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Response of broad bean (*Vicia faba* L.) and nitrogen availability to cobalt and nickel applications in the soil

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Abstract: Nitrogen is a fundamental macronutrient that can be renewable. Leguminous crops applied nitrogen to the soil, thereby improving soil fertility. Nickel and cobalt are pivotal in nitrogen metabolism and biological nitrogen fixation within legumes. This investigation determined two applications of nickel and cobalt to the soil, which were applied at three different rates (0, 50, and 100 mg of nickel and cobalt kg^{-1} soil) with three replicates. The treatment of 0 mg of nickel with 100 mg of cobalt kg^{-1} soil resulted in the highest seed yield value (3.65 Mg ha^{-1}). The treatment T5 (0 mg of nickel with 100 mg of cobalt kg^{-1} soil) achieved a fresh nodule weight of 304.30 kg ha^{-1}. The greatest mean soil nitrogen concentration value was found in T6 at the flowering stage, which showed a mean value of 34.30 mg N kg^{-1} soil. Additionally, the best correlation was observed between nitrogen uptake in seeds and broad bean seed yield, with an R^2 value of 0.88. The average concentrations of nickel and cobalt in the soil achieved values from 0.85 to 22.60 mg Ni kg^{-1} soil and 0.43 to 5.79 mg Co kg^{-1} soil, respectively, with all mean values below the acceptable toxicity thresholds. This research provides significant insight into the role of nickel and cobalt in enhancing legume nodulation to improve the yield of commercial broad bean plants.

Keywords: Leaf area, seed yield, protein, PCA, nitrogen uptake, leaf cobalt concentration.

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1. Introduction

Broad beans (*Vicia faba* L.) are cultivated worldwide as a source of nutrients such as protein for human consumption (AbdEL-Azeiz and Faiyad, 2024). Furthermore, broad beans facilitate replenishing soil nitrogen through atmospheric nitrogen fixation (Gu et al., 2024; Kibkab and Berhane, 2024). Additionally, improve agricultural sustainability in cropping systems via providing nitrogen, a major nutrient for all crops, through biological nitrogen fixation (Elsalam et al., 2021; Lin et al., 2024). Moreover, legumes enhance soil-root biodiversity, in turn enhancing the crop's resistance to different pests and diseases (Hammadi and Alwan, 2023; Hayati et al., 2024; Thapa et al., 2021). Broad bean also considered a source of protein, which is a required compound for human consumption (Alhammad and Seleiman, 2023). Food and Agriculture Organization of the United Nations (FAO), the total planted area for dry and green broad beans worldwide is approximately 3,603,846 hectares and 262,886 hectares, respectively. However, FAO reported that Iraq's broad bean cultivated area for dry and green is 540 hectares and 4,240 hectares, respectively. In terms of broad bean yield, the global production resulted in 151.16 and 262.886 Mg ha^{-1} for dry and green broad beans, respectively. On the contrary, Iraq's dry and green broad beans are 2.769 and 6.65 Mg ha^{-1}, respectively, which constitutes 1.83% and 2.53% of global broad bean production yields, respectively (FAOSTAT, 2025).

Nickel (Ni) is considered a micronutrient substantial for all field crops (Elbagory et al., 2022; Mendes et al., 2023). Nickel plays a vital role in the nitrogen metabolism within crops, remarkably impacting the processes of the urease enzyme, nitrogenase, and hydrogenase. This approach is decisive for the gain and conversion of atmospheric nitrogen into ammonia, predominantly in broad bean crops (Mendes et al., 2023; Parwez et al., 2021). Further, Ni improves the crop's capacity to withstand abiotic stresses. The Ni deficiency can adversely influence nitrogen metabolism, considering that it compromises growth, causes premature senescence, prevents nitrogen absorption, and diminishes iron consumption in crops (Saad et al., 2018).

In recent years, Ni has been applied to improve plant tolerance, in turn reinforcing the crops' defense system against abiotic and biotic stresses, enhancing biological nitrogen fixation, a critical process in legumes, and improving plant pigment metabolism, which considerably leads to increased crop yield (Bosse et al., 2024). Broad bean roots can absorb available nickel in the soil through mass flow or diffusion as Ni^{2+} ions from the soil pool. Mainly, the soil nickel sources are minerals, exchange sites, and organic substances. Nickel is translocated into the xylem and phloem predominantly as an anionic organic complex (Moreira et al., 2024). Organic substances represent the main components of this complex (Mahmood, 2016; Saad et al., 2018). Nevertheless, soil nickel levels, similar to other micronutrients, can be influenced by factors such as soil-water holding capacity, soil pH, soil temperature, physical and chemical properties, solid-phase nickel components, and biological processes (Bosse et al., 2024; Ibrahem and Naser, 2020). Nickel is rapidly absorbed by crop roots upon contact (Thapa et al., 2021).

Cobalt is one of the beneficial nutrients that plays a crucial role in legumes due to its function in nitrogen fixation (Barker and Pilbeam, 2015). Cobalt is essential for the cobalamin coenzyme, which is vital for the bacteroid process and nodulation (AbdEL-Azeiz and Faiyad, 2024; Anees et al., 2025; Baddour et al., 2021). Legume nodulation and nitrogen fixation require enzymes and coenzymes that are considered part of the nitrogen fixation system (Danish et al., 2024). Nitrogen fixation enzymes and coenzymes require vitamin B12 (Gad and El-habbak, 2024). The main enzymes involved in this process are ribonucleotide reductase and methylone coenzyme A mutase, activated through cobalt applications (Onte et al., 2019). Cobalamin serves as a vital cofactor for rhizobium enzymes that drive the catalysts transforming N_2 into NH_3 (Rai et al., 2025). Legume cobalt fertilization can improve the efficiency of legume-rhizobia interactions and enhance nitrogen in plant tissues(Ulukapı et al., 2024). Soil physicochemical characteristics can impact the bioavailability of cobalt in soil, including organic matter, water-holding capacity, soil pH, reduction process, sorption potential, adsorption, clay mineral content, and texture (Al Maamouri et al., 2025; Ulukapı et al., 2024). However, soil pH is the primary factor that influences the bioavailability of cobalt in the soils (Tomić et al., 2023). Soil pH decline can increase the cobalt availability, and vice versa (Ulukapı et al., 2024). Cobalt in moderate quantities, in conjunction with nitrogen, can impact several factors, including the number of nodules per plant, nitrogen levels in shoots and roots, seed mass, flowering count per plant, pod and nodule quantity per plant, and overall yield(Aguiar et al., 2025; Yeremko et al., 2025).

This study aims to evaluate the response of broad bean growth, yield, and seed quality to nickel and cobalt applications. It will also examine nitrogen uptake via broad bean leaves and seeds, and nitrogen soil availability.

