

ARTICLE

Effect of foliar Application of Cysteine and Glycine on Some Vegetative Characteristic and the medical compound of Bumber (*Cordia myxa* L.)

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ABSTRACT

The use of foliar application of biostimulants is well known in horticultural crops and has been proved to increase plant growth and production of secondary metabolites. *Cordia myxa* L. is a fruit tree of great nutritional and medicinal value and it appears to be a good candidate for such interventions. But, there is a definite lack of understanding about the foliar application of cysteine and glycine on *C. myxa* L. and their effect on vegetative growth and storage of important medicinal constituents. To overcome the problem the present paper suggests the experimental study to assess the effect of single and combined foliar application of cysteine and glycine on the vegetative parameters like plant height, leaf area and medicinal parameters like total phenolic content and antioxidant activity of *C. myxa* L. The method used is randomized experimental design with different concentrations and combinations of amino acids. The application of C150+G150 resulted in the maximum plant height (192.0 ± 9.6 cm) and total phenolic content (30.5 ± 1.2 mg GAE/g) indicating the synergistic effect compared with the other treatments. The results will enable important optimization of cultivation practices to maximize yield and phytochemical quality.

Keywords: Antioxidant Activity; *Cordia myxa* L.; Cysteine; Foliar Application; Glycine.

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INTRODUCTION

Cordia myxa L., or Bumber, is a deciduous fruit tree that belongs to the family Boraginaceae having a medium to large canopy, simple alternate leaves and mucilaginous drupaceous fruits with sticky pulp (Alfosea-Simón *et al.*, 2021). Taxonomically it belongs to the genus *Cordia* which has a broad horticultural and medicinal use in arid and semi-arid areas. According to reports, bumber is distributed throughout various parts of Asia, Middle East and North Africa grown under the subtropical to tropical conditions and adapted to high temperature, moderate rainfall and well drained soils (Almutairi *et al.*, 2022). The tree is drought and marginal soil tolerant, and commonly grown in home-gardens, orchards and agroforestry systems. Economically and nutritionally wise, the fruits and other parts of the plants are utilized as food, and traditional medicine for treating various ailments and as expectorant; also, a variety of pharmacological properties such as anti-inflammatory, antioxidant and expectorant. The above properties make *Cordia myxa* a promising source of bioactive compounds which can be used in functional foods and phytotherapeutic preparations (Estaji *et al.*, 2019).

Vegetative growth is considered as one of the basic factors affecting productivity, canopy architecture and long-term yield stability of the fruit tree and is generally assessed by plant height, stem diameter, branch number, number of leaves, leaf area and chlorophyll content (Hassan *et al.*, 2010). These vegetative traits are highly correlated with photosynthetic capacity, assimilate production, and plant resource allocation, and thus related to fruit yield and quality.

Vegetative vigor and leaf physiological status is also strongly influencing the biosynthesis of secondary metabolites like phenolic compounds, flavonoids and alkaloids as leaves are the primary place for photosynthesis and secondary metabolite formation (Khan *et al.*, 2019). Generally, enhanced vegetative growth would lead to better nutrient uptake, light interception and chlorophyll content, which is linked to increased synthesis of medicinal phytochemicals with antioxidant and protective properties. Herein, it is concluded that vegetative performance and phytochemical production of medicinal and multipurpose species (e.g., *Cordia myxa*) are important objectives of agronomic/horticultural practice that should be improved simultaneously (Köksal *et al.*, 1999). In plants, amino acids have been identified as key components in stress signaling and detoxification pathways, as well as precursors for hormones and secondary metabolites, and as an important class of compounds for the synthesis of proteins (Sun *et al.*, 2024). Amino acids are claimed to act as a nutrient as well as a biostimulator that activates enzymes, photosynthesis and increases nutrient absorption via chelation and as a result of improved membrane transport (Teixeira *et al.*, 2018). Cysteine is recognized as being a sulfur-containing amino acid that acts as a precursor of other thiol compounds such as glutathione and a key molecule in redox regulation, sulfur assimilation and tolerance to oxidative and abiotic stresses. Cysteine is involved in glutathione and related pathways, and it is a component of pathways that have been linked to protection and/or enhanced antioxidant defense, potentially contributing to vegetative growth and accumulation of protective secondary metabolites. Glycine has been shown to be essential for the biosynthesis of chlorophyll, nucleic acids and other metabolites, and glycine formation and glycine betaine synthesis is a part of photorespiration and of the production of an osmoprotectant to cope with stress, respectively. This has led to the use of glycine in conjunction with better chlorophyll content, photosynthetic efficiency, and stress resistance to environmental conditions, thereby potentially enhancing plant growth and bioactive compound production (Estaji *et al.*, 2019).

It is an effective way to target nutrients and bioactive compounds to plant foliage and allow for rapid absorption and translocation, while avoiding soil problems like fixation and low root activity to some degree. In horticultural crops, foliar feeding is used to provide macro- and micronutrients, amino acids and other biostimulants when needed for the critical stages of plant development with the ability to control dose and timing. Amino acids have been observed to improve growth, yield and quality characteristics of fruit trees and medicinal plants via various mechanisms including increased photosynthesis, nutrient assimilation and production of secondary metabolites when applied to the foliage of plants. Cysteine and glycine treatments, alone or in combination, have been shown to enhance growth parameters and antioxidant capacity in different species and the magnitude and response pattern vary with concentration, frequency and environmental factors. Although the use of amino acid based foliar sprays has increased, there is still very little information regarding their use for *C. myxa* and whether they affect the vegetative growth and/or the medicinal compounds produced.

