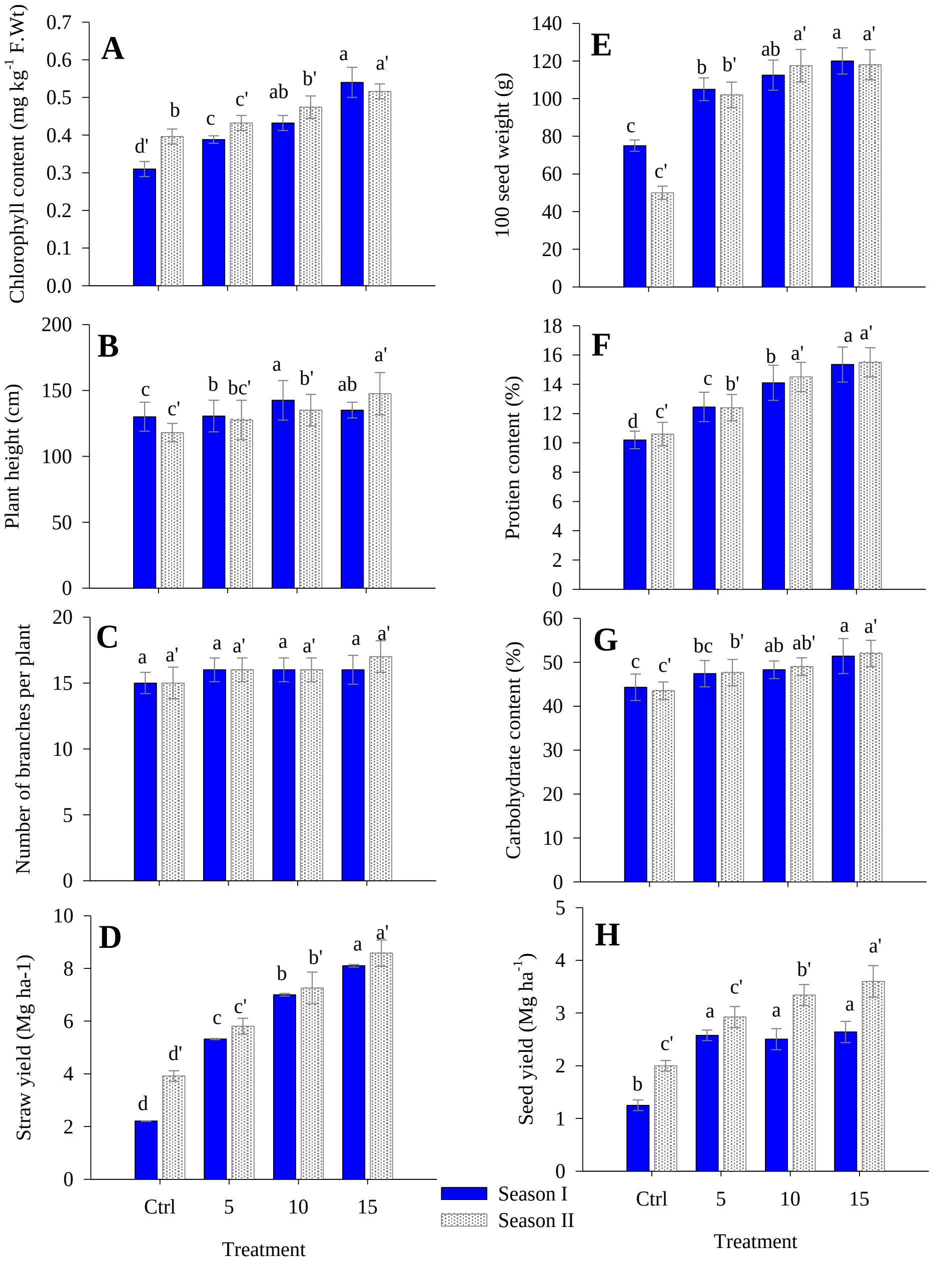
Fig. 1. A flowchart of the study.

3. Results

3.1. Plant growth parameters

Application of Co (irrespective of its source) significantly increased the following plant parameters: chlorophyll content (2 A), plant height (2 B), and straw yield (2D), across the two seasons of study. Such increases were more pronounced with increasing the dose of Co application, except for 100 seed weight, which did not vary significantly between the two higher doses of Co application (10 and 15 mg Co L^{-1}). Additionally, the source of cobalt significantly impacted the aforementioned growth parameters, with superiority for $CoSO_4$ over $CoCl_2$ ($P<0.05$). An exception was chlorophyll content in the first season, where no significant variations were detected between the two Co sources. Overall, these results indicated that cobalt enhanced both the biochemical (chlorophyll) and morphological (plant height) traits, resulting in higher straw yield. On the other hand, the number of branches per plant was not significantly affected by either the source of Co or the dose of its application.

