Physiological and morphophysiological responses of guar (*Cyamopsis tetragonoloba* L.) to cobalt and jasmonic acid

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Abstract

Cobalt (Co) toxicity is a significant barrier to agriculture, and it holds economic significance especially in guar (*Cyamopsis tetragonoloba* L.). The study evaluated whether jasmonic acid (JA) could alleviate cobalt-mediated stress on growth and physiological and morphophysiological parameters of guar plants. Guar seedlings were grown in different levels of Co (0, 50, 100, and 200 µM) with subseque nt application of JA (0, 5, 10, and 15 µM). Results revealed that as Co levels increased, growth and biomass, gum, and carbohydrate accumulation as well as water status reduced while proline, catalase, peroxidase, phenol, and superoxide dismutase increased. It appears that applications of JA at lower concentrations (5 and 10 µM) reduced Co toxicity through increases in water status, reduction in Co uptake, increase in antioxidant defense, and improvements in photosynthesis.

Keywords: antioxidant, heavy metal, photosynthesis, stress, toxicity

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Introduction

Environmental stresses, namely heavy metal pollutants, remain barriers to plant health and agricultural sustainability. Cobalt (Co) is a major contaminant restricting crop production and crop performance stability over time. Cobalt (Co) is required at trace levels by some plants. Cobalt (Co) can be extremely toxic if there is an accumulation of soil Co content above the native soil concentration (Selim et al., 2022; Zahid et al., 2024). Both mining in its artisanal or commercial form and industrial emissions are anthropogenic sources increasing the cobalt (Co) content in soils (Denton-Thompson and Sayer, 2022; Kosiorek and Wyszkowski, 2021). In plants, Co is absorbed as bi- and tri-valent cations and primarily concentrated in roots, and the soil pH affects Co toxicity (Kosiorek and Wyszkowski, 2021). High plant tissue concentrations of Co, correspond to high levels of cellular damage, decreased biomass, and changed root architecture (Hu et al., 2021; Zahid et al., 2024). Co toxicity symptoms are usually identified by chlorosis, decrease in stem height, and leaf wilting at early stage of growth (Genchi et al., 2023; Kosiorek and Wyszkowski, 2021). The oxidative stress is caused by Cobalt which initiates the formation of ROS that are basically destructive elements and in the end they harm the cellular

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lipids, membranes, and DNA (Hu et al., 2021; Zahid et al., 2024). Cobalt also interrupts chloroplast activity, and it restrains antimicrobial enzymes, leading to decreased carbon assimilation (Genchi et al., 2023; Zahid et al., 2024). The expression and severity of Co toxicity differs based on species, soil type and pH (Genchi et al., 2023). Recognizing these effects is essential in minimizing Co toxicity in agricultural and environmental settings. It has been discovered that in case plants get into trouble due to the change in the environment, they can release some elements of their defense in the form of a response. The plant hormone, jasmonic acid (JA), is a necessary signaling molecule in the process and the key to the plant's reaction (Wang et al., 2021), which supports the plant in managing pest and disease attacks, as well as abiotic sources of stress, e.g, heavy metal poisoning (Raza et al., 2021). Even external application of JA causes a rapid increase in the endogenous JA content in plants affected by heavy metal. This result confirms the role of JA in resistance to stress (Raza et al., 2021). Besides being one of the agents responsible for the detoxification of cobalt in the cell, JA still prevents ROS from being steady in concentration and, on the contrary, increases the level of antioxidant defenses (Hu et al., 2023). Heavy metal uptake and the resulting accumulation in the plant tissue are dramatically reduced by JA implying that the photosynthetic apparatus and the osmoregulative processes get protected. Preserving osmoregulation, JA also stimulates osmoprotectants to protect cellular structures (Ahmad et al., 2017). JA also stimulates the accumulation of protective secondary metabolites by triggering signal transduction pathways and inducing defense mechanism (Upadhyay et al., 2025; Wasternack et al., 2019). Therefore, there are practical usages of exogenous JA, as a plant growth regulator since it affords the plant viable alternatives to improve plant tolerance of cobalt toxicity, thereby supporting sustainable agricultural practices in contaminated soils.

Guar (*Cyamopsis tetragonoloba* L.), a drought-resistant legume with high industrial potential due to its galactomannan content, is not immune to these threats, and has a particularly susceptible to contaminants, specifically cobalt which inhibits growth and yield (Abdelgawad et al., 2024). Cobalt has been shown to inhibit functions like photosynthesis and nutrient absorption, and cause oxidative stress, affecting economic potential (Salam et al., 2023). Given the importance of guar in oil and gas, food and pharma industries, it is important to understand transgenerational risks to plan for stressors (Meftahizadeh et al., 2023).

Jasmonates have shown promise in mitigating cobalt stress by enhancing antioxidant defenses and improving physiological parameters in guar (Khurizadeh et al., 2024). Foliar applications of JA increase plant height, leaf area, and root development, leading to higher biomass and seed yield (Khurizadeh et al., 2024; Sheteiwy et al., 2021). Jasmonates also improve galactomannan production and nutrient use efficiency, vital for industrial applications (Khurizadeh et al., 2024). Therefore, this study investigates the potential of JA to alleviate cobalt toxicity in guar, aiming to enhance its physiological and morphophysiological responses in contaminated environments.