2. Materials and Methods

2.1. Study Site and Experimental Design

The study was implemented at the University of Baghdad, College of Engineering, Agricultural Sciences Station A. The study area is coordinated as 33.26914°N, 44.37908°E. In this study, twenty-seven treatments with three replicate datasets were performed during the 2022-2023 season; each replicate had nine treatments. The field experimental design was a randomized complete block. Each experimental unit distance was 2.40 meters in length, and the width was one meter. Six plants were planted in each row, the distance between plants was 0.4 meters, and the total number of plants at each experimental unit was twelve. Each treatment had two rows at each side of the treatment with a one-meter distance between both rows. One meter was the distance between treatments at the same replicate, and the same distance was maintained between replicates. The treatment designations are as follows:

Treatments	Description
T1	0 mg Nikg^{-1} soil + 0 mg Co kg^{-1} soil (control)
T2	0 mg Ni kg^{-1} soil + 50 mg Co kg^{-1} soil
T3	0 mg Ni kg^{-1} soil + 100 mg Co kg^{-1} soil
T4	50 mg Ni kg^{-1} soil + 0 mg Co kg^{-1} soil
T5	50 mg Ni kg^{-1} soil + 50 mg Co kg^{-1} soil
T6	50 mg Ni kg^{-1} soil + 100 mg Co kg^{-1} soil
T7	100 mg Ni kg^{-1} soil + 0 mg Co kg^{-1} soil
T8	100 mg Ni kg^{-1} soil + 50 mg Co kg^{-1} soil
T9	100 mg Ni kg^{-1} soil + 100 mg Co kg^{-1} soil

The study factors were set with cobalt applied as fertilizer using cobalt sulfate ($CoSO_4$) as a source at rates of 0, 50, and 100 mg Co kg^{-1} soil, and nickel, which is applied as fertilizer using nickel sulfate ($NiSO_4$) at rates of 0, 50, and 100 mg Ni kg^{-1} soil. Cobalt (Co) and nickel (Ni) applications were applied at one time, two weeks after the broad bean cultivation date. Nitrogen was applied using a urea (46% N) source, and the recommended application was 60 kg N ha^{-1}. Nitrogen (N) as a recommended application was applied from DAP (diammonium phosphate 18% N); then, the rest of the nitrogen amount, i.e., the N recommendation to be completed, was applied as a urea fertilizer. Phosphorus was applied as a diammonium phosphate (DAP 46% P_2O_5, 18%N) fertilizer source as a recommended 50 kg P ha^{-1} application. Potassium was applied as the recommended application, which is 150 kg K ha^{-1}, utilizing potassium sulfate (50% K_2O) as a fertilizer resource. Nitrogen and potassium were applied twice based on the growing season. The first application was made at the planting date, and the second was made four weeks after that. The physicochemical properties of the soil are described in Table 1.

Table 1. Physical and chemical characteristics and fertility attributes of the study's soil

Soil characteristic	Unit	Values
pH (1:1)	---------	6.91
Ec (1:1)	dS m^{-1}	3.06
Organic matter	%	1.72
Cl^-	Meq L^{-1}	13.00
Ca^{++}		14.00
Mg^{++}		6.00
Na+		7.30
Soluble K+		2.70
HCO_3		2.00
Nitrogen (NO_3^- + NH_4^+)	mg nutrient kg^{-1} soil	16.00
Phosphorus (P)		6.2
Available Potassium (K)		127
Soluble carbonate		Nil
Available Nickel		2.8
Available Cobalt		0.8
	Soil texture	
Sand	gm kg $soil^{-1}$	512
Silt		320
Clay		168
Soil texture type	loam	

2.2. Broad Bean Harvest

The broad bean was planted on October 10, 2022, and subsequently harvested on three occasions: March 4, March 11, and April 1, 2023. Six broad bean plants were randomly picked from 2.40 meters on each side for each treatment, with three plants selected from each row. The broad bean pods were peeled to count the number of seeds within each pod after determining the number of pods for each broad bean plant. The broad bean seed yield was weighed to ensure that the total seed yield (Mg ha^{-1}) for the three harvesting instances was determined. The broad bean variety indicated in Spain as "broad bean" (produced by Fito Care Co), particularly the LUZ DE OTONO type, with purity of 98% and a germination rate exceeding 85%, as indicated on the type's packaging label.

2.3. Broad Bean Parameters and Leaf Area Determination

The height of broad bean plants, the number of leaves, and the number of nods were collected at one time, 60 days after the planting date on December 15, 2022. Ten broad bean plants were selected for this matter. The leaf area (LA) was determined for five plants, which were randomly selected from each treatment. The LA was calculated by measuring the length and width of the broad bean leaves, fully expanded leaflets, as summarized by Erdoğan (2012) the following formula :

$$LAI = 1.6923 + (Le * 0.0161) + (Wi * 0.0929) + (0.0062 * Le * Wi) \text{ ----- } (1)$$

The Leaf Area (LA) is presented by the leaf area index. Furthermore, Wi and Le are noted as the width and length, respectively, of a fully expanded leaflet, measured in centimeters (cm).

2.4. Broad Beans Leaves and Seeds Nitrogen Uptake and Protein Determination

Broad bean seeds were randomly chosen for each treatment separately, and the produced crushed powder was passed through a 2 mm sieve. The total nitrogen content in leaves, which is ammonium (NH_4^+) and nitrate (NO_3^{-2}), was determined using the Kjeldahl method. The protein content in the broad bean seeds was calculated by multiplying the nitrogen concentration in the seeds (measured in percentage) by 6.25 (Alhammad and Seleiman, 2023). The total nitrogen uptake in the leaves and seeds of broad beans was determined separately, as calculated by Sharma et al. (2012) using the appropriate formula.

$$\text{Uptake of nitrogen leaves and seeds (kg ha}^{-1}\text{)} = \frac{\text{Nitrogen \%} * \text{Dry matter (Kg ha}^{-1}\text{)}}{100} \quad (2)$$

2.5. Chlorophyll index SPAD

The SPAD measurements were conducted utilizing the SPAD-502 PLUS chlorophyll meter, a product of KONICA MINOLTA SENSING, which is manufactured in Japan. Five broad bean plants were adopted from each treatment to determine chlorophyll contents on January 20, 2023.

2.6. Broad Bean Nodules Determination

Based on the study objectives, nodulation was designated according to the native nodules that naturally formed on broad bean roots, without the isolation of rhizobia. Broad bean nodules were collected from six plants on January 30th, 2023. Nodules were washed utilizing distilled water, then weighed as fresh nodules, which were converted to kg ha^{-1}. Nodules were dried in the oven at 65°C, and the results were also exhibited in kg ha^{-1}.

2.7. Broad Bean Leaves Preparation

Leaflets and petioles were selected from six plants for each treatment to calculate the number of compound leaves. Leaflets and petioles were cleaned and washed with distilled water to remove any dust or undesired materials that could interact with the analysis.

2.8. Soil Nickel and Cobalt Extraction and Determination

The nickel and cobalt in the soil were measured using the method detailed by Lindsay and Norvell (1978). The air-dried soil was sieved through a 2-millimeter mesh, and 10 g of this soil was extracted with 20 mL of diethylenetriaminepentaacetic acid (DTPA) solution, which was prepared from 0.005 M diethylenetriaminepentaacetic acid, 0.1 M triethanolamine, and 0.01 M $CaCl_2.2H_2O$. The solution was set at a pH of 7.3 at 25°C and then shaken for 2 hours. The soil-DTPA solution was filtered using Whatman filter paper grade 42. Nickel and cobalt content in the extracts were determined using an Atomic Absorption

Spectrophotometer (AAS), model PERKIN ELMER. Such nutrients were measured at wavelengths of 232.0 nm and 240.7 nm for nickel and cobalt, respectively.

2.9. Statistical Analysis

All datasets were examined for normality before the analysis, and the least significant difference (LSD) was determined at a significant difference level, $P \leq 0.05$. An analysis of variance (ANOVA) table was established to verify the differences between the two primary factors in this investigation, namely, nickel and cobalt, which are utilized as fertilizers. The Shapiro-Wilk (S-W) test was conducted in certain studies to validate the normality assumption, and no violations were detected among the datasets before the application of any analytical methods. The ANOVA was performed using RStudio software (Version RStudio 2025.09.2+418; Boston, MA, USA), coding the ExpDes function and fat2.rbd package to analyze the ANOVA table. Simple linear regression and additional parameters were assessed using RStudio software, utilizing the Rsq. adj package. Simple linear regression plots were performed using RStudio software, incorporating the ggplot2, patchwork, and magrittr packages.