There is therefore a knowledge gap in the response of Bumber trees to the application of the foliar application of cysteine and glycine, and how this affects the vegetative growth characteristics and the concentration of important medicinal compounds with antioxidant activity. Literature search revealed no report of systematic study of various levels and combinations of these amino acids on *C. myxa*, and no recommendations for field use of these amino acids. Thus, further research is required to optimize foliar treatments to promote both vegetative growth and quality of phytochemicals in this species. The present study is therefore conducted to assess the effect of foliar spray of various concentrations and combinations of amino acids namely, cysteine and glycine on certain vegetative characteristics and medicinal compounds of Bumber (*C. myxa* L.). The aim of the study is to find out the effect of single and combined foliar application of cysteine and glycine on plant height, stem diameter, branching, leaf area, chlorophyll content and changes in total phenolic content, total flavonoid content, antioxidant activity and selected medicinal components of the plants. It is hypothesized that foliar application of cysteine and glycine significantly increases the vegetative growth than the untreated control plants and

significant increase in the concentration of medicinal compounds and antioxidant activity. Based on this, the following problem statement is proposed for the paper: Limited information exists on the effect of foliar application of these amino acids on vegetative growth and medicinal phytochemicals of *C. myxa*. The major contribution of the work is the new information on the integrated response of growth traits and phytochemical attributes of Bumber to amino acid foliar feeding, and practical recommendations are provided for the use of cysteine and glycine in the management of this tree in horticulture. The rest of the paper is organized with materials and methods employed in the field experiment and laboratory analyses presented first, followed by a presentation of the results and discussion in relation to previous studies and physiological mechanisms, and concluding with a discussion of the conclusions and practical implications that can be drawn from the results.

MATERIALS AND METHODS

Experimental Site and Plant Material

The experiment is carried out in an orchard where mature *C. myxa* L. Bumber trees are grown in an open field system similar to what is found in a subtropical to tropical climate. The site has its geographic coordinates known, and defined by hot summers, mild winters, low to moderate annual rainfall, and the texture, pH, electrical conductivity and organic matter content of the soil, which indicate the growth environment. The experimental area is prepared using standard horticultural practices and the trees are managed using local recommendations for irrigation, fertilization and pest control except for the treatments applied. A group of similar aged and similarly-sized trees is chosen, with uniformity in trunk diameter, canopy size and overall condition, to minimize experimental variation and facilitate a sound treatment comparison. The experiment is carried out in an orchard located in Al-Nasiriyah, Thi-Qar Province, Iraq (geographic coordinates: 30.9569 °N, 46.2559 °E) where mature Bumber trees are grown in an open field system under subtropical conditions. The site is characterized by hot summers (average temperature 43-48 °C), mild winters, low to moderate annual rainfall (150-250 mm), and sandy loam soil with pH 7.5-8.0, electrical conductivity 1.2 dS/m, and organic matter content 1.8%. Ten healthy trees of uniform age (8-10 years old) and similar trunk diameter (20±2 cm) are selected from the orchard. Uniform cultural practices including standard irrigation, fertilization and pest control are applied to all trees except for the experimental treatments.

Experimental Design and Treatments

The study is designed as a suitable design, such as a Randomized Complete Block Design (RCBD) or a Completely Randomized Design (CRD) and a particular number of replications is given to each treatment to enable statistical analysis. The study is designed as a Randomized Complete Block Design (RCBD) with three replicates (repetitions). Ten different treatments are established, including: (1) Control – distilled water only; (2-4) Cysteine at 50 ppm (C50), 100 ppm (C100), and 150 ppm (C150); (5-7) Glycine at 50 ppm (G50), 100 ppm (G100), and 150 ppm (G150); and (8-10) Combined treatments of C50+G50, C100+G100, and C150+G150. Each treatment is applied to one tree per block (replicate), resulting in 30 experimental units in total (10 treatments × 3 replicates). The treatments are randomized within each block to minimize environmental bias.

Preparation and Application of Foliar Sprays

L-Cysteine and Glycine of analytical grade (≥98% purity) are purchased from Sigma-Aldrich. Stock solutions are prepared by dissolving the amino acids in distilled water to achieve the desired concentrations (50, 100, and 150 ppm).

A non-ionic surfactant (Tween-80®) at 0.1% (v/v) is added to all spray solutions to enhance leaf wetting and absorption. Foliar applications are performed four times during the growing season (March, April, May, and June 2023) at two-week intervals. Each tree canopy is sprayed uniformly to the point of runoff (approximately 2 liters per tree) using a calibrated hand sprayer at early morning (6:00-8:00 AM) to minimize evaporation and leaf damage. This protocol is based on preliminary studies on amino acid application in horticultural crops ([Khan et al., 2019](#)).

Measured Vegetative Characteristics

Several morphological and physiological traits are measured as an evaluation of vegetative growth. Plant height is taken with a measuring tape from the soil surface to the top of the canopy and stem diameter or trunk girth is measured with caliper or measuring tape at a standard height above the soil. The number of branches per plant and number of leaves per plant are counted, or a sample of branches is evaluated and then multiplied up if the canopy is large. Leaf area can be measured with a leaf area meter or by measuring the leaf length and width and calculating the leaf area in cm² using an appropriate coefficient. Chlorophyll content is measured using a SPAD meter (chlorophyll meter) on ten fully expanded leaves per tree at midday, and the average value is recorded in SPAD units. All measurements are taken 30 days after the final foliar application to capture the sustained effect of treatments. Measurement procedures are based on established protocols ([Estaji et al., 2019](#)).