Materials and Methods

Experimental setup and treatment application

Guar seeds of the RGC-1031 variety, obtained from the Agricultural Research Centre of Rajasthan State, India, were sanitized using 3% (v/v) hydrogen peroxide solution for 30-minutes, followed by a thorough rinse utilizing sterile, purified water. These seeds were subsequently germinated on humidified filter paper within Petri plates, incubated within a climate chamber. The environmental conditions within the chamber included a 22 °C / 18 °C day / night temperature regimen, 16-hour light / 8-hour dark photoperiod, light intensity of 225 ± 25 µmol m^{-2} s^{-1}, and 75% relative humidity level. At the two-leaf developmental stage, occurring 10 days after germination, uniformly developed seedlings were selected and relocated into 5-liter containers. These containers were covered with polystyrene sheets, each containing six equally spaced apertures, where two seedlings were planted per aperture, and then positioned within a greenhouse environment. Ten days following

transplantation, cobalt (Co, Merck, Darmstadt, Germany, 95%) stress was induced through the introduction of Co $(NO_3)_2$ into the irrigation water, resulting in final concentrations of 0, 50, 100, and 200 µM. The Co solution was consistently aerated and replenished at three-day intervals. The experimental framework utilized a completely randomized block design, incorporating four replicates for each treatment. Jasmonic acid (JA) solutions, formulated according to the methodology outlined by Yu et al. (2018), were prepared at concentrations of 0, 5, 10, and 15 µM. These JA solutions were administered via a handheld sprayer until runoff, at 08:00 a.m., and the containers were returned to their original positions 30 minutes following treatment application. Control specimens received only foliar applications of distilled water. After a treatment duration of 20 days, the plants were harvested, and the root and shoot components were separated.

Morphological evaluation

At 150 days following sowing, coinciding with physiological maturity as indicated by the yellowing of pods, the plants were harvested. For each experimental replicate, a random selection of three plants was made, and individual measurements were recorded, with subsequent calculation of pot means. Plant height, measured from the soil surface to the plant's apical point, was determined using a standard measuring ruler. Roots were carefully separated and washed, and their lengths were also quantified using a ruler. A range of morphological parameters were assessed, encompassing the number of branches per plant, flower count, cluster count, leaf count, seed count, and pod count. Five pods were randomly sampled to evaluate pod length and the number of seeds per pod. For the determination of biomass, plants were subjected to oven-drying at 72 °C for a 48-hour period before weighing. All linear measurements were recorded in centimeters. Leaf area was determined using the grid-based technique, as detailed by Meftahizadeh et al. (2019). Each leaf was placed on millimeter graph paper with a 1 cm grid. The leaf's perimeter was traced, and the number of complete and partial grid squares contained within the outline was enumerated. The total leaf area was then calculated using the following equation:

$$\text{Leaf Area (cm}^2\text{)} = N \times B$$

where:

N represents the total number of 1 cm² grid squares encompassed by the leaf outline and B represents the area of a single grid square, which is 1 cm².

Catalase enzyme activity assay

We evaluated and measured catalase (CAT) activities using an indirect spectrophotometric method modified from Hadwan et al. (2018). In brief, we added 50 µL of leaf extract to 1 ml of reaction solution (50 mM potassium phosphate buffer pH 7.0, 15 mM hydrogen peroxide H_2O_2), and recorded absorbance at 240 nm (A240) every 10 seconds for 60 seconds. By measuring the decrease in H_2O_2, we calculated catalase activity and expressed it as units per mg.

Superoxide dismutase activity assay

The activities of superoxide dismutase (SOD) were determined according to the modified method of Morgun et al. (2021) in short, 50 mL of leaf extract sample in the reaction mixture containing 75 mM NBT, 13 mM L-methionine, 0.1 mM EDTA, 2 mM riboflavin, and 50 mM potassium phosphate buffer at pH 7.8 were mixed and the reaction was allowed to start with the light of fluorescent bulb for 15 min. The absorbance was read at 560 nm with a spectrophotometer. The SOD activity was expressed in unit mg protein^{-1} by activating the absorbance reading of reduced NBT reduction inhibition expressed in unit mg protein^{-1}. One unit was defined as the amount of enzyme that elicited 50% inhibition of NBT reduction.

Peroxidase enzyme activity assay

Peroxidase (POX) activity was measured using spectroscopy (Ismail et al. 2022). Thirty-three (33) µL of leaf extract was taken and mixed with 1 mL of 13 mM guaiacol and 50 mM potassium phosphate buffer (7.0) with 5 mM H2O2 and 50

mM potassium phosphate buffer (pH 7.0). It was read at 470 nm for 60 s. Peroxidase activity was measured from the guaiacol oxidation rates, which were directly proportional to the change in orange-red color and expressed in units per mg protein.