3. Results

3.1. Means of datasets between nickel and cobalt effects on some broad bean growth parameters

Table 2 illustrates that the highest plant height of 39.80 cm was recorded with the T8 treatment of 100 mg Ni kg^{-1} soil and 50 mg Co kg^{-1} soil, compared to the control treatment, which recorded a plant height of 35.37 cm, reflecting an increase of 12.52%. The T8 treatment recorded a significant difference compared to the T6 treatment of 50 mg Ni kg^{-1} soil + 100 mg Co kg^{-1} soil, which achieved 34.79 cm, with a percentage increase value of 15.18% between both treatments. The highest mean value of the number of leaves $plant^{-1}$ was observed at treatment T9 (100 mg Ni kg^{-1} soil + 100 mg Co kg^{-1} soil), which recorded a value of 14.80 number of leaves $plant^{-1}$, which significantly differed from the T1 treatment (the control), which recorded a mean value of 12.00 number of leaves $plant^{-1}$, with a percentage increase value of 23.33%. The effect of nickel and cobalt applications on the number of nods $plant^{-1}$ was clearly observed at the maximum nickel and cobalt applications at T9 (100 mg Ni kg^{-1} soil + 100 mg Co kg^{-1} soil), which achieved a mean value of 14.60 number of nods $plant^{-1}$ valued a percentage increase of 25.86% compared to the control treatment, which achieved the lowest mean value of 11.60 number of nods $plant^{-1}$. No significant difference was found among the treatments regarding the number of nods. Leaf Area (LA) resulted in a maximum mean value, that of the observed at treatment T8, which achieved a mean value of 32.45. However, the minimum mean value was recorded at the control treatment, with a mean value of 22.00, exhibiting a percentage increase of 47.45% between the two treatments. The number of pods $plant^{-1}$ revealed the highest mean value observed at the T3 treatment (0 mg Ni kg^{-1} soil+ 100 mg Co kg^{-1}soil), which attained a mean value of 24.78 number of pods $plant^{-1}$ with a percentage increase value of 36.83% compared to the control treatment that achieved a mean value of 18.11 number of pods $plant^{-1}$. The number of seeds pod^{-1} showed the greatest mean value observed at the T6 and T8 treatments, which achieved mean values of 5.33 number of seeds pod^{-1} and 5.33 number of seeds pod^{-1}, respectively. However, the lowest mean value was recorded at the control treatment, which achieved a value of 4.50 number of seeds pod^{-1}, which was significantly different from the T6 and T8 treatments. However, among other treatments, it did not yield a significant difference. Results exhibited no significant differences between treatments regarding the weight of 100 seeds g plant-

[1]. However, the maximum mean value was observed at the treatment T7 (100 mg Ni kg $soil^{-1}$ + 0 mg Co kg $soil^{-1}$), which achieved a mean value of 22.35 g $plant^{-1}$.

Table 2. Effect of nickel and cobalt applications on broad bean growth parameters

Treatments	Plant Hight (cm)	Number of leaves plant $^{-1}$	Number of nods plant $^{-1}$	Leaf Area (LA)	Number of pods $plant^{-1}$	Number of seeds pod^{-1}	Weight of 100 seeds g $plant^{-1}$
T1	35.37 ab	12.00 c	11.60 c	22.00 c	18.11 b	4.50 b	17.69 a
T2	36.47 ab	12.00 c	12.07 bc	26.10 bc	20.64 ab	4.53 b	18.87 a
T3	34.79 ab	12.60 bc	12.60 bc	22.29 c	24.78 a	4.93 ab	18.87 a
T4	36.24 ab	13.47 abc	13.80 ab	24.98 c	20.22 ab	4.70 ab	20.54 a
T5	35.97 ab	12.20 c	12.53 bc	26.11 bc	21.56 ab	4.70 ab	20.26 a
T6	34.57 b	13.07 bc	13.60 ab	29.31 ab	16.86 b	5.33 a	20.47 a
T7	35.44 ab	14.00 ab	13.80 ab	25.20 bc	21.31 ab	4.97 ab	22.35 a
T8	39.80 a	13.40 abc	13.13 abc	32.45 a	22.17 ab	5.33 a	19.58 a
T9	39.37 ab	14.80 a	14.60 a	23.80 c	20.56 ab	4.77 ab	18.07 a
CV	8.26	6.89	8.33	9.56	17.36	9.35	19.41

CV: Coefficient of variation. Based on Duncan's test ($P \leq 0.05$), means followed by different letters indicate statistically significant differences between the treatments.

3.2. Means of datasets between nickel and cobalt effects on SPAD, fresh nodule, weight, petioles, and dry leaflets weight

The SPAD (chlorophyll meter) indicates the nitrogen content by assessing the greenness of the broad bean leaves. Chlorophyll molecules in the leaves contain four nitrogen atoms, which form the principal structure of chlorophyll. As illustrated in Table 3, the maximum mean value of SPAD was observed at treatment T5 (50 mg Ni kg^{-1} soil + 50 mg Co kg^{-1} soil), which attained a mean value of 44.93 with a percentage increase of 11.91% compared to the control treatment, which achieved a mean value of 40.15. However, the SPAD parameter did not show a significant difference among the treatments. The fresh nodule weight results showed that the highest mean value was observed at treatment T4 (50 mg Ni kg^{-1} soil + 0 mg Co kg^{-1} soil), which achieved a value of 456.77 kg ha^{-1} with a percentage increase of 226.19% compared to T1 treatment (0 mg Ni kg^{-1} soil + 0 mg Co kg^{-1} soil), which recorded a mean value of 140.03 kg ha^{-1}. However, data analysis indicated significant differences between the T4 treatment and the control treatment. Conversely, the lowest mean value was observed in the treatment with the highest application of nickel and cobalt (100 mg Ni kg^{-1} soil + 100 mg Co kg^{-1} soil), which achieved a mean value of 117.03 kg $ha^{-1,}$ significantly different from the maximum mean value obtained in other treatments. In terms of dry petiole and leaflet weights, no significant differences were noted across treatments; however, the highest mean values were recorded in treatment T9 (100 mg Ni kg $soil^{-1}$ + 100 mg Co kg $soil^{-1}$) and treatment T6 (50 mg Ni kg^{-1} soil + 100 mg Co kg^{-1} soil), with dry petiole and leaflet weights of approximately 9.83 kg ha^{-1} and 65.10 kg ha^{-1}, respectively.

Table 3. Impact of nickel and cobalt applications on SPAD, fresh nodule weight, Dry petioles, and leaflets weight kg ha^{-1}.

Treatments	SPAD	Fresh nodule weight kg ha^{-1}	Dry petioles weight kg ha^{-1}	Dry leaflets weigh kg ha^{-1}
T1	40.15 a	140.03 b	8.87 a	56.63 a
T2	44.56 a	171.40 b	8.97 a	60.77 a
T3	43.21 a	280.37 ab	9.07 a	59.40 a
T4	39.68 a	456.77 a	9.40 a	56.40 a
T5	44.93 a	304.30 ab	9.20 a	58.13 a
T6	38.35 a	274.50 ab	8.23 a	65.10 a
T7	38.33 a	221.43 ab	8.77 a	55.83 a
T8	41.87 a	249.77 ab	8.73 a	62.80 a
T9	44.68 a	117.03 b	9.83 a	62.27 a
CV	16.58	28.96	22.00	14.62

CV: Coefficient of variation. Based on Duncan's test ($p \le 0.05$), means followed by different letters indicate statistically significant differences among the treatments.

3.3. Means of datasets between the nickel and cobalt effect on available nickel, available cobalt in the study soil, leaf nickel concentration, and leaf cobalt concentration.