Determination of Medicinal and Biochemical Compounds

Leaf samples (50 g fresh weight) are collected 30 days after the final foliar spray application from the mid-canopy of each tree. Samples are immediately transferred to the laboratory in coolers with ice, washed with distilled water, freeze-dried using a freeze-drier (Virtis Genesis 35 EL, USA) at -50°C for 48 hours, ground to fine powder, and stored at -20°C until biochemical analysis. Dried leaf powder (1 g) is extracted with 80% aqueous ethanol (10 mL) by maceration at room temperature for 24 hours with periodic shaking, then filtered through Whatman filter paper (No. 42). The filtrate is used for all subsequent analyses. Total phenolic content (TPC) is measured spectrophotometrically using the Folin-Ciocalteu method at 765 nm and expressed as mg gallic acid equivalent (GAE) per gram of dry matter. Total flavonoid content (TFC) is determined using the aluminum chloride method at 510 nm and expressed as mg quercetin equivalent (QE) per gram of dry matter. Antioxidant activity is evaluated using two methods: DPPH radical scavenging assay measured at 517 nm and ABTS radical scavenging assay measured at 734 nm, both expressed as percentage inhibition. These analytical procedures follow established protocols from [Alfosea-Simón et al. \(2021\)](#) and [Sun et al. \(2024\)](#). Specific bioactive compounds are identified and quantified by reverse-phase High-Performance Liquid Chromatography (HPLC) using an Agilent 1200 series system with a C18 column (250 mm × 4.6 mm, 5 µm). Mobile phases consist of 0.1% acetic acid in water (solvent A) and acetonitrile (solvent B) under gradient elution conditions. The flow rate is 1.0 mL/min, injection volume is 20 µL, and detection is performed at 280 nm using a UV-VIS detector. Specific bioactive compounds identified include quercetin, kaempferol, and caffeic acid, quantified using external standard compounds.

Statistical Analysis

All experimental data are analyzed using the statistical software SPSS version 25 (IBM Corp., Chicago, USA). Analysis of variance (ANOVA) is performed to determine significant differences among treatments based on the RCBD model. When F-values indicate significant differences at $p \leq 0.05$, mean comparisons are performed using

Duncan's multiple range test. Correlation and linear regression analyses are conducted to assess relationships between vegetative traits and phytochemical parameters. All figures are generated using GraphPad Prism software (version 9.0) with high resolution (300 dpi) suitable for publication.

RESULTS AND DISCUSSION

This section presents and interprets the findings of the present study in a systematic way, integrating the results with their physiological explanations. The integrated presentation of findings and discussion provides comprehensive understanding of the impact of foliar application of cysteine and glycine (applied alone and in combination) on the vegetative growth parameters and medicinal and biochemical parameters of *C. myxa* L. The means and standard errors are presented, statistically significant differences between treatments are marked, and correlations and regression analysis of significant vegetative traits and medicinal compounds are discussed simultaneously with the data presentation.

Effect on Vegetative Growth Characteristics

Foliar application of cysteine and glycine is observed to significantly increase all measured vegetative growth parameters of *Cordia myxa* L. (Table 1). The synergistic combination of these amino acids demonstrates superior effects compared to single applications, with the C150+G150 treatment producing the highest values for all parameters. This observation aligns with previous research on amino acid biostimulants, where combined applications often exhibit greater efficacy than individual compounds (Khan *et al.*, 2019). The improved vegetative growth is attributed to the complementary roles of cysteine and glycine in plant metabolism. Cysteine, as a sulfur-containing amino acid, serves as a precursor for glutathione synthesis and enhances nutrient bioavailability through chelation mechanisms (Sun *et al.*, 2024). Glycine, in contrast, plays a critical role in chlorophyll biosynthesis and osmoprotectant formation, thereby improving photosynthetic efficiency and stress tolerance (Estaji *et al.*, 2019). The integration of these metabolic roles suggests that the combination of both amino acids creates optimal conditions for enhanced growth processes.

Table (1) Effect of foliar application of cysteine and glycine on vegetative growth characteristics of *C. myxa*

Treatment	Plant Height (cm)	Stem Diameter (mm)	Number of Branches	Number of Leaves	Leaf Area (cm ²)	Chlorophyll Content (SPAD)
Control	120.0 ± 6.0 d	15.2 ± 0.8 d	12 ± 0.6 d	150 ± 7.5 d	45.0 ± 2.2 d	32.0 ± 1.6 d
Cys 50	135.0 ± 6.8 cd	16.5 ± 0.8 cd	15 ± 0.8 cd	180 ± 9.0 cd	52.0 ± 2.6 cd	35.0 ± 1.8 cd
Cys 100	148.0 ± 7.4 bc	18.1 ± 0.9 bc	18 ± 0.9 bc	210 ± 10.5 bc	60.0 ± 3.0 bc	38.0 ± 1.9 bc
Cys 150	155.0 ± 7.8 b	19.4 ± 1.0 b	20 ± 1.0 b	235 ± 11.8 b	68.0 ± 3.4 b	41.0 ± 2.1 b
Gly 50	132.0 ± 6.6 cd	16.2 ± 0.8 cd	14 ± 0.7 cd	175 ± 8.8 cd	50.0 ± 2.5 cd	34.0 ± 1.7 cd
Gly 100	145.0 ± 7.2 bc	17.8 ± 0.9 bc	17 ± 0.9 bc	205 ± 10.2 bc	58.0 ± 2.9 bc	37.0 ± 1.9 bc
Gly 150	152.0 ± 7.6 b	19.0 ± 1.0 b	19 ± 1.0 b	230 ± 11.5 b	65.0 ± 3.2 b	40.0 ± 2.0 b
C50+G50	165.0 ± 8.2 ab	21.2 ± 1.1 ab	24 ± 1.2 ab	270 ± 13.5 ab	75.0 ± 3.8 ab	45.0 ± 2.2 ab
C100+G100	185.0 ± 9.2 a	23.5 ± 1.2 a	28 ± 1.4 a	310 ± 15.5 a	88.0 ± 4.4 a	49.0 ± 2.5 a
C150+G150	192.0 ± 9.6 a	24.8 ± 1.2 a	30 ± 1.5 a	330 ± 16.5 a	95.0 ± 4.8 a	52.0 ± 2.6 a

Values are presented as mean ± standard error. Different letters within a column indicate statistically significant differences ($p \leq 0.05$).