Proline content assay

Proline content was spectrophotometrically determined, by the method of Kubi et al. (2021). Homogenates were obtained after grinding 0.5 g of fresh leaf tissue in 10 mL 3% (w/v) sulfosalicylic acid in a mortar and pestle. The homogenate was filtered on Whatman No. 2 filter paper. An aliquot (2 mL) of the filtrate was treated with acid ninhydrin reagent (dilute 1.25 g of ninhydrin in 30 mL of glacial acetic acid) and was allowed to incubate in the dark at 50 °C for 2 h. The reaction solution was heated in a 100 °C water bath for 60 min. The reaction ended by placing the tubes in an ice bath. The chromophore was extracted with 4 mL of toluene and vortexed. The toluene phase was quantified by UV absorption at 520 nm with a spectrophotometer. Proline level was measured employing a standard curve of known proline concentrations, and presented as µmol/g (g fresh weight) material.

Chlorophyll content assay

The chlorophyll a, chlorophyll b, and total chlorophyll content of the guar leaves was ascertained using the equations set forth by Arnon (1949):

Chlorophyll a (mg g^{-1} FW) ¼ (0.0127 × A663) - (0.00269 × A645)

Chlorophyll b (mg g^{-1} FW) ¼ (0.0229 × A645) - (0.00468 × A663)

Total Chlorophylls (mg g^{-1} FW) ¼ (0.0202 × A645) þ (0.00802 × A663)

Relative water content assay

The relative water content (RWC) was assessed utilizing the protocol established by Neshat et al. (2022).

Gum percentage assay

To quantify the gum content, a separation procedure, adapted with minor modifications from Meftahizadeh et al (2023), was implemented. Initially, the seeds were hydrated for 8 hours. Following seed husks removal by surface abrasion, mechanical pressure was applied to expel the gum from endosperm and embryo. The resultant gum was subsequently oven dried at 50 C for 8 hours. The weight of the dried gum was then weighed and calculated as a percentage against the embryo and endosperm.

Total phenolic compounds assay

Folin-Ciocalteu method was used to determine the total phenolic content. To this end, 0.1 g dry leaf tissue sample was homogenized in 1 ml deionized water. Then, 0.1 ml of the homogenate was adjusted in 2 ml-2% sodium carbonate (Na_2CO_3), and 2.8 ml deionized water. Next, 0.1 ml-50% Folin-Ciocalteu reagent was added. The solution was mixed and left for 30 minutes at room temperature and absorbance was read at 765 nm using a spectrophotometer (Bright Technologies Inc, Chandkheda, India) with the blank being distilled water. A standard curve using gallic acid was created following Miliauskas et al (2004). Concentrations are presented in mg gallic acid equivalent/g dried weight.

Total carbohydrates assay

The total carbohydrate concentration was identified using the modified method described in Meftahizadeh et al (2022). In short, powdered samples (500 mg) were placed into 250 mL volumetric flasks and acid hydrolysis was performed using 1 g of powder and 4 mL of 70% sulfuric acid. The flasks were then placed into a water bath (90 °C) for a period of three hours. The filtrate collected in Erlenmeyer flasks were diluted to a mark of 100 mL. A 1 mL aliquot of this 100 mL dilution was added to 1 mL of double-distilled water making a blank. Next, 0.2 mL of phenol reagent was added to both the blank and sample tubes, and then 5 mL of concentrated sulfuric acid was quickly added and mixed thoroughly. The absorbance of the samples was then measured at 480 nm. Total carbohydrate concentrations were calculated based on a calibration curve from

varying concentrations of glucose, and reported as percent.

Measurement of viscosity

In order to assess the viscosity of the extracted gum, a preliminary purification procedure was performed (as detailed in Brummer, et al.2003). After dissolving the gum powder in water, to produce a hydrated gum base, the gum powder-water suspension was centrifuged for 15 minutes at 5000 rpm to eliminate sub-cellular ionic and polymeric debris. An ethanol solution was introduced into the purified gum solution at a ratio of 3:1 and allowed to sit for 50 minutes. At this stage, the ethanol disrupted gum-water interactions, which facilitated the setting of white coils within the solution. These coils of purified gum were collected and dried in an oven at 40 °C for 6 hours. For viscosity assay, solutions at several concentrations were prepared from the purified gum. These included the preparation of a 1% solution (1 gram of gum powder diluted in 99 mL of deionized water). The 1% gum powder solution was mixed with a magnetic impeller for 24 hours for complete hydration. Subsequently, the viscosity of the gum solutions was determined at the prepared concentrations, using a calibrated D-V-3 ultra-viscometer (Lamy Rheology, Champagne-sur-Marne, France).

Data Analysis

The statistical analysis of the collected data was conducted using SAS software, version 9.4, developed by SAS Institute, Cary, North Carolina, USA. Prior to any analytical procedures, the dataset, consisting of three replications, was evaluated for adherence to normality assumptions. Subsequently, an analysis of variance (ANOVA) based on a general linear model was employed to examine both the main and interaction effects. Results that could allow for a more extended interpretation of results in different apple-types, were also stratified by variety. In cases where the F-test was significant at the p-risk level, the mean of the level of primary effects was compared by Duncan's Multiple Range Test (DMRT) at 0.01prob value.