The four-panel Fig 3 illustrates the interactive effects of two Co salts ($CoCl_2$ and $CoSO_4$) applied as a foliar spray at three concentrations (5, 10 and 15 mg Co L^{-1}) on key growth parameters of faba bean plants over two successive seasons. This interaction was only significant for straw yield (Fig 3A), and the highest increases were observed due to the application of $CoSO_4$ at a rate of 15 mg Co L^{-1} (9.32 Mg ha^{-1} in the first season and 9.95 Mg ha^{-1} in the second season), while the lowest values were recorded for the application of $CoCl_2$ at a rate of 5 mg Co L^{-1} (4.55 and 5.25 Mg ha-1 in the 1st and 2nd seasons, respectively). For other growth parameters, the interaction between Co source and dose was not significant. For example, slight insignificant variations were detected in plant height, i.e., the overall increase was (~3–5 cm) among treatments.



	Plant growth parameters							
	Straw Yield		Chlorophyll content		Plant height		Number of branches per plant	
	SI	SII	SI	SII	SI	SII	SI	SII
P_{Type}	**	**	ns	*	*	*	ns	ns
P_{Dose}	**	**	**	**	**	**	ns	ns
$P_{Type*Dose}$	**	**	ns	ns	ns	ns	ns	ns
	Yield and yield components							
	Seed yield		100 seed weight		Protein content		Carbohydrate content	
	SI	SII	SI	SII	SI	SII	SI	SII
P_{Type}	ns	*	**	ns	**	**	*	**
P_{Dose}	**	**	**	**	**	**	**	**
$P_{Type*Dose}$	ns	ns	**	**	ns	ns	ns	ns

ns: not significant

Fig. 2. Grand means of the impacts of applied Co doses on the growth and productivity of faba bean plants. Similar letters indicate no significant variations among treatments.

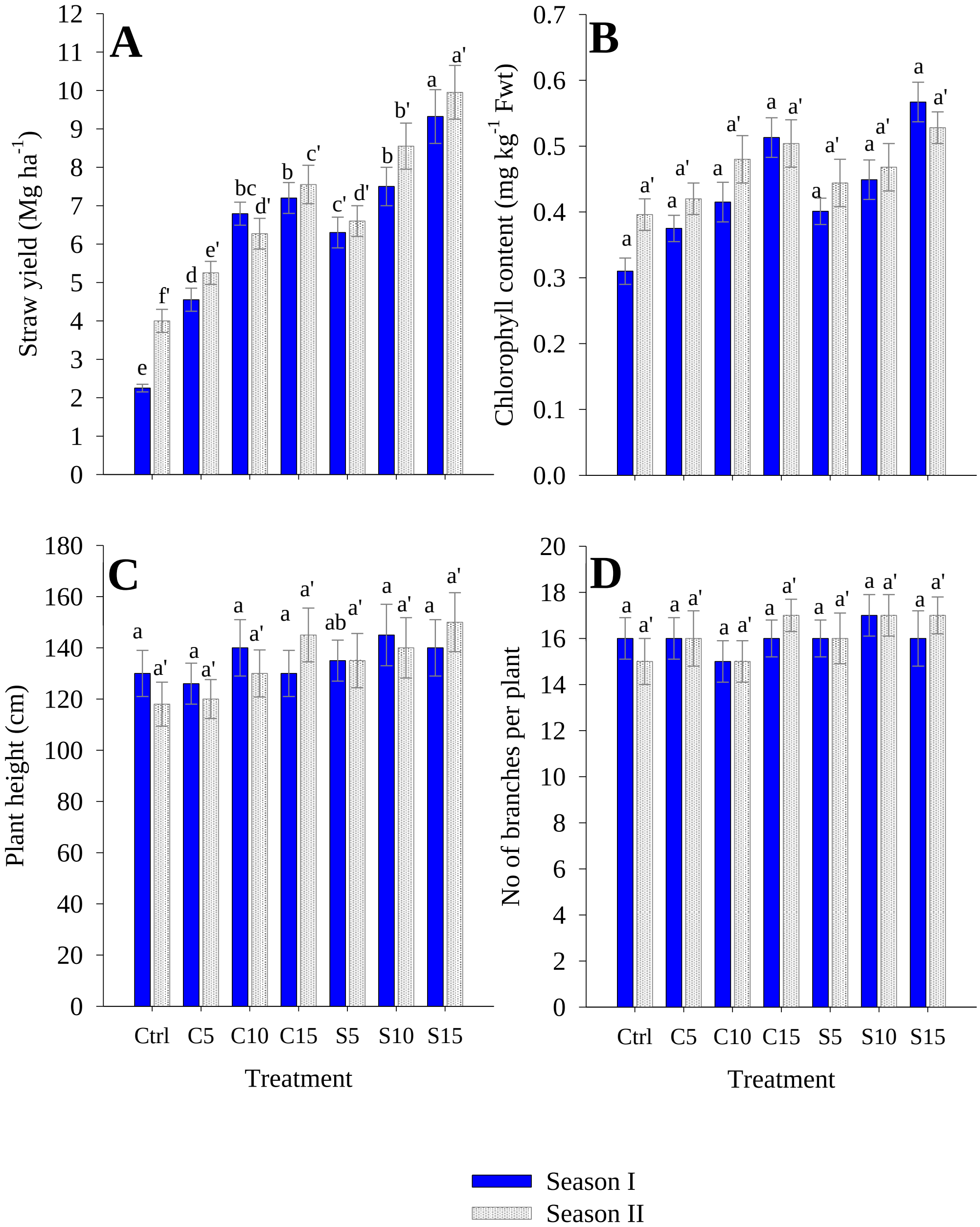


Fig. 3. Plant growth parameters as affected by the form and rate of applied Co. Note :$CoCl_2$ applied rates were 5 mg L^{-1} (C5), 10 mg L^{-1} (C10) and 15 mg L^{-1} (C15), while $CoSO_4$ applied rates were 5 mg L^{-1} (S5), 10 mg L^{-1} (S10) and 15 mg L^{-1} (S15). Similar letters indicate no significant variations among treatments.