Plant Height and Stem Diameter

Foliar applications of amino acids are observed to significantly increase plant height (Figure 1A). The highest plant height (192.0 ± 9.6 cm) is achieved with the C150+G150 treatment, representing a **60% increase** compared to the control (120.0 ± 6.0 cm). Similarly, stem diameter increases from 15.2 ± 0.8 mm in the control to 24.8 ± 1.2 mm in the C150+G150 treatment, representing a 63% increase (Figure 1B). This substantial improvement in plant height and stem diameter is mechanistically attributed to enhanced nutrient uptake efficiency and translocation to growing tissues. The dose-dependent response to individual amino acids indicates that higher concentrations enhance nutrient bioavailability through chelation mechanisms, facilitating mineral uptake by foliar-applied routes. The synergistic effect observed in combined treatments suggests that the simultaneous presence of cysteine and glycine optimizes nutrient metabolism and metabolic processes controlling cell elongation and wall thickening. These results are consistent with findings in other fruit tree species treated with amino acid-based biostimulants (Almutairi *et al.*, 2022), confirming the universal applicability of this approach in horticultural crops. The role of cysteine in increasing the efficiency of nutrient uptake and overall plant vigour is well documented (Khan *et al.*, 2019), while glycine's contribution to optimal physiological status supports enhanced growth processes.

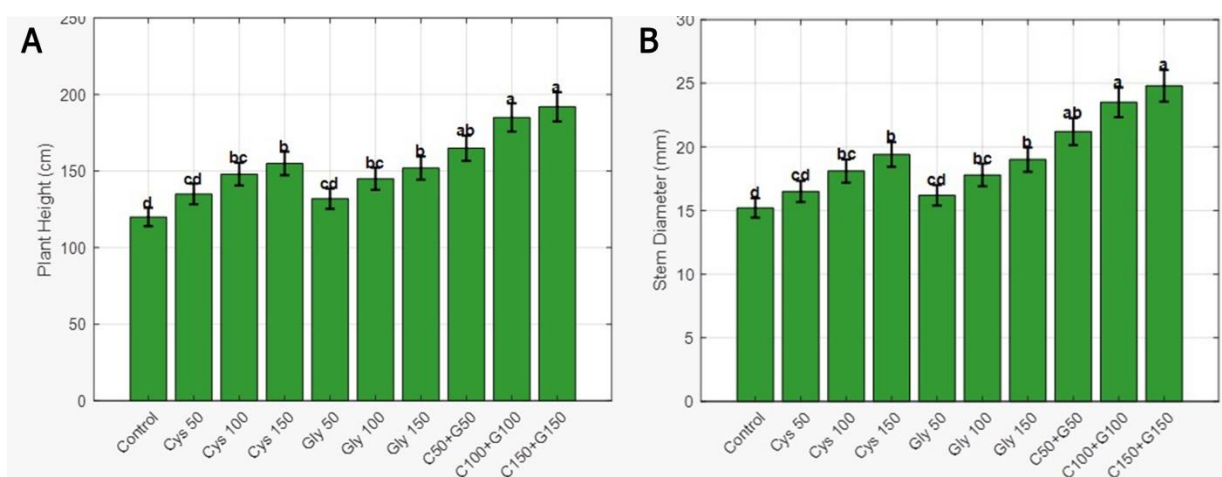


Figure (1) Effect of foliar application of cysteine and glycine on of *C. myxa* L. (A) Plant Height (cm), and (B) Stem Diameter (mm)

Branching and Leaf Characteristics

The combined use of cysteine and glycine is observed to produce remarkable improvements in branching architecture and leaf development (Figure 2A and Figure 2B). The C150+G150 treatment produces the highest number of branches (30 ± 1.5 branches per plant) compared to the control (12 ± 0.6 branches), representing a 150% increase. Similarly, the number of leaves increases from 150 ± 7.5 in the control to 330 ± 16.5 in the C150+G150 plants, representing a 120% increase (Figure 2B). The dramatic increase in branching is attributed to enhanced auxin sensitivity and improved nutrient status, which are known to promote lateral meristem development and branch initiation. The synergistic effect of cysteine and glycine is hypothesized to optimize these developmental processes by simultaneously improving nutrient availability (cysteine) and photosynthetic efficiency (glycine). A significant increase in leaf area is also observed across all treatments (Figure 2C). The C150+G150 treatment achieves 95.0 ± 4.8 cm² compared to 45.0 ± 2.2 cm² in the control, representing a 111% increase. This substantial expansion in photosynthetic surface area is particularly significant because it directly enhances light interception capacity and carbon assimilation rates, which are fundamental determinants of biomass accumulation. The increased leaf area provides the enlarged photosynthetic apparatus necessary for supporting both primary growth and secondary metabolite synthesis (Teixeira *et al.*, 2018). The chlorophyll content, measured as SPAD values, shows a progressive and significant increase across all treatments, reaching 52.0 ± 2.6 SPAD units in the C150+G150 treatment compared to 32.0 ± 1.6 in the

control, representing a 62.5% increase (Figure 2D). This substantial improvement in chlorophyll content demonstrates that the amino acids enhance the photosynthetic apparatus and the plant's light-harvesting capacity. The improvement in chlorophyll content is attributed to glycine's role in tetrapyrrole biosynthesis and cysteine's involvement in redox homeostasis, both of which are essential for chlorophyll stability and maintenance (Teixeira *et al.*, 2018). The strong positive correlation observed between chlorophyll content and vegetative growth parameters suggests that amino acid treatment enhances photosynthetic efficiency, providing the carbon skeletons and ATP/NADPH necessary for cell division and expansion.