Results

Variance analysis

The application of ANOVA revealed statistically significant alterations across all measured morphological and morphophysiological traits within the guar variety. These variations were observed in the main effects, as well as in two-way interactions among the diverse levels of jasmonic acid JA and Co stress. The following section details these interactions and main effects, presented in a hierarchical order of statistical significance, starting with the most intricate interactions and concluding with the effects of individual treatments. In instances where two interactions were identified for a specific parameter, interpretation of the main effects was either partially addressed or entirely excluded.

Physiological responses of guar to cobalt and jasmonic acid

The results of the study showed that as concentrations of Co increased from 50 µM to 200 µM, all morphological traits studied for guar plants were significantly and progressively decreased. This demonstrated the impact of cobalt stress on overall plant growth and development (Table 1). Plant height, an important measure of vegetative growth, decreased with increasing concentrations of cobalt. The control plants, which were 60 cm tall, compared to a severe reduction in height for plants at 200 µM Co. There was a similar reduction in number of branches per plant, which is another important measure of vegetative growth, with increasing amounts of cobalt co. Compared to the control plants, which showed the highest branch count (12.4), There was a significant reduction at 50 µM Co, with further reductions at 200 µM Co, which indicated that cobalt inhibited branching in guar. In addition, Mirina had a notable impact on the reproductive function of guar. The number of flowers, which are necessary for the production

Table 1
Mean comparison for the morphological traits of guar variety (RGC-1031) under different cobalt stress

CO (µmol)	High	Branch/plant	Flower No	Cluster No	Leaf No	Leaf area	Pod length	Seed No	Pod No
Control	60.2±2^{a}	12.4±1.2^{a}	85.4±2^{a}	11.5±1.5^{a}	110.4±3^{a}	21.5±1^{a}	7.5±1^{a}	7.3±1.2^{a}	5.3±0.75^{a}
50	55.3±1^{b}	11.2±1.3^{b}	55.2±3^{b}	10.5±1^{ab}	85.6±2^{b}	15.7±2^{b}	6.5±1^{b}	6.7±2^{a}	4.5±0.5^{b}
100	45.1±2^{c}	11.5±1.1^{b}	45.3±1^{c}	8.6±1^{b}	46.7±1^{c}	16.5±1^{ab}	5.4±0.5^{bc}	5.6±1.1^{b}	4.3±0.5^{b}
200	44.6±2^{c}	10.7±1.2^{c}	42.5±2^{c}	5.6±1^{c}	45.6±2^{c}	14.8±1^{b}	4.5±0.5^{c}	5.5±1.2^{b}	4.8±0.5^{ab}

The Duncan's Multiple Range Test (DMRT) test (p≤0.05) was used to identify significant differences between means within columns; dissimilar letters denote these differences.

of seeds and gum, significantly decreased with increasing cobalt levels. Control plants had a maximum of 85.4 flowers produced, but they declined at 50 µM Co with further declines with increased Co. Similarly, the clusters, which relate to yield, followed the same trend, with control plants having the most clusters (11.5) and decreasing under cobalt stress (Table 1). Several of the vegetative parameters showed a negative response to cobalt exposure. The leaf area, which is essential for photosynthesis showed a significant reduction at higher cobalt levels. The control plants had the largest leaf area (110.4). The pod length was also significantly smaller under cobalt stress. Control plants had the longest pod length (21.5) and the highest number of pods (7.5). Therefore, the number of seeds per pod, which is the direct measure of yield, also dramatically reduced when cobalt concentrations increased. The control plants had the highest number of seeds (7.3), reaffirming the negative impact cobalt has on reproductive potential. The number of pods, however, presented a unique pattern. The highest number of pods was noted in the control plants (5.3) and then it decreased with exposure to increasing cobalt stress and finally increased slightly at 200 µM Co. After reviewing the cumulative effect of increasing cobalt stress, the impact of cobalt was clearly significant across all measured morphological traits, suggesting that cobalt detrimentally affected the growth, development, and yield of guar (Table 1).

Morphological responses of guar to cobalt and jasmonic acid

A considerable decrease in the morphophysiological performance of guare was observed across the two evaluated levels of increasing cobalt concentrations, 50 to 200 µM. The variable stress of cobalt significantly reduced root length, biomass, gum and carbohydrate amount, % water and chlorophyll amount (Table 2). The stress of cobalt haltered plant growth and also increased proline, catalase, peroxidase, phenol and superoxide dismutase activity indicating these plants defense mechanism has been activated. Application of jasmonic acid (JA), in particular the lower concentrations (5 µM and 10 µM), demonstrated some effectiveness in alleviating the adverse effects of cobalt stress. For example, JA mostly at 5 µM still maintained or improved biomass, carbohydrate content and relative water content at all cobalt stress levels (Table 2). Additionally, JA application, primarily at 5 µM and 15 µM, decreased proline accumulation, and for the 5 µM JA, increased catalase activity under cobalt stress indicating its potential for lowering cellular oxidative damage (Table 2). JA also increased viscosity at the 200 µM Co treatment level, possibly suggesting a great beneficial impact on gum quality following severe stress events. However, JA increased peroxidase activity to a more limited degree and decreased phenol content under cobalt stress. Additionally, provided the concentration increase in cobalt stress, at high cobalt concentrations, it was notable to see those effects of JA application on root length, as well as superoxide dismutase activity, decreased. Generally, JA application, especially at lower concentrations, improved plant resilience under cobalt stress, thus indicating its potential as a bio stimulant to mitigate heavy metal toxicity to guar plants (Table 2) 2). This investigation also focused on evaluating how cobalt (Co) and jasmonic acid (JA) influence glutathione reductase activity within guar plants. Enzyme activity was relatively low in the control