3.2. Grain Yield and Quality Attributes

Application of Co salts ($CoSO_4$ or $CoCl_2$) significantly enhanced grain yield quantity (Fig 2 H) and quality (100 grain weight, proteins and carbohydrates grains, Figs 4 E, F and G) up to a rate of 10 mg Co L^{-1}; nevertheless, no further significant increases occurred in these parameters owing to application of the higher Co dose (15 mg Co L^{-1}). The form of Co ($CoSO_4$ vs $CoCl_2$) seemed to be less effective than the dose of application. Nevertheless, $CoSO_4$ application recorded significant enhancements versus $CoCl_2$, particularly in the seed yield of the second season, 100 seed weight of the first season, proteins and carbohydrates in seeds of the two seasons. Such increases were consistent across the two successive growing seasons. Overall, faba grain yield increased due to the application of 10 mg Co L^{-1} by 1.67-1.75-fold for $CoSO_4$ and 1.615 and 1.67-fold for $CoCl_2$. It can therefore be deduced that enhanced grain yield quantity and quality are derived mostly from Co's role in increasing the overall photosynthesis during grain filling. The interaction between the source and dose of applied was only significant for the 100 seed weight (Fig 4), with the highest increases recorded for $CoSO_4$ at all applied rates.

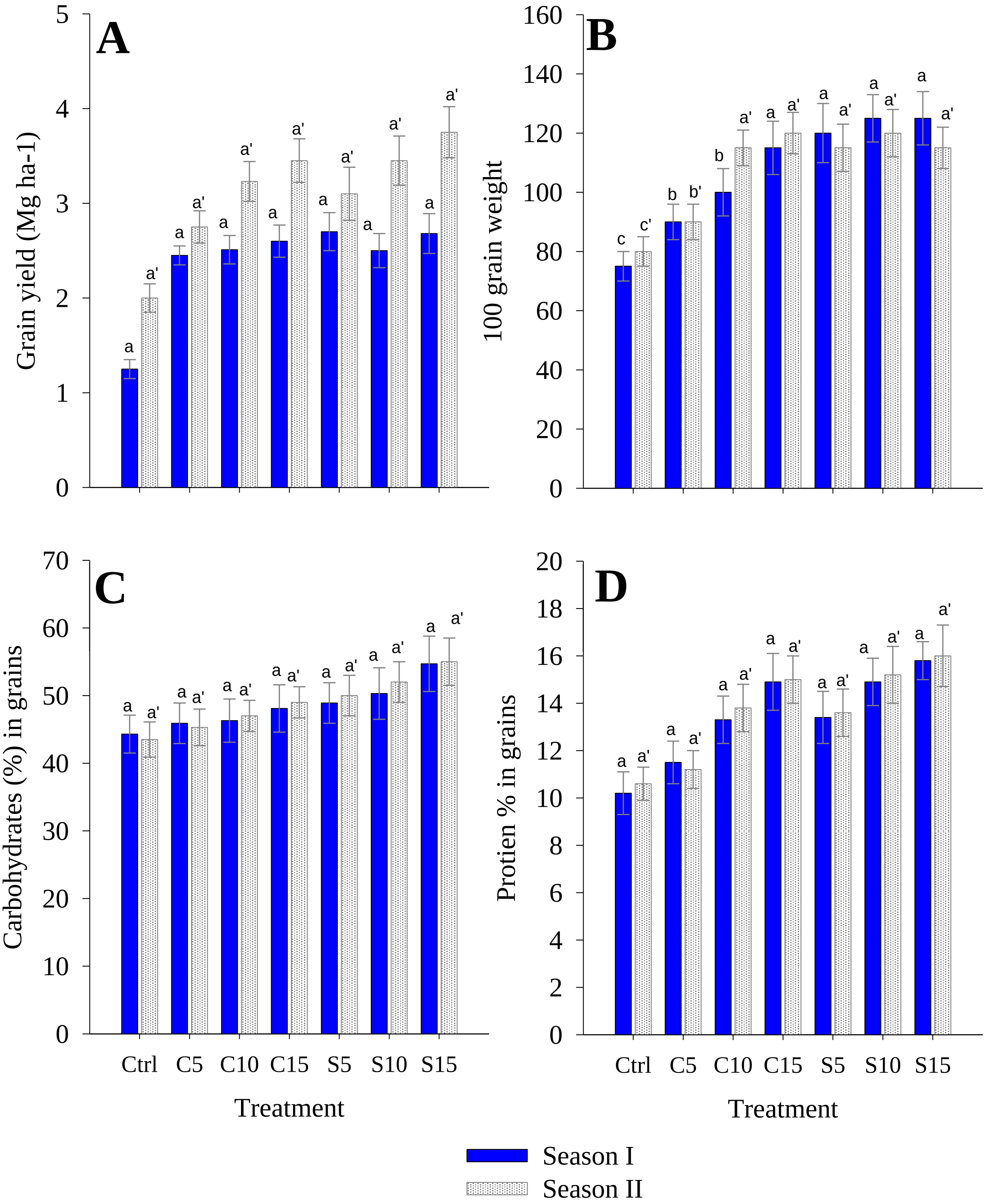
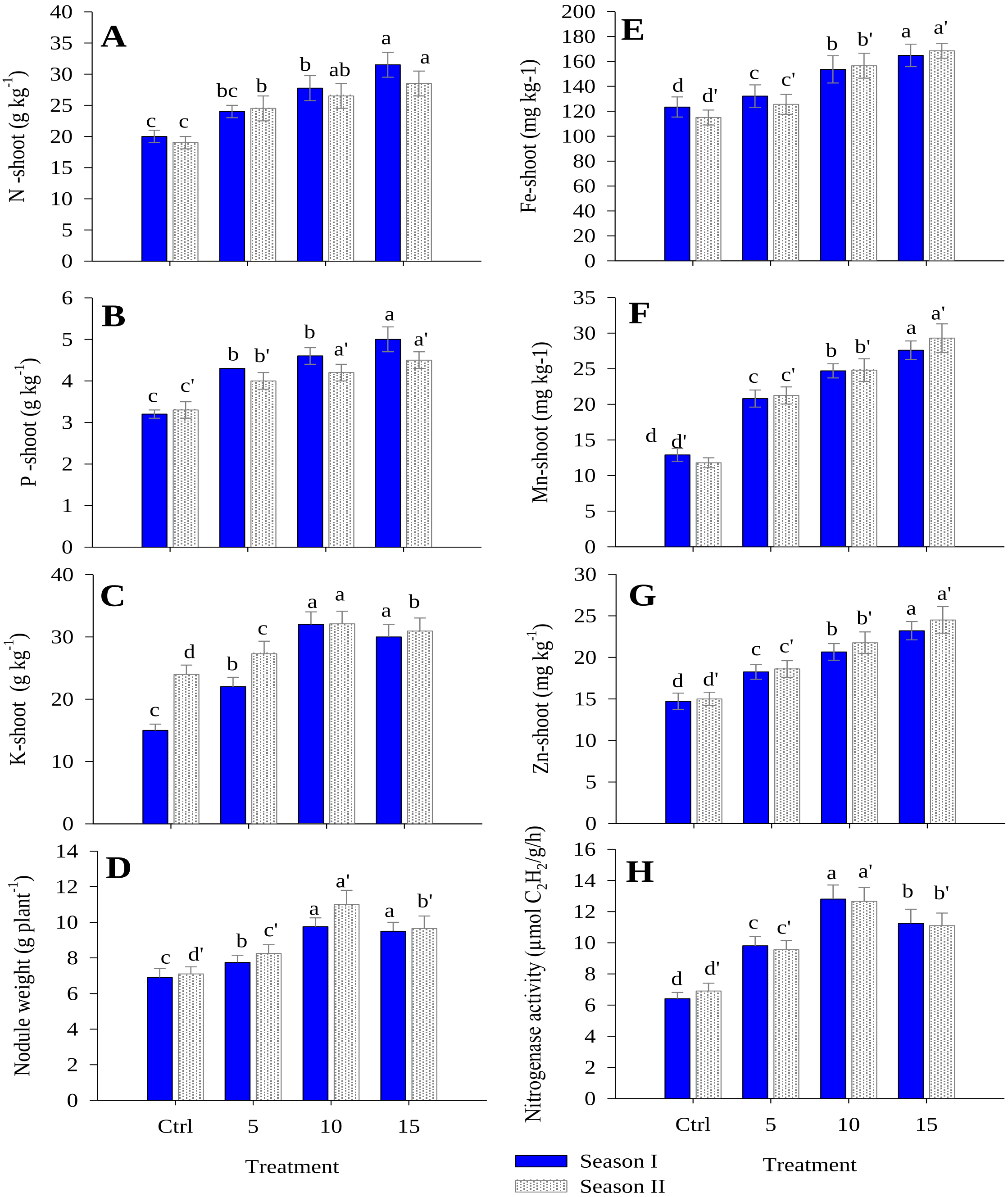


Fig. 4. Grain yield and quality attributes as affected by the form and rate of applied Co. See footnote Fig. 3. Similar letters indicate no significant variations among treatments.

3.3. Macro and micro-nutrient accumulation in faba bean shoots

Foliar application of Co, either in the form of $CoCl_2$ or $CoSO_4$, significantly improved the nutritional status of the treated plants as denoted by the marked increases in macro and micronutrient contents within plant shoots (Fig 5 A, B, C, E, F and G). These improvements became obvious with increasing the dose of applied Co, with superiority for $CoSO_4$ versus $CoCl_2$ in enhancing P, Fe, Mn and Zn contents within plant shoots.