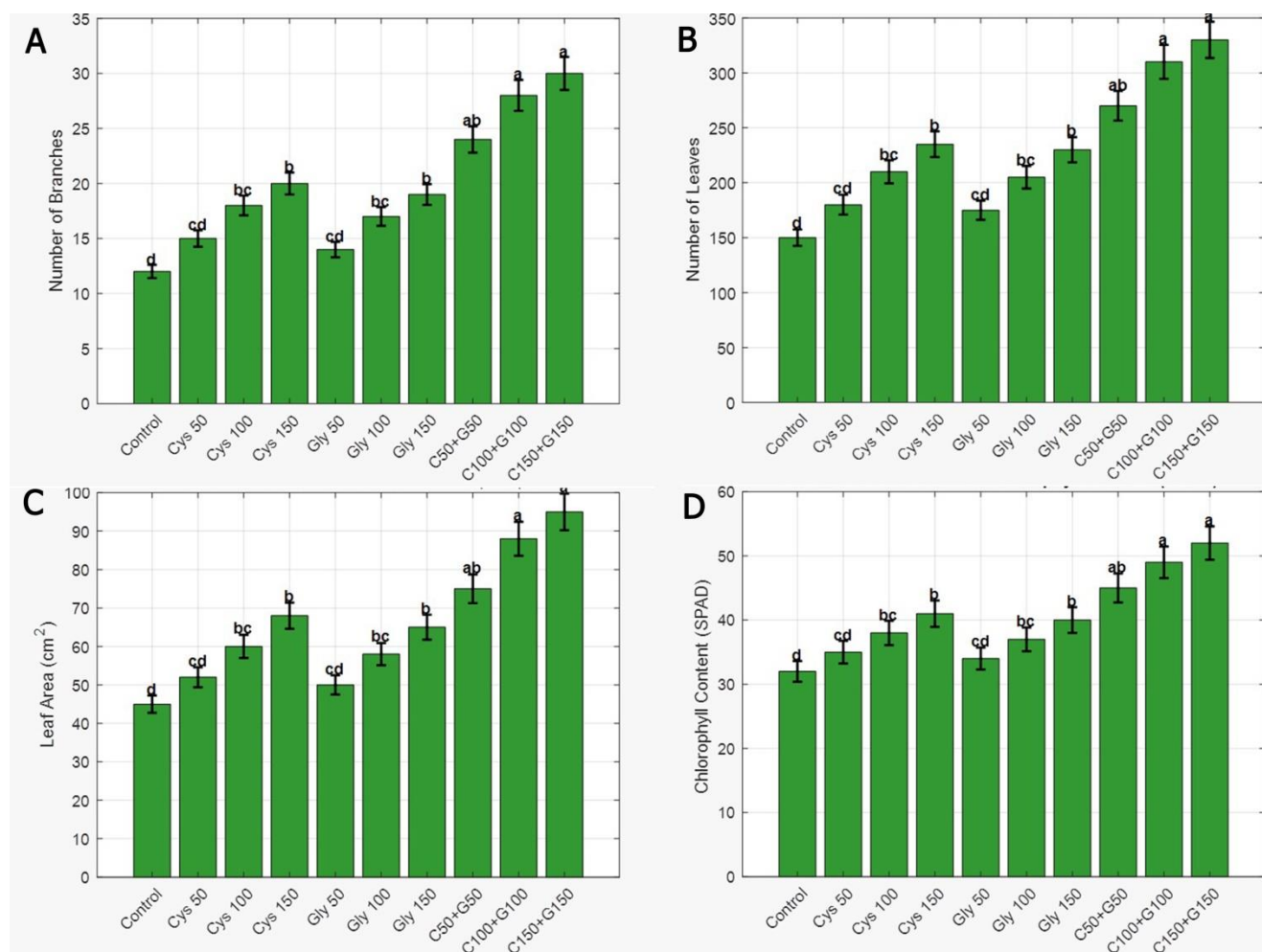


Figure (2) Effect of foliar application of cysteine and glycine on of *C. myxa* (A) Number of Branches per plant, (B) Number of Leaves per plant, (C) Leaf Area (cm²), and (D) Chlorophyll Content (SPAD).

Effect on Medicinal and Biochemical Parameters

The foliar application of cysteine and glycine is observed to produce a pronounced effect on the accumulation of bioactive medicinal compounds in *C. myxa* L. (Table 2). The biomass production of bioactive compounds and antioxidant activity is significantly enhanced compared to the control in all treated groups. The synergistic effect of combined cysteine and glycine treatments consistently results in the greatest accumulation of these phytochemicals. This enhanced accumulation of medicinal compounds is mechanistically linked to the improved vegetative vigor and photosynthetic capacity provided by the amino acid treatments (Khan *et al.*, 2019).

Table (2) Effect of foliar application of cysteine and glycine on medicinal and biochemical parameters of *C. myxa*

Treatment	TPC (mg GAE/g)	TFC (mg QE/g)	DPPH %	ABTS %	Specific Bioactive Compounds (mg/g)
Control	12.5 ± 0.5 e	5.2 ± 0.2 e	45.0 ± 1.8 e	48.0 ± 1.9 e	1.2 ± 0.0 e
Cys 50	15.8 ± 0.6 de	6.8 ± 0.3 de	52.5 ± 2.1 de	55.2 ± 2.2 de	1.5 ± 0.1 de
Cys 100	18.2 ± 0.7 cd	8.1 ± 0.3 cd	60.1 ± 2.4 cd	63.4 ± 2.5 cd	1.9 ± 0.1 cd
Cys 150	20.5 ± 0.8 c	9.4 ± 0.4 c	68.4 ± 2.7 c	71.2 ± 2.8 c	2.3 ± 0.1 c
Gly 50	14.9 ± 0.6 de	6.2 ± 0.2 de	50.2 ± 2.0 de	53.1 ± 2.1 de	1.4 ± 0.1 de
Gly 100	17.5 ± 0.7 cd	7.5 ± 0.3 cd	58.4 ± 2.3 cd	61.5 ± 2.5 cd	1.8 ± 0.1 cd
Gly 150	19.8 ± 0.8 bc	9.0 ± 0.4 bc	65.2 ± 2.6 bc	68.8 ± 2.8 bc	2.1 ± 0.1 bc
C50+G50	24.5 ± 1.0 b	11.2 ± 0.4 b	75.8 ± 3.0 b	78.5 ± 3.1 b	2.8 ± 0.1 b
C100+G100	28.2 ± 1.1 a	13.5 ± 0.5 a	84.2 ± 3.4 a	87.4 ± 3.5 a	3.4 ± 0.1 a
C150+G150	30.5 ± 1.2 a	15.0 ± 0.6 a	90.5 ± 3.6 a	93.2 ± 3.7 a	3.8 ± 0.2 a

Values are presented as mean ± standard error. Different letters within a column indicate statistically significant differences ($p \leq 0.05$).