Table 4 indicates that the available soil nickel is significantly influenced by nickel applications. The highest value was recorded in the T7 treatment, corresponding to (100 mg Ni kg^{-1} soil + 0 mg Co kg^{-1} soil), with a value of 22.60 mg Ni kg^{-1} soil, representing an increase of 1134% relative to the control treatment (T1), which exhibited a value of 1.83 mg Ni kg^{-1} soil. In contrast, the lowest mean value was observed in the T3 treatment (0 mg Ni kg^{-1} soil + 100 mg Co kg^{-1} soil). Based on Duncan's test, statistically significant differences were recorded between the treatments, T2 and T3, which did not show a significant difference, as indicated by the same letters in the mean values. The available soil cobalt results showed a similar behavior to those of soil nickel. The highest mean value of soil available cobalt was observed in the treatment T3 (0 mg Ni kg^{-1} soil + 100 mg Co kg^{-1} soil), which achieved a mean value of 5.79 mg Co kg^{-1} soil with a percentage increase of 898.28% compared to the control treatment, which achieved a mean value of 0.58 mg Co kg^{-1} soil. The lowest value was recorded in the T4 treatment (50 mg Ni kg^{-1} soil + 0 mg Co kg^{-1} soil). However, most treatments achieved significant differences in the soil, except for T1, T4, and T7, which did not differ significantly from each other. The maximum concentration of nickel in leaves was observed in treatment T8 (100 mg Ni kg^{-1} soil + 50 mg Co kg^{-1} soil), with a mean value of 35.00 mg Ni kg^{-1} DM. In contrast, the minimum mean value was recorded in the control treatment, which achieved 0.49 mg Ni kg^{-1} DM. The T8 and treatments T1, T2, T3, and T5 particularly had significant differences. Treatments T1, T2, T3, and T5 did not differ significantly from each other. Additionally, T8 and T7 did not show a significant difference. The cobalt concentration in broad bean leaves demonstrated that the highest mean value was observed in T3 (0 mg Ni kg^{-1} soil + 100 mg Co kg^{-1} soil), with a value of 54.20 mg Co kg^{-1} DM, which was significantly different from T1 (control), the treatment with the lowest mean value of 0.20 mg Co kg^{-1} DM.

Table 4. Impact of nickel and cobalt applications on available nickel, available cobalt in the study soil, leaf nickel concentration, and leaf cobalt concentration.

Treatments	Available Nickel mg kg^{-1} soil	Available Cobalt mg kg^{-1} soil	Leaf Nickel Concentration	Leaf Cobalt Concentration

			mg kg-1 DM	mg kg-1 DM
T1	1.83 g	0.58 g	0.49 d	0.20 e
T2	1.09 h	2.27 d	1.72 d	22.62 c
T3	0.85 h	5.79 a	1.90 d	54.20 a
T4	3.40 f	0.43 h	20.76 c	0.39 e
T5	6.25 e	2.00 e	22.66 bc	0.37 e
T6	12.65 d	3.91 b	21.26 c	45.10 b
T7	22.60 a	0.55 gh	30.45 ab	0.29 e
T8	18.16 b	1.58 f	35.00 a	23.04 c
T9	16.06 c	2.65 c	15.74 c	10.89 d
CV	3.84	3.81	30.94	20.22

DM: Dry matter. CV: Coefficient of variation. Based on Duncan's test ($p \leq 0.05$), means followed by different letters indicate statistically significant differences among the treatments.

3.4. Influence of nickel and cobalt applications on the nitrogen concentration in broad bean leaves, seeds, and soil at flowering and harvesting stages.

The nitrogen concentration in leaves is stated in Figure 1a. Results showed that the highest mean value was observed in the T5 treatment (50 mg Ni kg^{-1} soil + 50 mg Co kg^{-1} soil), which achieved a concentration of 5.01%, significantly different from other treatments. The T5 treatment achieved a percentage increase of 16.24% compared to the control treatment, which achieved a mean value of 4.31%. The lowest nitrogen concentration in the leaf mean values was observed in T4, with a recorded mean value of 3.64%, with a percentage decrease value of 27.35% and 18.41% compared to the T5 and T1 treatments, respectively. However, significant differences were noticed between the treatments. Figure 1b showed a considerable difference in the nitrogen concentration in the broad bean seeds (yield). The results indicated that the maximum mean value was observed in T6 treatment (50 mg Ni kg^{-1} soil + 100 mg Co kg^{-1} soil), reaching a concentration of 3.57% nitrogen in broad bean seeds, and achieved a percentage increase of 33.21% compared to the control treatment, which had a mean value of 2.68. The highest value significantly differed from other treatments except T8 and T9. Similar results were achieved for T6 treatment, which demonstrated the greatest mean value for nitrogen concentration in the investigation's soil at the flowering stage (Figure 1c). The T6 showed a mean value of 34.30 mg N kg^{-1} soil, with a percentage increase of 19.51% compared to the control treatment (T1), which recorded a mean value of 28.70 mg N kg^{-1} soil. Nonetheless, no significant differences were observed between the T6 and other treatments, except for the T8 treatment, which recorded the lowest value. Figure 1d illustrates that the maximum mean value of soil nitrogen at the harvest date was recorded in the T5 treatment, which achieved a value of 24.50 mg N kg^{-1} However, significant differences were observed between T5, which recorded the highest mean value, and the other treatments, except of treatments T4 (50 mg Ni kg^{-1} soil + 0 mg Co kg^{-1} soil) and T9 (100 mg Ni kg^{-1} soil + 100 mg Co kg^{-1} soil), which did not show significant differences. The results illustrated in Figures 1c and

1d demonstrate a lower total nitrogen concentration in the soil across all treatments at both time points, namely, the flowering stage and the harvest date.