Table 2
Mean comparison for the morphophysiological traits of guar variety (RGC-1031) under different cobalt stress and jasmonic acid

JA (µM)	RL (cm)	BIO (kg/ha)	CAT (mg protein)	SOD (Unit mg $^{-1}$ protein. Min $^{-1}$)	POX (mM H_2O_2 mg^{-1})	PRO (µ mol g^{-1} FW)	CH (mg/g FW)
control	32.8±0.2 f	6523±3 b	23.4±0.5 h	98.2±2 h	4.2±0.2 h	1.4±0.1 g	36.3±2 b
5	26.5±0.3 g	6752±4 ab	23.2±0.25 h	95.8±2 i	3.6±0.4 i	1.6±0.2 f	37.9 ±2 ab
10	23.5±0.4 h	7565±5 a	21.4±0.5 i	85.9±3 j	3.5±0.3 i	1.7±0.4 ef	38.2±2 a
15	20.0±0.2 i	7465±4 a	22.9±0.3 hi	82.6±4 j	3.7±0.4 hi	1.6±0.1 f	39.4±4 a
control	47.8±1 c	6425±5 b	27.4±0.5 e	125.3±2 f	4.6±0.4 f	1.8±0.3 d	34.8±2 d
5	42.6±2 d	5896±4 c	26.3±0.4 f	118.5±2 fg	4.3±0.4 g	1.7±0.1 d	35.2±2 cd
10	39.6±2 de	5669±6 cd	25.4±0.5 fg	110.6±4 g	4.1 ±0.3 g	1.5±0.2 e	36.2±1 c
15	20.6±1 i	5879±5 c	24.6±0.6 g	120.1±2 fg	5.1±0.4 ef	1.5±0.2 e	35.5±2 cd
control	70.1±2 a	4146±3 e	31.2±0.4 c	160.3±1 d	6.8±0.1 d	2.2±0.1 b	33.2± e
5	62.2±2 ab	5896±2 c	32.4±0.5 bc	135.6±2 e	6.5±0.4 d	2.1±0.4 b	33.5±3 e
10	35.2±1 e	5268±5 d	29.4±0.4 d	166.5±1 c	5.8±0.4 e	2.4±0.4 b	31.7±4 f
15	35. 7±1 e	5196±6 de	29.7±0.5 d	155.3±2 d	5.5±0.4 e	1.9±0.3 c	31.5±2 f
control	55.3±1 b	3652±4 f	31.2±0.5 c	180.2±2 a	8.8±0.4 a	3.1±0.2 a	25.5±1 h
5	48.3±2 c	4125±4 ef	33.2±0.4 a	175.8±1 b	8.5±0.4 ab	2.3±0.4 b	26.3±2 g
10	30.7±1 fg	2963±2 g	32.5±0.6 bc	170.6±2 bc	8.2±0.4 b	2.2±0.3 b	24.2±4 i
15	22.6±0.5 h	2863±3 h	33.1±0.4 b	165.2±2 c	7.3±0.1 c	2.7±0.4 ab	25.3±2 h

Table 2 (continued)
Mean comparison for the morphophysiological traits of guar variety (RGC-1031) under different cobalt stress and jasmonic acid

CO (µM)	JA (µM)	RWC (%)	Gum (%)	PH (µg GAE ml^{-1})	V (cps)	Carb (%)
control	control	91.8±2 ab	25.6±1 bc	14.5±1 g	3500±8 b	32.5±2 f
	5	90.2±3 b	26.7±2 b	13.4±2 h	3620±5 a	36.2±1 a
	10	93.4±2 a	28.6±1 ab	12.8±1 i	3714±7 a	36.4±4 a
	15	91.5±4 ab	30.4±2 a	13.2±2 h	3536±6 b	35.6±3 b
50	control	85.3±2 cd	24.4±1 d	18.5±2 d	2850±4 c	33.2±2 e
	5	86.7±2 c	25.7±2 c	16.7±2 e	2760±5 c	34.2±1 de
	10	83.5±1 e	24.3±3 d	16.4±2 e	2640±4 cd	33.5±2 e
	15	84.2±2 d	24.8±1 d	15.4±1 f	2520±3 d	34.6±2 c
100	control	75.2±2 fg	23.4±1 e	22.4±3 b	2350±4 e	29.5±1 g
	5	76.8±4 f	22.4±2 ef	21.6±2 bc	2330±5 e	29.6±2 g
	10	78.3±2 e	23.1±1 e	20.1±1 c	2245±6 f	27.2±1 e
	15	75.8 ±3 fg	22.7±2 f	21.5±2 bc	2300±8 e	25.3±2 ef
200	control	73.5±1 h	21.7±1 h	24.5±1 a	1650±8 i	22.1±3 g
	5	75.2±3 g	22.4±2 g	22.3±1 b	1850±5 h	23.6±1 f
	10	72.5±4 hi	22.7±2 g	21.5±2 bc	2050±6 g	23.5±1 f
	15	72.4±2 hi	20.3±3 hi	22.6±2 b	1860±7 h	22.3±2 g