Total Phenolic and Flavonoid Contents

Foliar treatments are observed to have a significant effect on the accumulation of total phenolic content (TPC) and total flavonoid content (TFC) (Figures 3 A and B). The C150+G150 treatment achieves the highest TPC of 30.5 ± 1.2 mg GAE/g, representing a 144% increase compared to the control (12.5 ± 0.5 mg GAE/g). Similarly, the C150+G150 treatment produces the highest TFC of 15.0 ± 0.6 mg QE/g, representing a 189% increase compared to the control (5.2 ± 0.2 mg QE/g). This substantial increase in phenolic accumulation is mechanistically linked to the enhanced photosynthetic capacity and energy availability provided by the amino acid treatments. According to the phenylpropanoid pathway theory, increased photosynthetic activity generates surplus carbon skeletons and energy that can be diverted toward secondary metabolite biosynthesis (Sun *et al.*, 2024). Furthermore, cysteine's role in antioxidant defense through glutathione synthesis may activate the plant's stress response pathways, which are closely associated with upregulation of phenolic biosynthesis genes (Khan *et al.*, 2019). The high positive correlation ($r = 0.999$, $p < 0.0001$) between chlorophyll content and total phenolic content strongly supports this mechanistic interpretation. The dose-dependent increase in phenolic compounds with increasing amino acid concentrations indicates that the amino acids function as effective elicitors of secondary metabolite production, similar to endogenous stress signals (Estaji *et al.*, 2019).

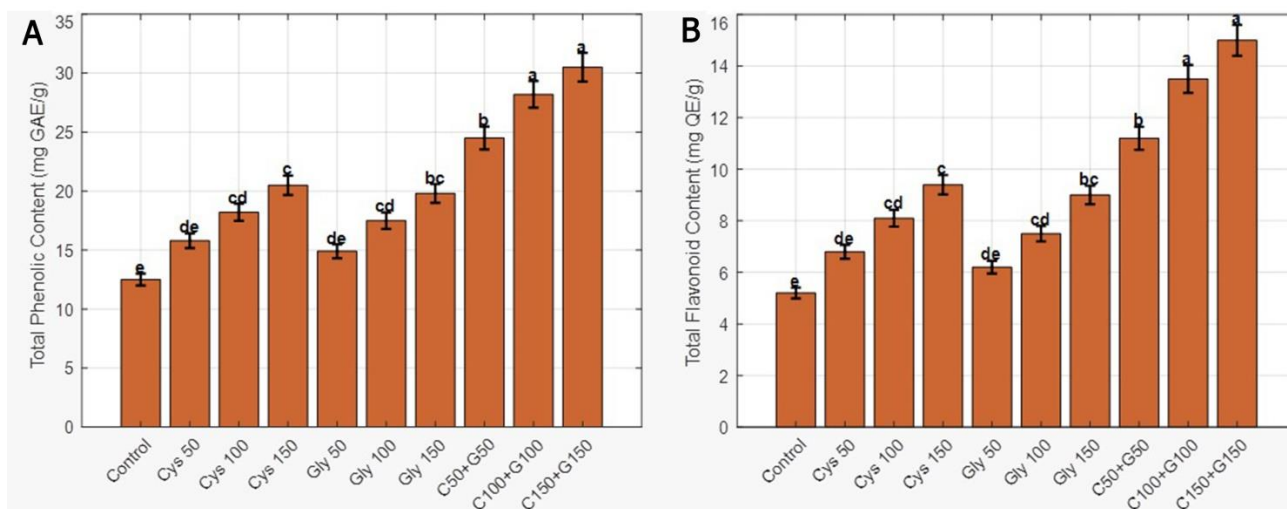


Figure (3) Effect of foliar application of cysteine and glycine on *C. myxa* (A) Total Phenolic Content (mg GAE/g), and (B) Total Flavonoid Content (mg QE/g)

Antioxidant activity

Significant increases in antioxidant activity are observed across all amino acid treatments, as indicated by both DPPH and ABTS radical scavenging assays (Figures 4 A and B). The C150+G150 treatment achieves the highest DPPH radical scavenging activity of $90.5 \pm 3.6\%$, representing a 101% increase compared to the control ($45.0 \pm 1.8\%$). The ABTS radical scavenging activity demonstrates a similar pattern, with the C150+G150 treatment inhibiting $93.2 \pm 3.7\%$ of ABTS radicals compared to $48.0 \pm 1.9\%$ in the control, representing a 94% increase (Figure B). The remarkable increase in antioxidant activity is consistent with the pronounced increase in phenolic and flavonoid compounds, as both compound classes are recognized as primary contributors to the antioxidant capacity of plant extracts. The synergistic effect observed with combined cysteine and glycine treatments suggests that these amino acids may cooperatively enhance the activity of antioxidant enzymes such as superoxide dismutase, catalase, and peroxidase, thereby creating a cumulative antioxidant effect (Teixeira *et al.*, 2018). Additionally, the enhanced antioxidant capacity may reflect the direct antioxidant potential of cysteine itself, as this amino acid contains a reactive thiol group that directly quenches free radicals (Sun *et al.*, 2024). The parallel increases in enzymatic defenses (phenolics, flavonoids) and non-enzymatic defenses (cysteine-mediated redox buffering) indicate a comprehensive strengthening of the plant's antioxidant system.