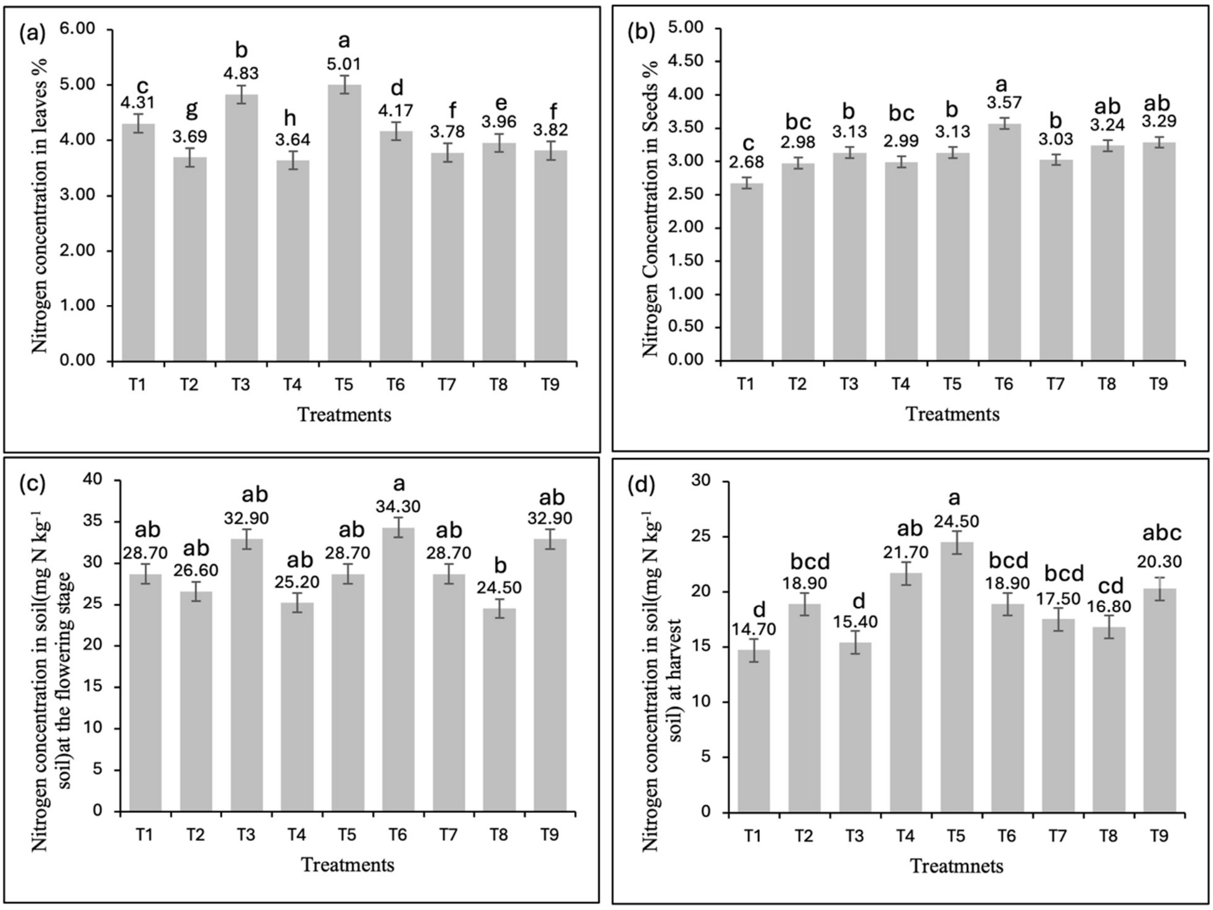

Figure 1. The influence of nickel and cobalt application on (a) nitrogen concentration in leaves; (b) nitrogen concentration in seeds (%); (c) nitrogen concentration in soils (mg N kg^{-1} soil) at the flowering stage; (d) nitrogen concentration in soils (mg N kg^{-1} soil) at harvest. Based on Duncan's test ($p \leq 0.05$), means followed by different letters indicate statistically significant differences among the treatments.

3.5 The influence of nickel and cobalt application on (a) seed yield (Mg ha^{-1}), nitrogen uptake in leaves (kg ha^{-1}), nitrogen uptake in seeds (kg ha^{-1}), and the protein percentage in seeds.

The broad bean seed yield is illustrated in Figure 2a. The seed yield results indicated that the maximum mean value was observed in the T3 treatment (0 mg Ni kg^{-1} soil + 100 mg Co kg^{-1} soil), which recorded a seed yield of 3.69 Mg ha^{-1}, with a percentage increase of 37.69% compared to the control treatment, which achieved a mean value of 2.68 Mg ha^{-1}. However, the seed yield did not show a significant difference among the treatments. Conversely, the nitrogen uptake in leaves elucidated a significant difference between the treatments (Figure 2b). The maximum nitrogen uptake in leaves' mean value was observed at the T5 treatment, which achieved a value of 3.31 kg ha^{-1}, and a percentage increase of 19.50% and 40.42% compared to the T1 and T4, which recorded a mean value of 2.82 kg ha^{-1} and 2.20 kg ha^{-1}, respectively. However, the T5 treatment only differed from T4 and T7. In contrast, the T3 treatment (0 mg Ni kg^{-1} soil + 100 mg Co kg^{-1} soil) recorded the maximum mean value of 115.70 kg ha^{-1}, with a percentage increase of 60.69% compared to the control treatment (T1), which recorded a mean value of 71.96 kg ha^{-1}. The protein content in seeds is depicted in Figure 2d. The T6 treatment recorded the maximum protein in the seeds, with a mean value of 22.31%. However, the lower value was observed at the control treatment, which achieved a mean value of 16.73% and significantly differed from the T6 treatment.

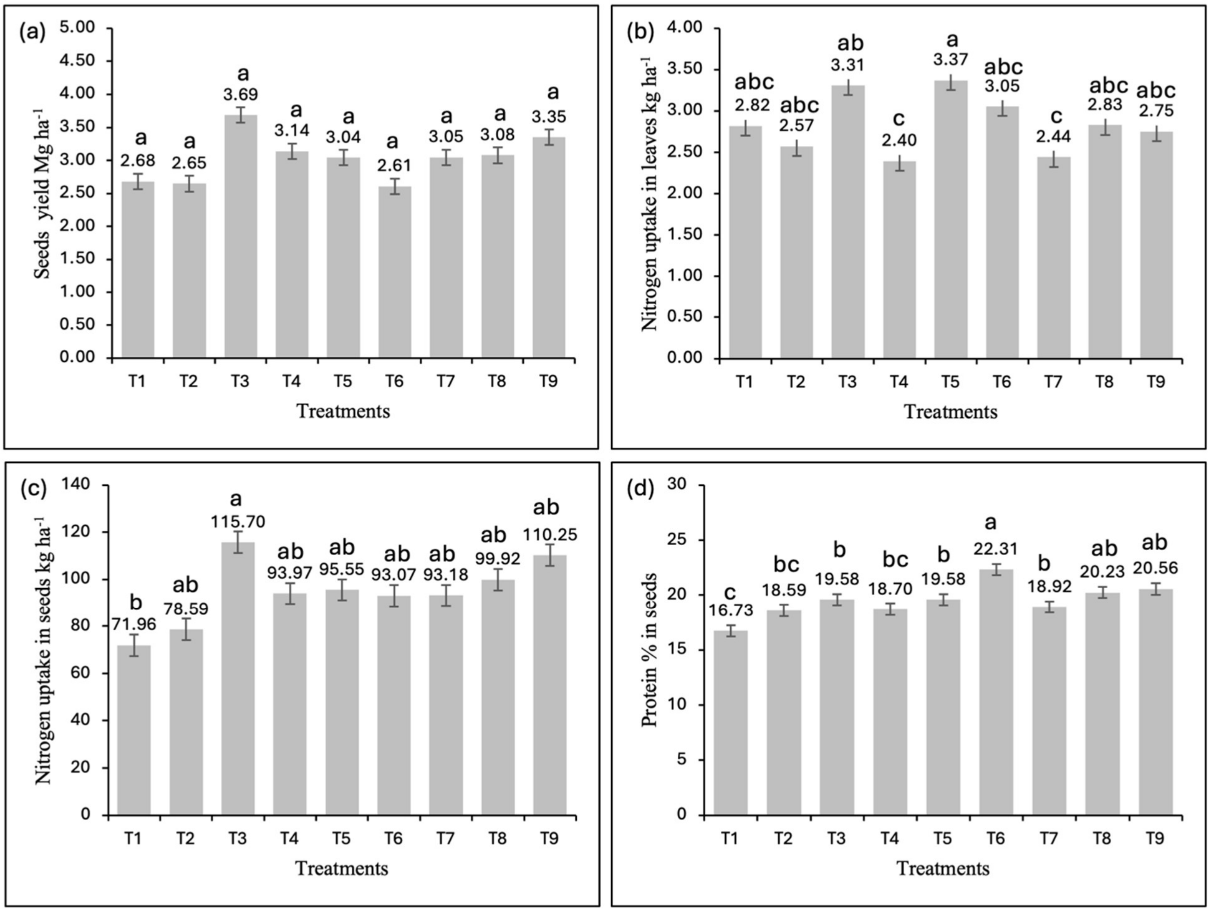

Figure 2. The impact of nickel and cobalt application on (a) seed yield; (b) nitrogen uptake in leaves (kg ha^{-1}); (c) nitrogen uptake in seeds (kg ha^{-1}); (d) protein percentage in seeds. Based on Duncan's test ($p \leq 0.05$), means followed by different letters indicate statistically significant differences among the treatments.

3.5. Simple linear regression between broad bean seed yield, the number of pods plant^{-1}, and nitrogen uptake in seeds (kg ha^{-1})

The simple linear regression illustrates the relationship between the dependent variable, which is the seed yield, and the explanatory variable, indicated by the number of pods per plant (Figure 3a). The linearity showed a positive correlation, yielding a coefficient of determination value of R^2=0.78. On the contrary, a more robust relationship was confirmed between the responsive variable (seed yield) and the predictor variable, nitrogen uptake in seeds, which exhibited a remarkable statistical difference and correlation, achieving an R^2 value of 0.88 (Fig. 3b). Furthermore, the relationships depicted in both linear models, labeled a and b in Figure 3, underscore the strong correlation regarding the influences of the number of pods per plant and nitrogen uptake in seeds, both of which significantly enhance the seed yield of broad beans.