samples (culture maintained without cobalt 0 µM Co) -around 20 units. A progressive increase in enzyme activity was observed with increasing cobalt concentrations, reaching a maximum at

approximately 60 units with 10 µM Co. However, the activity diminished to about 50 units with 15

µM Co, suggesting a potential toxic effect at high cobalt levels (Fig. I). Subsequently, there was not significantly different activity from the control JA treatment at 15 µM Co, indicating a potential maximum of the protective effect of JA against extreme levels of stress.

This research analyzed the impact of cobalt (Co) and jasmonic acid (JA) on the respiration rate of guar plants. Under control conditions (0 µM Co), the rate of respiration was at a minimum, approximately 25 units. As Cobalt levels increased to 5 µM and then to 10 µM, the respiration rate increased and then peaked at 60 units. The respiration rate was normalized again at 15 µM Co at a rate of approximately 50 units (Fig. II). The addition of jasmonic acid (JA) at 5 µM and 10 µM Co, increased the respiration rate, showing that JA can enhance metabolic activity in cobalt response. JA did not show any influence in the respiration rate at the 15 µM Co site.

Correlation analysis of morphophysiological traits in guar plants under cobalt stress

Enzyme activity was relatively low in the control samples (culture maintained without cobalt 0 µM Co) -around 20 units. A progressive increase in enzyme activity was observed with increasing cobalt concentrations, reaching a maximum at

approximately 60 units with 10 µM Co. However, the activity diminished to about 50 units with 15 µM Co, suggesting a potential toxic effect at high cobalt levels. Finally, members showed that gum (Gum) was positively correlated to carbohydrate (CH), relative water content (RWC), and viscosity (V) (Table 3).

This means that greater gum production is associated with more carbohydrate build-up, water retention, and viscosity. There was also a significant positive correlation between relative water content (RWC) and viscosity (V) and phenol content (PH). The activity of catalase (CAT) and peroxidase (POX) also had a significant positive

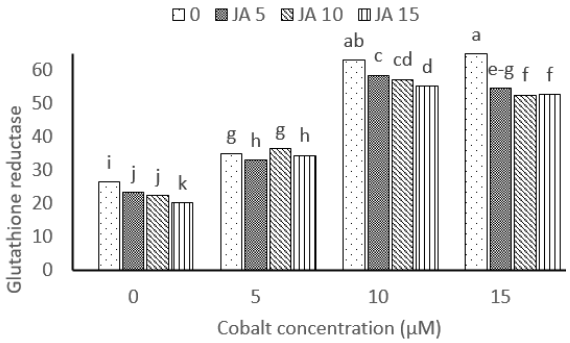

Fig. I. Modulation of glutathione reductase activity in guar plants under cobalt stress by jasmonic acid

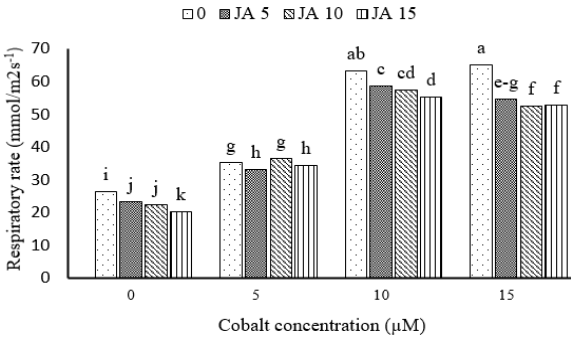

Fig. II. Impact of cobalt stress and jasmonic acid on respiration rate in guar plants

correlation with SOD activity, suggesting that oxidative damage may follow a coordinated route in plant stressing events. However, root length (RL), biomass (Bio), gum production (Gum), and relative water content (RWC) were significantly negatively correlated with proline (PRO) content, catalase (CAT) activity, and peroxidase (POX) activity.