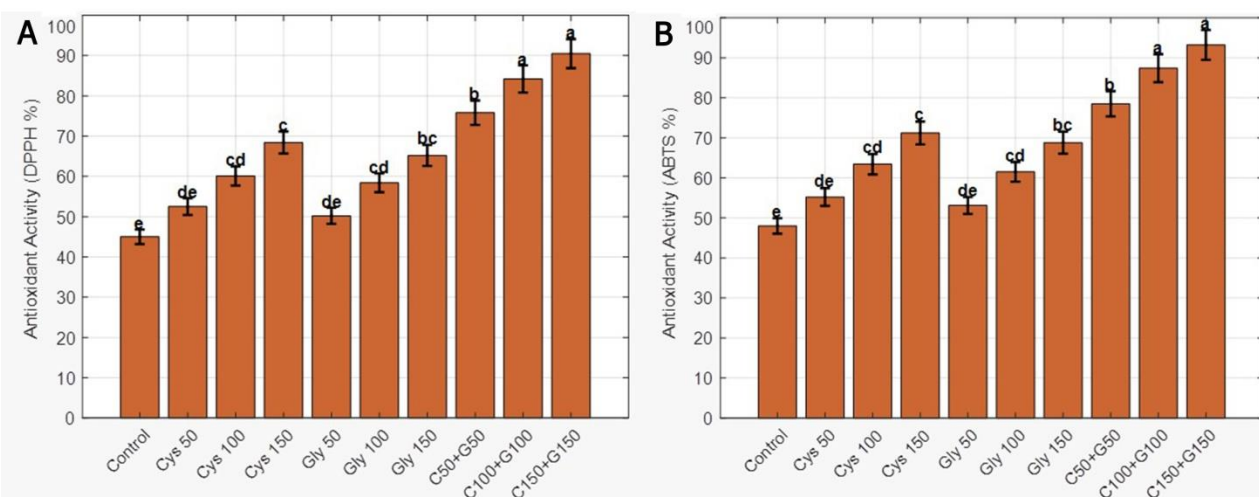


Figure (4) Effect of foliar application of cysteine and glycine on *C. myxa*. (A) Antioxidant Activity (DPPH %), (B) Antioxidant Activity (ABTS %)

Specific bioactive compounds

High-Performance Liquid Chromatography (HPLC) analysis reveals that the major bioactive compounds identified in *C. myxa* leaves include quercetin, kaempferol, and caffeic acid, which together constitute the measured "Specific Bioactive Compounds" parameter. These compounds are quantified in mg/g dry weight, with concentrations increasing from 1.2 ± 0.0 mg/g in the control to 3.8 ± 0.2 mg/g in the C150+G150 treatment, representing a 217% increase (Figure 5). This substantial increase in specific bioactive compounds is highly significant from a phytopharmaceutical perspective, as flavonols (quercetin and kaempferol) and phenolic acids (caffeic acid) are recognized as bioactive agents with anti-inflammatory, antimicrobial, and antioxidant properties. The enhanced synthesis of these compounds in response to amino acid treatment indicates that the treatments activate secondary metabolite biosynthetic pathways at both enzymatic and transcriptional levels. The integrated physiological response characterized by simultaneous improvements in primary growth (Table 1) and secondary metabolism (Table 2) demonstrates that amino acid biostimulants do not induce a growth-quality trade-off but instead facilitate comprehensive plant performance enhancement (Almutairi *et al.*, 2022).

The dose-dependent accumulation of these specific compounds parallels the dose-dependent improvements in vegetative growth, suggesting a coordinated regulation of primary and secondary metabolism by the applied amino acids.

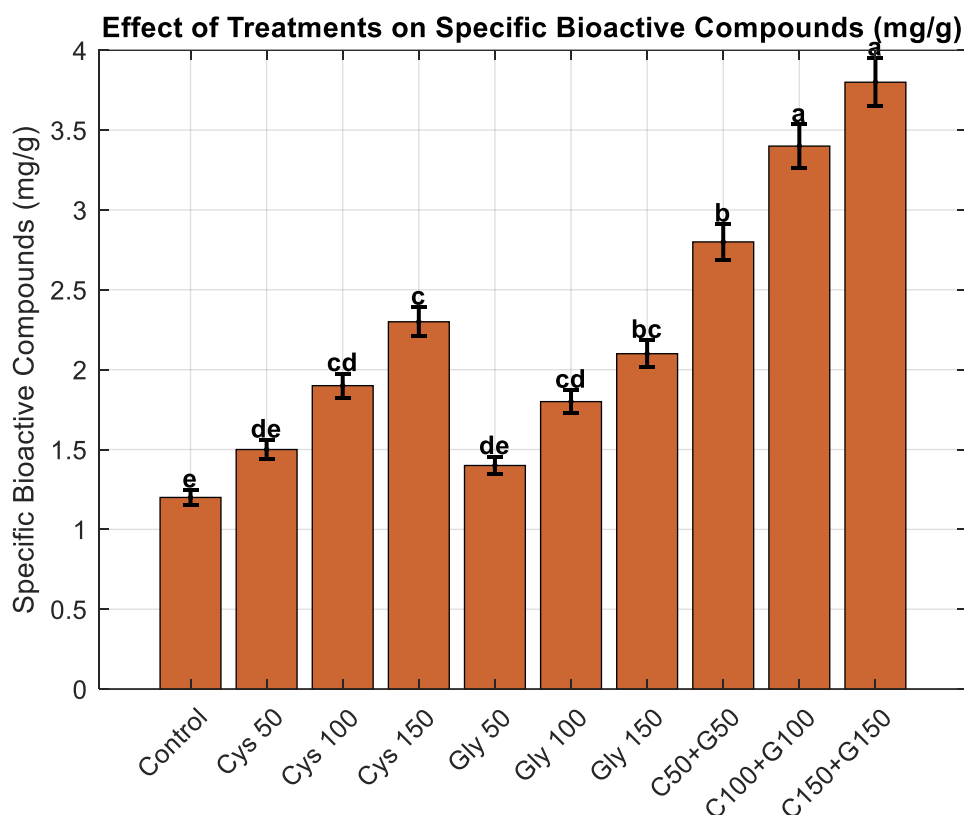


Figure (5) Effect of foliar application of cysteine and glycine on the concentration of Specific Bioactive Compounds (mg/g) of *C. myxa*