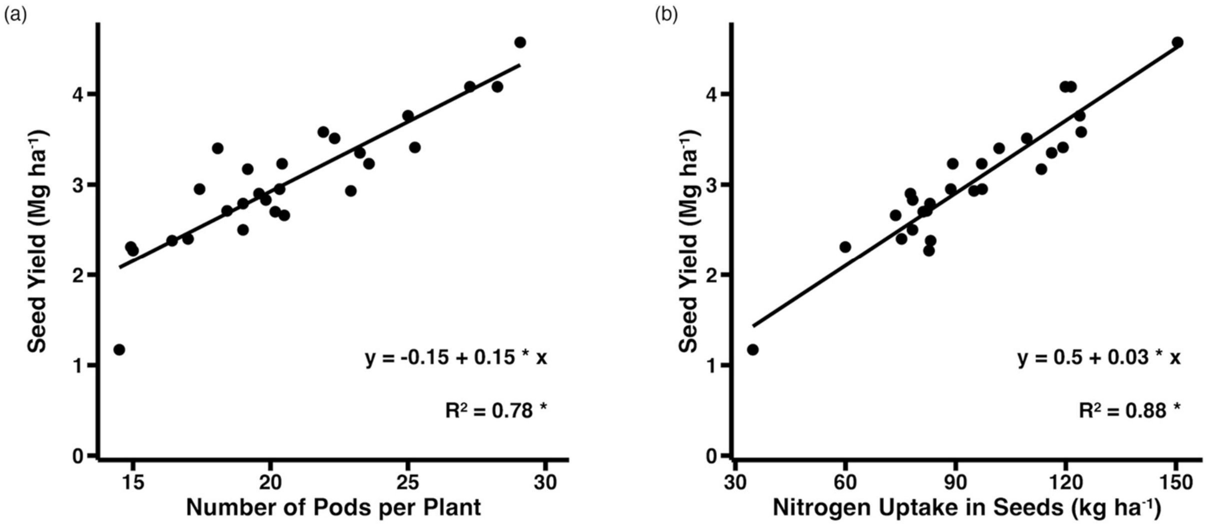

Figure 3. The relation between seed yield (Mg ha^{-1}) and (a) the number of pods per plant, and (b) nitrogen uptake in seeds (kg ha^{-1}). The significant probability $P \leq 0.05$

3.6. Analysis of Simple Linear Regression Relationships Between Nitrogen Concentration in Leaves and Seeds, and Nitrogen Uptake in Leaves and Seeds

To comprehensively understand nitrogen concentration and uptake in broad beans throughout the growing season, the correlations between leaves and seeds were analyzed at both the flowering stage and the harvest date. However, Figure 4 illustrates the linear relationship between nitrogen concentration and nitrogen uptake in broad bean leaves. A simple linear regression analysis revealed that an increase in nitrogen levels within the leaves positively and significantly correlates with an increase in nitrogen uptake, achieving a coefficient of determination (R^2) of 0.39. Likewise, the relationship between the explanatory variable (nitrogen concentration in seeds) and the response variable (nitrogen uptake in seeds) was found to be significantly positive, resulting in an R^2 value of 0.22, as depicted in Figure 4.b.

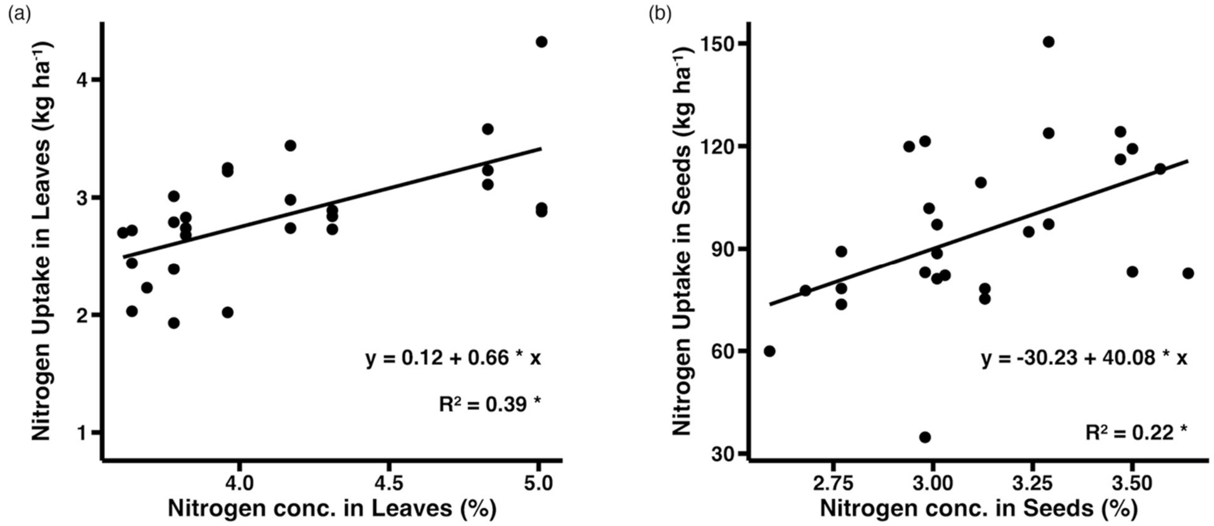

Figure 4. The relationship between (a) nitrogen concentration and nitrogen uptake in leaves; (b) nitrogen concentration and nitrogen uptake in seedlings. The significant probability $P \leq 0.05$

3.7. Biplot of Principal Component Analysis (PCA) Results for the Parameters Evaluated Under Nickel and Cobalt Application

The Principal Component Analysis was performed as an alternative process to assess and modify conflicts among the distinct treatments and trials. This methodology emphasized significant differences across treatments and trials, categorized as high, moderate, and low (Figure 5). However, the results show a conspicuously positive and considerable difference regarding the number of pods per plant, seed yield, nitrogen uptake in seeds, and plant height. Nonetheless, the application of nickel and cobalt has been performed to increase both the number of leaves and the number of nods plant^{-1}, along with nitrogen uptake in seeds. The correlation between the number of pods per plant and nitrogen uptake in leaves and seeds was likely related to the broad bean seed yield. The PCA revealed that nitrogen concentration in seeds and protein percentage was closely affiliated with the PCA, showing a positive correlation. Similarly, nitrogen uptake in leaves, the number of pods per plant, and seed yield demonstrated a significant positive relationship. However, nitrogen uptake in seeds, the number of leaves per plant, and the number of nodes per plant emerged as the most critical and influential variables associated with the PCA, contributing maximally to the interrelationship among the datasets. Notably, leaf area, SPAD readings, and available nitrogen concentration in soils during the flowering stage were identified as a lower dominant, negatively correlated variable, responsible for differentiating among observations along the PCA.