Discussion

Guar physiological responses under cobalt and jasmonic acid treatments

Cobalt in high doses is toxic, this occurs mainly as a consequence of induction of oxidative stress or cellular homeostasis disruption (Meftahizadeh et al. 2023). This results in the accumulation of reactive oxygen species which affects central enzymatic activities and the photosynthetic process as well as chlorosis and decreases in chlorophyll content (Kosiorek et al., 2021). The effect of cobalt on roots and nodulation is suppressed, especially at low cobalt stress (Hu et al.,2023). Cobalt also interferes with the uptake of

Table 3
Correlation analysis of morphophysiological traits in guar plants under cobalt stress

	RL	Bio	Gum	CH	RWC	PRO	CAT	POX	PH	V	SOD
RL	1										
Bio	0.812**	1									
Gum	0.829**	0.762**	1								
CH	0.611**	0.434**	0.622**	1							
RWC	0.732**	0.610**	0.615**	0.601**	1						
PRO	-.348**	-0.545**	-.521**	0.001	-.341**	1					
CAT	-.372**	-0.503**	-.314**	-0.090	-.351**	0.415**	1				
POX	-.437**	-0.425**	-.418**	-0.147	-.479**	0.574**	.270**	1			
PH	0.800**	0.734**	0.718**	0.533**	0.690**	-.437**	-.299**	-.510**	1		
V	0.740**	0.766**	0.899**	0.378**	0.554**	-.736**	-.388**	-.514**	.702**	1	
SOD	-.797**	-0.781**	-.804**	-.450**	-.739**	.737**	0.517**	0.665**	-.820**	-.880**	1

Correlation matrix showing Pearson's correlation coefficients (r) between various morphophysiological traits in guar plants under cobalt stress; significant correlations ($p<0.05$) are indicated with asterisks (*). RL: Root Length, Bio: Biomass, Gum: Gum Content, CH: Carbohydrate Content, RWC: Relative Water Content, PRO: Proline Content, CAT: Catalase Activity, POX: Peroxidase Activity, PH: Phenol Content, V: Viscosity, SOD: Superoxide Dismutase Activity.

nutrients like iron and copper with the latter interference on chlorophyll production being worse (Elshamly, 2023). Furthermore, it interferes with plant hormone balance and water transport, impacting overall growth and development of the plant (Hatamman and Abdullah, 2021). Studies consistently report significant growth inhibition, reduced seed germination, and compromised photosynthetic capacity in legumes under cobalt stress (Kosiorek et al., 2021). These findings align with our observations of reduced morphological traits in guar, reinforcing the detrimental impact of excessive cobalt on legume health and productivity.

The morphological effects of cobalt and jasmonic acid on guar

In guar plants under cobalt stress, JA application helps overcome the detrimental consequences through a multipronged approach. JA also incrementally affected water relations by increasing relative water content (RWC), thus maintaining turgor and function under stress (Ahmad et al. 2021; Hanaka et al. 2015). In addition, as in fava bean, JA exerts as a non-selective uptake and accumulation phytotoxic cobalt in plant tissues which decrease the direct metal toxicity (Ahmad et al., 2017). Also, JA counteracts the oxidative damage of H_2O_2 and MDA by increasing antioxidant enzymes and thus enhances the plant cellular elements defense system (Ahmad et al., 2017, Zhu et al., 2021). Moreover, JA activates stress-responsive genes which help the plant cope and survive cobalt-induced stress using intricate signaling pathways (Yavaş et al., 2022; Liu et al., 2021; Pandey et al., 2023). Furthermore, JA has shown to alleviate the negative influences of cobalt on vital processes by increasing the photosynthetic capacity and gas exchange parameters associated with chlorophyll (Rehman et al., 2023). Evidence has also shown that JA preserves cellular balance and function during stress by enhancing the antioxidant functions of organelles (Ashraf and Siddiqi, 2024; Swain et al., 2023). Finally, foliar JA application enhances ROS defense by regulating water content and photosynthetic parameters, improving plants' overall performance under cobalt stress (Ashraf and Siddiqi, 2024). These mechanisms collectively demonstrate how JA application in guar plants effectively counteracts cobalt stress, aligning with our findings of improved morphological and physiological traits.

The use of Jasmonic acid increases relative water content (RWC) and augments water relations in plants under heavy metal stress (Ahmad et al., 2021; Hanaka et al., 2015). JA improves efficiency of water and photosynthetic use due to the enhancement of chlorophyll and gas exchange parameters (Ashraf and Siddiqi, 2024; Swain et al., 2023). JA strengthens cellular antioxidant systems by lowering H_2O_2 and MDA, increasing protective enzymes' activity (Ahmad et al., 2021). Ipomoea aquatica, a water plant, accumulates proline which helps as an osmoregulatory mechanism and JA