	Nutrient concentrations in plants						Biological nitrogen fixation activity	
	N		P		K		Nodule weight	
	SI	SII	SI	SII	SI	SII	SI	SII
P_{Type}	**	*	*	*	Ns	Ns	**	**
P_{Dose}	**	**	**	**	**	**	**	**
$P_{Type*Dose}$	*	nd	nd	ns	*	*	**	ns
	Fe		Mn		Zn		Nitrogenase activity	
	SI	SII	SI	SII	SI	SII	SI	SII
P_{Type}	*	*	**	*	*	**	**	**
P_{Dose}	**	**	**	**	**	**	**	**
$P_{Type*Dose}$	ns	*	**	**	**	*	ns	**

Fig. 5. Grand means of the impacts of applied Co doses on nutritional status in faba bean plants and the biological nitrogen fixation activity in soil. Similar letters indicate no significant variations among treatments.

On the contrary, plants treated with $CoCl_2$ exhibited significantly higher N-concentrations in shoots than those treated with $CoSO_4$. In case of K^+, no significant differences were detected between the two cobalt sources. The interaction between the source of Co and its application dose were more significant on micronutrients than the macro-nutrients (Fig 6). Although, the increases in micronutrients were generally dose-dependent; yet the increases due to application of $CoSO_4$ at a rate of 10 mg Co L^{-1} were significantly comparable to those recorded for $CoCl_2$ at the highest application dose (15 mg Co L_{-1}). Economically, applying $CoSO_4$ at 10 mg L^{-1} is cost-efficient as recorded comparable micronutrient increases to higher $CoCl_2$ doses, and environmentally it reduces the risk of excess cobalt accumulation in plant tissues. Overall, these results suggest that Co applications stimulate the uptake of micronutrients which contribute in increasing plant metabolism.

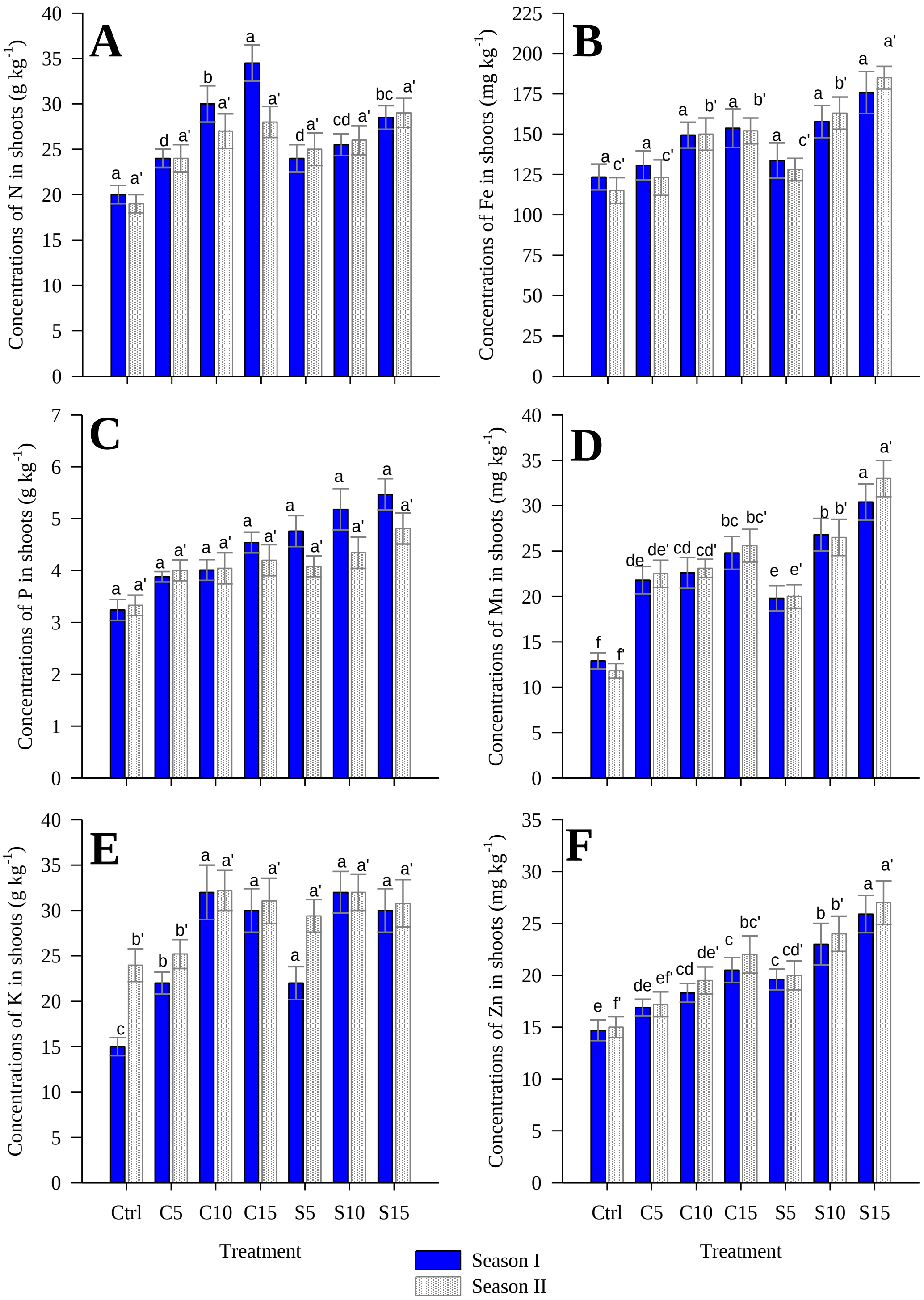


Fig. 6. Macor and micronutrients in faba bean shoots as affected by the form and rate of applied Co. See footnote Fig. 3. Similar letters indicate no significant variations among treatments.