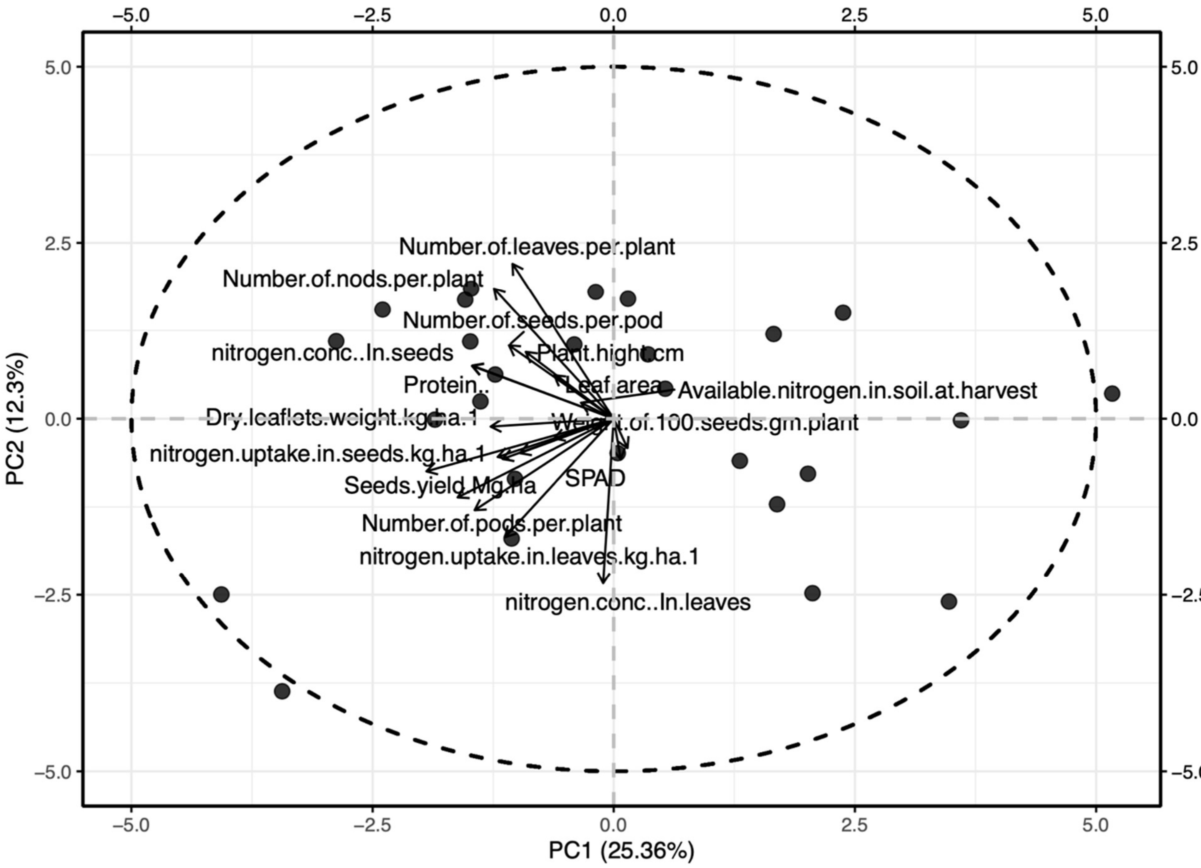

Figure 5. presents the biplot of principal component analysis (PCA) pertaining to individual traits as determined under the influence of nickel and cobalt on broad bean cultivated in the study area.

3.8. The Influence of Nickel and Cobalt Applications on the Contribution of the Variables in the Bar Plot of the Study's Parameters

The bar plot presents the impact of each variable on the variance as determined within principal component analysis (Figure 6). However, the variables exhibiting strong values in the contribution bar chart are considered the most effective in describing the directions of the PCA dimensions (Dim 1 and Dim 2). The outputs showed that nitrogen uptake in seeds achieved the highest contribution value of 10.89%, explaining its importance in presenting the variance within the dataset in relation to PCA. This observation shows that the quantity of nitrogen absorbed by broad bean seeds plays a pivotal role in designation among the observed outcomes. On the contrary, the available nitrogen in the soil during the flowering stage showed the lowest contribution value of 0.28%, suggesting an insignificant effect on the observed inconsistency within the dataset. The number of leaves of plant^{-1} recorded the second-highest contribution value of 9.31%, proving it as a significant variable that impacts the variations in the study results. A moderate contribution value of 8.68% was observed in the seed yield variable, designating that while it exerts a substantial influence on the dataset within PCA, it does not qualify as a dominant factor among the critical variables. Remarkably, minimal contribution values for available nitrogen in the soil at harvest, SPAD, and weight of 100 seeds were recorded at 0.38%, 0.45%, and 1.13%, respectively.

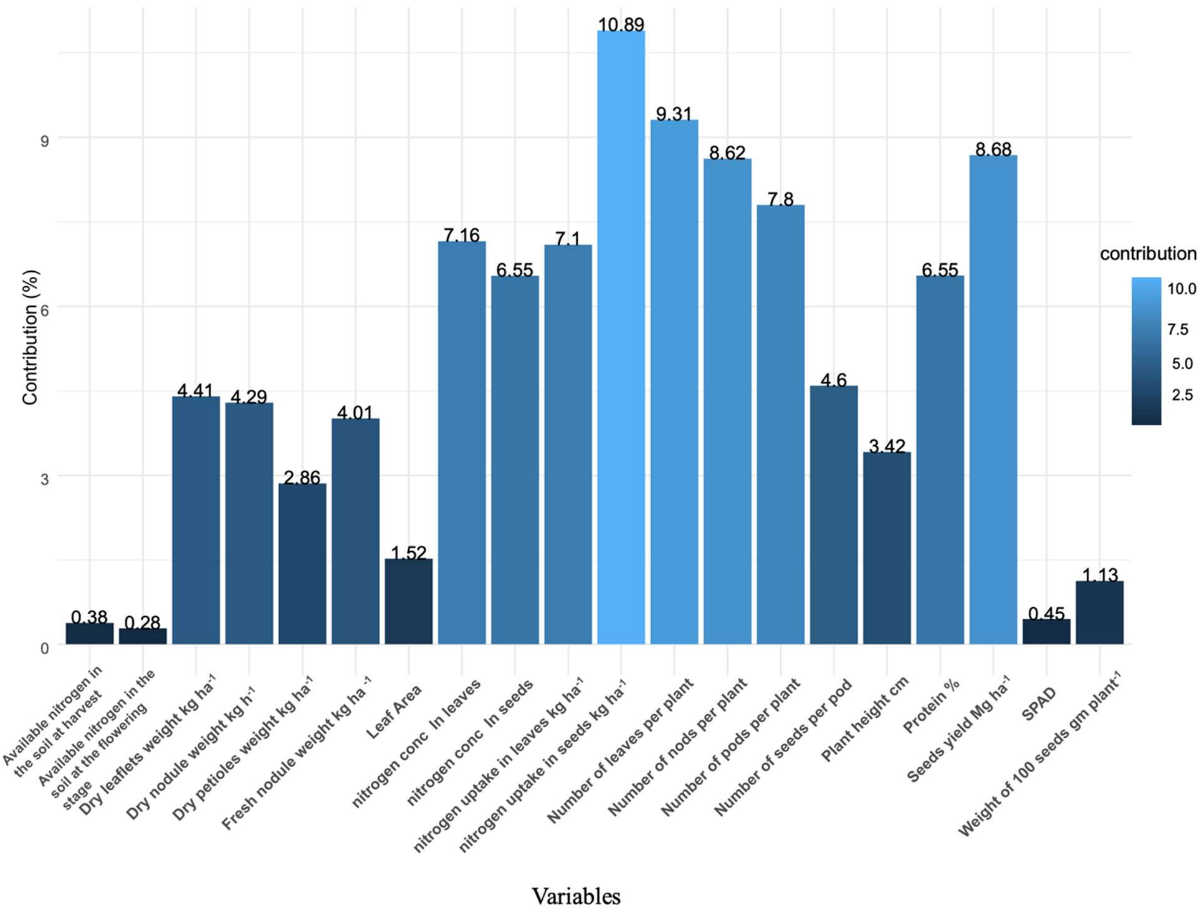

Figure 6. The relationship between the contribution and the independent variables in the bar plot

4. Discussion

Numerous investigations have emphasized the significance of legumes as rotational crops (Al-Obaidi and Abdul-Ratha, 2021). Legumes boost soil nitrogen content and simplify the absorption of such nutrients, in turn, enhancing the nitrogen fixation process. However, the treatment designated as T8, which contains 100 mg Ni kg^{-1} soil + 50 mg Co kg^{-1} soil, has significantly increased plant height, leaf area, and the number of seed pods^{-1}. The maximum values of leaves and nods plant^{-1} were accompanied by the maximum applications of nickel and cobalt (Table 2). This increase can be attributed to the application of nickel and cobalt fertilizers, which play a crucial role in nitrogen metabolism, supplemented by nickel's role in ureide synthesis (Bosse et al., 2024; de Moraes et al., 2022; Saad et al., 2018). Cobalt is an important nutrient for the increase of symbiotic microorganisms such as Rhizobia, which are free-living, nitrogen-fixing bacteria (Anees et al., 2025; Danish et al., 2024; Gad and El-habbak, 2024; Onte et al., 2019). Correspondingly, nickel and cobalt enhance SPAD readings, fresh nodule weight (kg ha^{-1}), dry petiole weight (kg ha^{-1}), and dry leaflet weight (kg ha^{-1}) (Table 3). These findings are consistent with outcomes from other researchers