positively influences its production (Hosseinifard et al., 2022, Inayat et al., 2024). It has been shown that jasmonic acid also regulates levels in hormones, which helps increase abscisic acid (ABA), gibberellic acid (GA), and endogenous jasmonic acid (JA) (Sheteiwy et al., 2021; Yavaş et al., 2022; Liu et al., 2021). The relationship with soil mineral is improved with JA, because nutrient homeostasis is returned to plants by improving mineral balance in chromium stress treatments of soil minerals (Rehman et al., 2023, Vega et al., 2022). In palynological plants, JA increased drought tolerance by promoting antioxidant enzyme activity and proline levels (Đurić et al., 2023). Cobalt is critical for nitrogen fixation systems of leguminous plants affecting enzyme systems as well as leghemoglobin (Banerjee et al. 2021, Kumari et al., 2022, Tomić et al., 2020). JA, together with foliar application of cobalt, optimizes cobalt status in the roots and nodules (Tomić et al., 2020). In addition, JA alleviates metabolic disorders caused by heavy metal exposure, and increases the activities of antioxidant enzymes (Raza et al., 2021). JA enhances drought related gene expression in soybeans treated with cobalt nanoparticles (Linh et al., 2020, Tamindžić et al., 2024). JA enhances efficiency of cobalt in moringa plants (Elshamly, 2023; Gad et al., 2019). In addition, jasmonic acid application improved the morphological characteristics of plant grown under heavy metal stress. Foliar application of JA reduced chromium stress on plants, and at higher concentrations, of 5, 10 and 15 µM was most effective, and improvements were seen in morphological parameters (Rehman et al., 2023). JA treatment recovers decreases in both root length and plant height, and thereby biomass production, induced by heavy metals (Vega et al., 2022). In detail, JA neutralizes detrimental stress to plant growth caused by cobalt stress specifically (Mahmood et al., 2023, Rehman et al., 2023). JA decreased metal accumulation while allowing growth to occur through morphological improvements and reducing chromium uptake (Vega et al., 2022; Rehman et al., 2023). JA protects the structure of cells, as demonstrated in an experiment with zinc oxide nanoparticles in maize (Silva et al., 2022). JA also encourages root formation which improves overall growth and plant architecture (Vega et al., 2022). These enhancements are attributed to JA ability to lower oxidative stress, which prevents cellular stress resulting in the prolonged morphological development in the plant (Silva et al., 2022). These findings support our results where JA treatment was able to alleviate harmful impacts of cobalt stress on morphology and physiology of guar plants.

The increasing activity of glutathione reductase under cobalt concentrations (up to 10 µM) demonstrates the plant defense system responding to cobalt stress. Since this enzyme is an important part of the antioxidant system of a plant, which is responsible for decomposing free radicals to alleviate oxidative stress, the observed increase in activity can reflect the plant's efforts to reduce any oxidative damage imposed by cobalt (Farasati Far 2024, Jobe et al., 2023). The diminished activity of glutathione reductase with 15 µM Co indicates the toxic effects of high concentrations of cobalt in the plant. Cobalt can affect the antioxidant defense of the plant, potentially with effects on glutathione reductase activity (an unintentional cofactor). Increased glutathione reductase activity via JA application at 5 µM and 10 µM Co demonstrated increased tolerance to stress from cobalt, demonstrating that JA mediates plant tolerance to cobalt stress. JA is a biologically active compound involved in plant stress responses. Ja studies have focused on changes in antioxidant enzyme activity (which may be a direct or indirect consequence of antioxidant signaling); in the case of cobalt, JA may stimulate antioxidant enzyme activities and the activities of antioxidant enzymes increase the plant’s tolerance. It is noted that low to moderate concentrations of cobalt stimulate glutathione reductase activity, activating plant antioxidant defense mechanisms. High concentrations of cobalt are toxic and will reduce any enzyme activity. Jasmonic acid will enhance plant tolerance stress under cobalt stress treatment at low concentrations (Farasati Far, 2024, Jobe et al., 2023).

The rising respiration rates observed with increasing cobalt concentrations up to possibly 10 µM indicate a metabolic response to cobalt stress, reflecting a high energy requirement possibly due to lower metabolic efficiency to combat cobalt-

induced oxidative stress or, possibly, a completely different metabolic disturbance. The respiratory decreases at 15 µM Co shows that cobalt at such high levels causes toxic effects which in turn could damage the metabolic machinery of the plant. The strong stimulation of respiration rate by JA at both 5 µM and 10 µM Co demonstrates its capacity for stimulating metabolic activity, likely through influencing metabolic enzyme activity and energy levels (Salam et al., 2023; Sarraf et al., 2023). Overall, low to moderate cobalt concentrations stimulated the respiration rate, indicating an activated metabolic response while high concentrations compromised the respiration rate, indicating toxicity. Yet jasmonic acid was robustly able to stimulate metabolic activity in the presence of cobalt stress, particularly at lower concentrations (Salam et al., 2023; Sarraf et al., 2023).

Conclusion

This study thoroughly substantiated the harmful effects of cobalt stress on the morphophysiological behavior of guar plants, which indicated decreasing growth, yield, and physiology. However, JA, particularly at lower concentrations (5 and 10 µM), mitigated the effects of cobalt on plant behavior. In particular, JA improved plant water relations, reduced cobalt uptake, increased antioxidant activity, and improved the photosynthesis efficiency. As a result, biomass, total carbohydrates, and relative water content improved. Additionally, JA was shown to increase gum viscosity under severe cobalt stress, suggesting a likelihood of increased gum quality. Data corroborates the effectiveness of JA to protect the guar plant against cobalt toxicity. Although higher rate cobalt was shown to limit the protective nature from JA, its application (as with the lower dose) still represents a good option for sustainable production of guar in cobalt polluted areas. Future research should strive to improve the application of JA for guar and identify the mechanisms underlying the molecular dynamics leading to improved cobalt tolerance for guar and potentially other economically viable crops.

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