3.4. Biological nitrogen fixation activity

Application of cobalt as a foliar spray- whether in the form of $CoCl_2$ or $CoSO_4$- significantly boosted nodule weights (Fig 5D) and also augmented the activity of nitrogenase enzyme in soil (Fig 5H). In this regard, the highest increases were achieved for the application of 10 mg Co L^{-1}, beyond which the response slightly declined. Among the two cobalt sources, $CoSO_4$ was generally more effective than $CoCl_2$ in promoting biological nitrogen fixation, and treatment effectiveness could be arranged as follows: S10>S15>C10>C15>S5>C5 ≈ control (Fig 7). These results suggest that cobalt application significantly enhances several physiological and biochemical parameters within plants up to a certain level, exceeding this dose may lead to diminishing returns, probably due to its toxicity or its interference with other nutrients on uptake by plants.

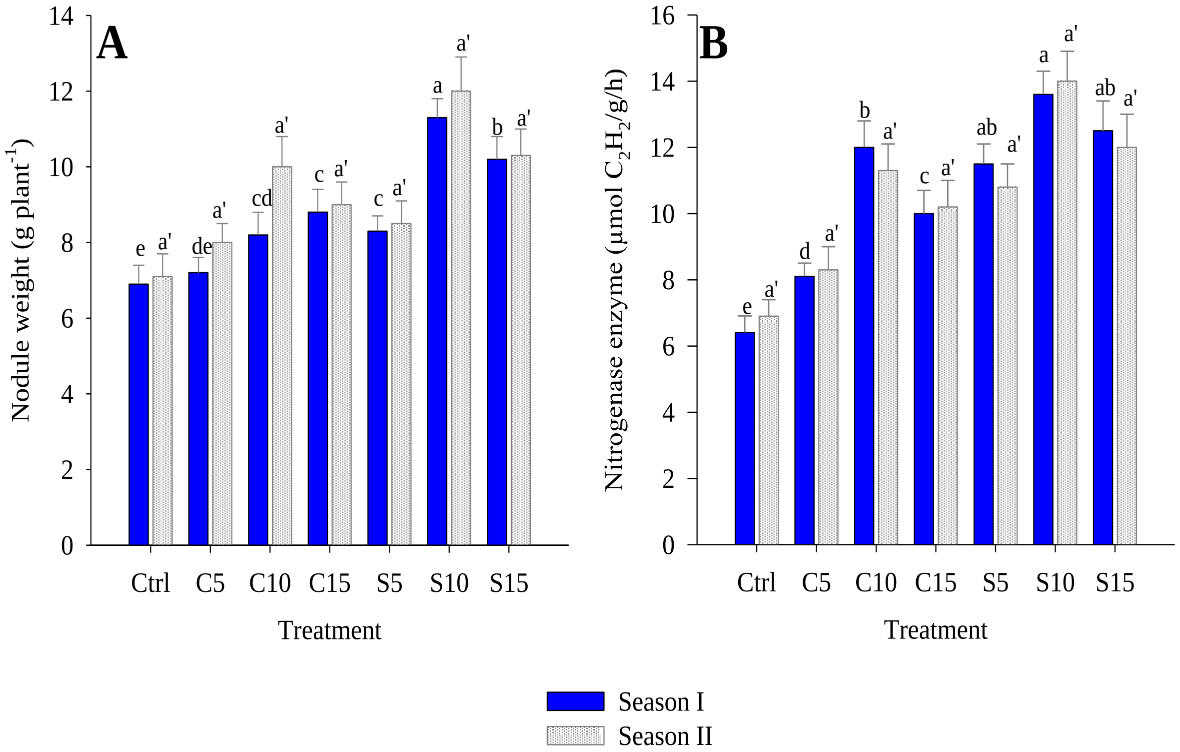


Fig. 7. Nodule weight and nitrogenase activity in soil as affected by the form and rate of applied Co. See footnote Fig. 3. Similar letters indicate no significant variations among treatments

3.5. Correlation relationships between plant nutritional status, physiological parameters, and yield Attributes in faba bean

Positive significant correlations were observed between faba bean growth parameters and yield components with concentrations of macro (nitrogen, phosphorus, and potassium) and micronutrients (iron, manganese, and zinc)—within plant shoots (Table 2). Thus, balanced nutritional status is important in promoting plant growth and productivity. Notably, concentrations of these macro- and micronutrients were also positively and significantly correlated with chlorophyll content. These contributions resulted in greater assimilation production, thereby improving plant growth and yield outcomes. Furthermore, significant positive correlations were identified between nutrient contents in shoots and the dry weight of root nodules, except phosphorus (P), which showed no significant correlation with root nodulation. This exception may be related to the relatively mobile nature of phosphorus in plants. Regarding grain quality, both protein and carbohydrate contents in faba bean seeds were significantly and positively correlated with the chlorophyll content in shoots. Remarkably, protein content was positively correlated with nitrogenase activity in soil and also with nodule dry weight. Thus, biological nitrogen fixation is the key for supporting plant growth and protein accumulation in legume grains.