(Elbagory et al., 2022; Gad and El-habbak, 2024; Saad et al., 2018) who noted a correlation with elevated shoot nitrogen content. However, the results obviously illustrate those increasing applications of nickel and cobalt lead to an elevation in their concentrations within the soil, which is reflected in the nitrogen content of the broad bean leaves (Table 4). The mean values of nickel in the soil ranged from 0.85 to 22.60 mg Ni kg^{-1} soil, whereas cobalt mean values ranged from 0.43 to 5.79 mg Co kg^{-1} soil. However, all nickel and cobalt mean values remain below the toxicity threshold. The nickel and cobalt contents in the leaves of broad beans were consistent with these soil thresholds and deemed non-toxic to the plants (Kapustka et al., 2006; Shivali and Kumar, 2024; K et al., 2025; Barker and Pilbeam, 2015).

Nickel plays a major role in the legume crops (Allito et al., 2020; Lin et al., 2024; Liu et al., 2023). Ureide-transporting types possess metalloenzymes that utilize nickel optimally as a cofactor (Saad et al., 2018). Furthermore, evidence shows that nickel reinforces nitrogen use efficiency (Mahdi and Omran, 2021; Mendes et al., 2023). This study revealed that the application of nickel and cobalt treatments boosted nitrogen content in the leaflets and seeds of broad beans (Figures 1a and 1b). The form of ATP through the tricarboxylic acid (TCA) cycle is important to Rhizobium bacteria, which require organic carbon to obtain such energy (de Moraes et al., 2022). The application of nickel can stimulate the TCA cycle by raising the levels of citrate and malate (Barker and Pilbeam, 2015). Cobalt is vital for nodule formation and is necessary for the synthesis of vitamin B12, which is crucial for hemoglobin development in nodules and optimal nitrogen fixation (Danish et al., 2024). Further, the soil test nitrogen plays a vital role in broad beans growing, especially at the flowering and harvest stages, which are affected by the cobalt and nickel fertilization in the soil (Figures 1c and 1d). The nickel and cobalt fertilizers used in this study contained nickel and cobalt sulfates. However, sulfates could lessen soil pH within the rhizosphere, which improves the availability of essential nutrients, such as nitrogen (AL-Tameemi et al., 2025; El-Fahdawi et al., 2020; Gad and El-habbak, 2024; Jasim, Sharma, et al., 2020). Moreover, sulfur can improve legume nodulation (Gad and El-habbak, 2024).

Figure 2 shows that treatments containing cobalt application resulted in the maximum yield of broad beans. The highest nitrogen uptake values were observed in leaflets and seeds when nickel and cobalt were combined. These observations are in agreement with the findings of previous studies (Gad and El-habbak, 2024; Gu et al., 2024; Thapa et al., 2021), which noted that legumes respond positively to the application of nickel and cobalt, whether applied to the soil or via foliar applications. The researchers elucidate that the application of nickel and cobalt significantly increased all mineral content compared to the control treatment. These findings suggest that nickel mainly affects the yield and quality of crops where protein content is critical. For instance, in soybeans, the application date of nickel enhances the nitrogen concentration in the plant shoot system (de Moraes et al., 2022). The protein content was additionally ameliorated with the application of nickel and cobalt, with the maximum value recorded at the T6 treatment (50 mg Ni kg^{-1} soil + 100 mg Co kg^{-1} soil). This phenomenon can be attributed to the necessity of cobalt for the growth of rhizobia, which play a pivotal role in the nodulation of legumes and the fixation of nitrogen in the atmosphere into amino acids (Gad and El-habbak, 2024; Onte et al., 2019; Saad et al., 2018; Yeremko et al., 2025).

The linear result is illustrated in Figures 3a and b, which indicate that the predictor, specified as the number of pods $plant^{-1}$, resulted in a positive correlation with the response variable, broad bean seed yield, achieving an R^2 value of 0.78. Moreover, the relationship between the explanatory variable, nitrogen uptake in seeds, and the responsive variable, broad bean seed yield, is specified by a positive correlation, as reflected by an R^2 value of 0.88. This can be attributed to the functions of nickel and cobalt in enhancing the seed's biochemical properties, including amino acids, total carbohydrates, starch, and total soluble sugars (Alkurtany et al., 2018). These findings are consistent with published literature (Daur et al., 2011). The leaf area serves as a vital indicator of the plant's vitality and overall health (Hafez et al., 2021). The relationship between nitrogen concentration and uptake in leaves achieved a coefficient of determination (R^2) value of 0.39. Further, the correlation between nitrogen concentration and uptake in broad bean seeds yielded an R^2 value of 0.22, as explicated in Figures 4a and 4b. These findings are in agreement with previous studies by Elshamly (2023); Mendes et al. (2023), who suggested that applying nickel and cobalt can facilitate the conversion of urea into ammonia, which is crucial for the biosynthesis of amino acids that are, in turn, converted into proteins. Other research showed that nickel boosts an increase in urease activity by decreasing urea concentration and increasing ammonia concentration in the leaves, showing a biphasic response to nickel. Moreover, nickel helps the transformation of amino acids into proteins, as evidenced by a reduction in the amounts of amino acids and an increase in protein contents in the leaves (Khazaei and Vandenberg, 2020). Additionally, the application of cobalt results in improved nutrient absorption, including nitrogen, in both leaves and seeds.

The PCA has clarified the positive correlation between the number of leaves and nods per plant (Amoako et al., 2023; Debnath et al., 2025; Pastor et al., 2024). However, a positive correlation was observed between nitrogen content and protein in seeds, as stated in Figure 5. Further, the yield of broad bean seeds, nitrogen uptake in seeds, and the number of pods exhibit a positive correlation with the PCA biplot. In contrast, an inverse relationship is observed between the weight of 100 seeds and the SPAD readings. Figure 6 shows that the variables contributing most significantly are nitrogen uptake in seeds, the number of leaves per plant, broad bean seed yield, and the number of pods per plant. This correlation is mainly attributable to the positive relationships among these parameters, as derived by their interactions with seed yield, which had been notably impacted (Aguiar et al., 2025; Al-Tameemi et al., 2024; Elsalam et al., 2021; Girgel, 2021; Jasim, 2025; Jasim et al., 2020; Saad et al., 2018).

5.Conclusions

This study shows that the nickel and cobalt fertilizer applications can significantly enhance the broad bean seed yield, nitrogen uptake in leaves and seeds, and seed protein content. Mainly, the strongest correlation noticed was between the nitrogen uptake in broad bean seeds and the yield. The principal component analysis of the research variables revealed the most substantial correlations among the number of leaves, pods per plant, and seeds per pod. Additionally, the application of such fertilizers can be beneficial by reducing the economic cost of the need for chemical nitrogen fertilizers, as they increase the activity of nitrogen-fixing bacteria, as evidenced by the analysis of nitrogen availability in broad beans. This investigation shows a viable strategy to improve broad bean productivity. Further studies are required to investigate the effect of nickel and cobalt on other legume species with varying soil physiochemical characteristics.

Author Contributions: "Conceptualization, A.J. methodology, A.J. and K.M; software, A.J.; validation, A.J., K.M., and.; formal analysis, A.J.; investigation, A.J.; resources, K.M.; data curation, A.J.; writing—original draft preparation, A.J.;

writing—review and editing, A.J.; visualization, A.J.; supervision, K.M.; project administration, A.J.; funding acquisition, A.J. All authors have read and agreed to the published version of the manuscript."

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