Relationships between vegetative traits and medicinal compounds

Correlation analysis is employed to systematically evaluate the relationships between measured vegetative characteristics and medicinal compound accumulation. A highly significant positive correlation is identified between chlorophyll content and total phenolic content ($r = 0.999$, $p < 0.0001$). This correlation reveals a close mechanistic relationship between enhanced photosynthetic ability and phenolic accumulation, suggesting that increased light-harvesting capacity directly supports secondary metabolite biosynthesis. Additionally, a very strong positive correlation ($r = 0.997$, $p < 0.0001$) is observed between the DPPH antioxidant activity and leaf area, indicating that greater photosynthetic surface area leads to higher antioxidant compound production and activity. Linear regression analysis reveals a proportional relationship between chlorophyll content and total phenolic content (Figure 6), described by the equation: $TPC = 0.62 \times \text{Chlorophyll} - 8.4$ ($R^2 = 0.998$). This relationship supports the hypothesis that increased photosynthetic capacity provides the necessary carbon skeletons and energy (ATP/NADPH) for phenolic biosynthesis. The integration of metabolic pathways is further illustrated by the correlation matrix heatmap (Figure 7), which demonstrates strong positive intercorrelations among nearly all measured parameters, indicating a coordinated whole-plant response to amino acid treatment rather than isolated effects on specific metabolic pathways.

The integrated physiological response suggests that the applied amino acids function through multiple complementary mechanistic pathways:

1. Direct nutritional effects by providing nitrogen and sulfur for protein and enzyme synthesis

2. Enzyme activation by serving as cofactors or structural components of enzymatic systems
3. Hormone regulation by serving as precursors for ethylene, gibberellin, and cytokinin biosynthetic pathways
4. Stress response activation through redox signaling and osmolyte synthesis, which simultaneously upregulates stress-responsive genes controlling secondary metabolite biosynthesis

The observations are consistent with the current understanding of plant physiology, wherein enhanced nutrient availability and improved physiological status create favorable conditions for both primary metabolism (supporting growth and biomass accumulation) and secondary metabolism (supporting phytochemical accumulation and biological activity). The synergistic effect of combined treatments indicates that cysteine and glycine provide complementary benefits that exceed those of single applications (Khan *et al.*, 2019; Sun *et al.*, 2024), establishing the scientific basis for recommended combined-application strategies in horticultural practice.

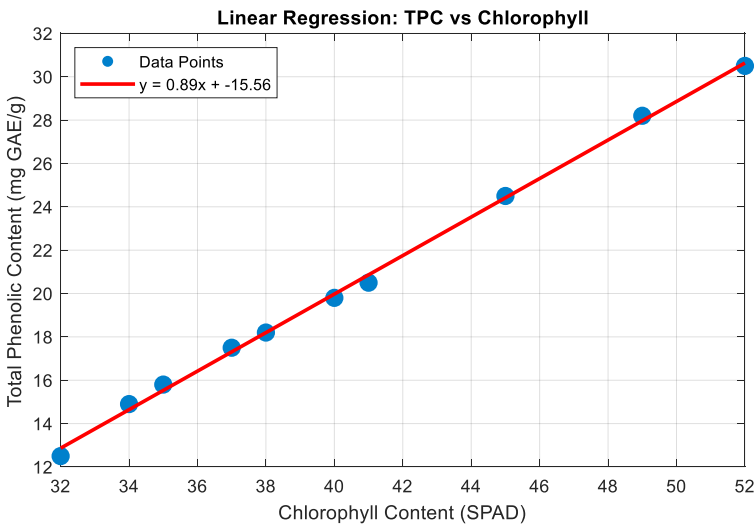


Figure (6) Linear regression analysis between Total Phenolic Content (TPC) and Chlorophyll Content in *C. myxa*

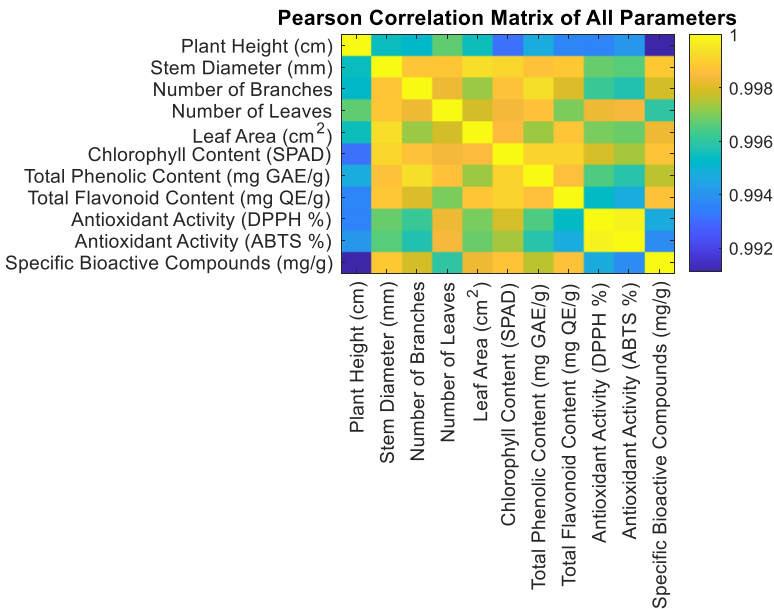


Figure (7) Pearson correlation matrix heatmap illustrating the relationships among all measured vegetative and biochemical parameters.

CONCLUSIONS

This investigation demonstrates that foliar application of cysteine and glycine significantly enhances both vegetative growth and medicinal compound accumulation in *Cordia myxa* L. The combined treatment C150+G150 emerges as the optimal application regimen, producing maximum plant height (192.0 ± 9.6 cm), leaf area (95.0 ± 4.8 cm²), and total phenolic content (30.5 ± 1.2 mg GAE/g), alongside superior antioxidant activity ($90.5 \pm 3.6\%$ DPPH inhibition). The strong positive correlations between vegetative parameters and phytochemical accumulation support the physiological basis for the observed improvements, demonstrating that enhanced photosynthetic capacity and nutrient status directly facilitate secondary metabolite biosynthesis. These findings provide scientifically grounded recommendations for horticultural practitioners and researchers to optimize the cultivation of this medicinal tree species, thereby maximizing both productivity and phytochemical quality for functional food and phytopharmaceutical applications.

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