Table 2. Correlation coefficients among plant nutrient status, physiological traits, and yield components in faba bean.

	Straw yield	Seed yield	100 grain weight	Protein in grains	Carbohydrates in grains	No of branches per plant	Plant height	chlorophyll	N2ase	Nodule Weight	N-shoot	P-Shoot	K-shoot	Fe-shoot	Mn-shoot	Zn-shoot
Straw yield																
Seed yield	0.795**															
100 grain weight	-0.545**	0.722**														
Protein in grains	-0.538**	0.751**	0.933**													
Carbohydrates in grains	-0.314	0.652**	0.802**	0.477*												
No of branches/plant	0.446*	0.447*	0.236	0.585**	0.760**											
Plant height	0.702**	0.055	0.131	0.816**	-0.150	0.789**										
chlorophyll	0.757**	0.370*	0.438*	0.809**	0.693**	0.368*	0.566**									
N2ase	0.673**	0.71**	0.679**	0.562**	0.096	0.151	0.479**	0.679**								
Nodule Weight	0.763**	0.569**	0.455**	0.540**	0.299	0.147	0.298	0.417*	0.405*							
N-shoot	0.703**	0.551**	0.663**	0.780**	0.573**	0.353*	0.568**	0.797**	0.528**	0.739**						
P-Shoot	0.814**	0.499*	0.864**	0.846**	0.833**	0.547**	0.684**	0.855**	0.721**	0.227	0.611**					
K-shoot	0.808**	0.757**	0.159	0.816**	0.603**	0.466*	0.593**	0.607**	0.552**	0.757**	0.226	0.585**				
Fe-shoot	0.687**	0.635**	0.454*	0.518	0.559	0.559**	0.810**	0.485*	0.467**	0.541**	0.132	0.790**	0.214			
Mn-shoot	0.617**	0.746**	0.362*	0.676**	0.789**	0.518**	0.687**	0.757**	0.476*	0.406*	-0.135	0.819**	0.028	0.609**		
Zn-shoot	0.554**	0.738**	-0.253	0.248	0.214	0.604**	0.788**	0.546**	0.514*	0.462*	0.022	0.859**	0.511**	0.432*	0.718**	

P>0.05 *P<0.05 (positive) **P<0.01 (positive) *P<0.01 (negative)

4. Discussion

4.1. Plant Growth parameters and Photosynthetic pigments a+b

Foliar application of Co significantly enhanced chlorophyll content within faba bean shoots, and also plant height as well as the overall straw yield with consistent results over two successive growing seasons. Such increases were dose-dependent, where the most substantial increases were recorded at 15 mg Co L^{-1}. These findings align with those of Elsonbaty and Elsherpiny (2024), who observed significant increases in chlorophyll content and photosynthetic efficiency in faba bean plants treated with Co. This may be attributed to the critical role of cobalt in several physiological and biochemical processes when being applied at optimum doses (Hu et al., 2021), e.g., protecting chloroplast integrity (Begović *et al.*, 2016; Roychoudhury and Chakraborty, 2022) and membrane stability under stress (Tourky *et al.*, 2023; Inayat *et al.*, 2024); thereby increasing plant metabolism and supporting biomass growth. Similarly, the form of applied Co recorded further significant impacts on both shoot chlorophyll (season II), plant height and shoot biomass, with superiority for $CoSO_4$ versus $CoCl_2$. Probably, (1) sulphate is incorporated in glutathione synthesis (Kopriva and Koprivova , 2005; Peng *et al.*, 2025), which takes part in mitigating abiotic stress (Shah *et al.*, 2022). Additionally, SO_4^{2-} ions promote indirectly the uptake of other micronutrients (e.g., Fe, Zn, Mn) (Chorianopoulou and Bouranis, 2022), while Cl^- antagonizes NO_3^- (Zhang et al., 2021). Finally, excess Cl^- increases ion toxicity in plants (Hasanuzzaman and Fujita, 2022). This suggests that cobalt primarily targets morphological development patterns (plant height), physiological (chlorophyll) and metabolic functions leading to plant growth.

4.2. Grain Yield and Yield Components

Grain yield and its components (100-grain weight, protein, and carbohydrate content) were significantly enhanced by the application of Co up to 10 mg Co L^{-1}. These improvements may be attributed to the role of Co as a key cofactor in various metabolic and enzymatic processes, e.g. biosynthesis of proteins and carbohydrates **(Ulhassan et al., 2023)**. Further increase in Co application dose, probably induced slight phytotoxic effects or partially suppressed the uptake of other essential micronutrients (such as iron and zinc) **(Ali et al., 2025)**, thereby hindering the beneficial effects of Co observed at lower concentrations. Accordingly, the improvements in both yield and qualitative components of faba beans at 15 mg L^{-1} were not significantly pronounced and 10 mg Co L^{-1} appears to represent a physiological threshold beyond; thereafter applied Co-dose efficiency decreased. Also, $CoSO_4$ tended to outperform $CoCl_2$ in all yield and yield components, except for seed yield (season one only) and 100-seed weight (second season only), where no significant differences were observed between the two forms of cobalt ($CoCl_2$ vs $CoSO_4$) on these yield metrics. At relatively high Co dosages, plants may direct their resources mainly toward maintaining shoot growth and vegetative development as a survival strategy to dilute Co within plant tissues under stress; thus, vegetative growth responds positively to increasing Co concentration. However, reproductive processes require a finely balanced supply of nutrients and assimilate such as flowering, grain formation, and filling.

4.3. Impact on nutrient accumulation within faba bean shoots

Co application enhanced concentrations of both macro (N, P, K) and micro (Fe, Mn, Zn) nutrients in shoots in a dose-dependent manner. Notably, while K accumulation in shoots did not vary significantly between the two cobalt forms ($CoSO_4$ versus $CoCl_2$), foliar application of $CoSO_4$ resulted in higher P, Fe, Mn, and Zn accumulation in shoots, whereas $CoCl_2$ foliar application resulted in higher N content in shoots. Such increases were simply the consequences of improving both the root system upon spraying plants with Co (Singh *et al*., 2025) as well as the enhancement in the symbiotic performance due to this foliar spray (Hu et al., 2021), which improved nutrient assimilation (Das et al., 2025). The above results validate the 1st and 2nd hypotheses, indicating that Co effectiveness were dose dependant in boosting faba bean physiological and biochemical performance, and consequently boosting plant growth and productivity, with superiority for its chemical form of CoSO4 versus $CoCl_2$.

4.4. Biological Nitrogen Fixation and Nodule Function

Nodule weight and soil nitrogenase activity significantly increased due to the application of Co salts, with maximum effectiveness at 10 mg Co L^{-1}, where $CoSO_4$ consistently outperformed $CoCl_2$. This nutrient is beneficial for plants and may have indirect impacts on boosting the biological nitrogen fixation and nodule function. Notably, Co exhibits intermediate mobility in the phloem (Riesen and Feller, 2005). A portion of the sprayed Co might also reach soil, entering plant roots and nodules through the traditional uptake pathway. In all cases, Co is essential for the activity of rhizobia because it is a constituent of vitamin B_{12}, which supports nitrogenase enzyme activity (Hu *et al*., 2021; Li *et al*., 2024).

Higher nodule weights were found with increasing the dose of applied Co, likely showed better functioning (Ott *et al*., 2025), including better leghemoglobin levels and steady nitrogenase activity (Sudhakar *et al*., 2016). However, at higher cobalt dose (15 mg Co L^{-1}), both nodulation and enzyme activity dropped. An important question arises here on how these toxic concentrations reached nodules. There are two possible pathways, the first one is through the downward movement of Co in the phloem from shoots to roots, then to nodules; yet considerable Co might become immobilized or compartmentalized within vegetative tissues to mitigate toxicity (Lotfy and Mostafa, 2014); then only a small fraction of Co could reach nodules. The alternative pathway is that portions of the foliar-sprayed Co leaked on the soil surface; however, this amount might not be enough to induce Co toxicity in root nodules. Then, the first assumption might be more acceptable, particularly if we assume that Co is needed only within the parts of nodules (cortical and vascular tissues) that are involved in atmospheric N, while being toxic if accumulated in other nodule parts. Interestingly, despite the reductions that occurred in nitrogenase activity at the highest Co dose, overall plant growth did not decline significantly. Mostly, cobalt toxicity impacted mainly nodule function while plants adapt (Li *et al*., 2018) via, i.e. (1) adjusting nitrogen metabolism (Ledermann et al., 2021; Kumari et al., 2022). (2) recycling N in old plant tissues to support new growth, and/or (3) compensate N-needs by absorbing available soil-N (Carlsson et al., 2003). The above results validate the 3rd, while did not support 4th hypotheses, indicating that Co indirectly promotes biological nitrogen fixation by increasing nodule biomass and enhancing nitrogenase activity within the rhizosphere. Yet, high cobalt doses could be toxic to both plants and N-fixers, as reported above.

5. Conclusion

Foliar application of cobalt (Co) up to **10 mg Co L^{-1}** significantly improved morphological (plant height), physiological (e.g. chlorophyll content), biochemical (e.g. nutrient accumulation in shoots and biological nitrogen fixation), and yield-related parameters in faba bean. These improvements were dose-dependent; yet at relatively high Co dosages, plants may direct their resources mainly towards increasing shoot growth as a survival strategy to dilute Co within plant tissues, while grains decreased due to disrupted nutrient and assimilation allocation. Also, nodule biomass and nitrogenase activity dropped considerably. Overall, $CoSO_4$ outperformed $CoCl_2$ in most physiological and biochemical parameters, though it exhibited comparable effects on yield components. This superiority could be because of sulfate effects in cellular redox balance, glutathione biosynthesis, and micronutrient uptake; on the other hand, excess chloride induces ionic stress and interferes with nitrate absorption. Future research should focus on molecular and transcriptomic responses of cobalt under field conditions, including Co impacts on biological nitrogen fixation symbiosis, nutrient uptake regulations, and stress mitigation routes. Also, more work should quantify Co uptake and track its mobility within different plant parts, especially from shoots into roots and nodules.

Acknowledge the gap

Cobalt accumulation in roots was not determined, which restricts a direct assessment of its translocation from leaves to nodules; yet our **interpretation** was based on the known Co mobility in phloem and its indirect indicators, such as nodulation and nitrogenase activity.

Declarations

Ethics approval and consent to participate

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