



Foliar application of L-phenylalanine, sodium selenate, and nitroxine biological fertilizer can improve antioxidant and phytochemical properties of goji berry (*Lycium barbarum* L.)

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ABSTRACT

Purpose: *Lycium barbarum* berries can be a source of natural antioxidants for human food production. **Research method:** To increase the antioxidant activity of secondary metabolites in goji berry seedlings, we applied amino acid L-phenylalanine (Phe: 0.5, 1, and 1.5 mM), sodium selenate (Se: 0.25, 0.5, and 1 mg. L⁻¹), and nitroxine biological fertilizer (170, 330, and 500 µL.L⁻¹) at three levels. Distilled water was the control treatment. The experiment took place at the research farm of Ferdowsi University of Mashhad during 2021-2022. **Findings:** The results revealed that the treatments significantly affected goji berry plants regarding physiological and chemical attributes. Phenylalanine, selenium, and nitroxine substantially affected photosynthetic pigments, including chlorophyll and carotenoid, antioxidant, and catalase during the two years of foliar application. The results showed that phenylalanine with selenium increased the amounts of flavonoids, anthocyanins, and carbohydrates in goji berry plants. Applying phenylalanine alone had a positive, more potent effect on the amount of phenylalanine ammonia-lyase enzyme, which shows the impact of this substance on the phenylpropanoid pathway. Using it with nitroxine enhanced the phenol content and superoxide dismutase activity significantly. **Research limitations:** There was no limitation. **Originality/Value:** According to the results of this experiment, during the two years of 2021 and 2022, phenylalanine improved antioxidant enzyme activity and other traits significantly. Using phenylalanine and sodium selenate at low concentrations increased all antioxidant compounds and improved plant growth.

INTRODUCTION

The *Lycium* genus of the Solanaceae family has excellent nutritional and medicinal value. Two species *Lycium barbarum* L. and *Lycium chinense* comprise the majority of the genus. The plant bears a fruit often called wolfberry or goji berry (Jiang et al., 2021). The red goji berry (*Lycium barbarum* L.) is a deciduous perennial shrub that grows in northwest China and the Mediterranean region (Xin et al., 2017). It has oval leaves with a curved spear-shaped tip. Its leaves and shoots appear in opposite groups on the branches. Its leaves are 7 cm long and 3.5 cm wide (Oğuz et al., 2022). Goji berry fruits can be a source of natural antioxidants in producing functional foods. So far, most studies have focused on phenolic compounds in *Lycium* leaves and root bark (Qian et al., 2017; Jiang et al., 2021). Studies have shown that fruits contain polysaccharides, carotenoids, flavonoids, vitamins, and essential oils. These compounds cause hepatic, hypoglycemic, lipid-lowering, anticancer, immune-stimulant, anti-fatigue, and neuroprotective properties (Ozkan et al., 2018). Among the dozens of *Lycium* species distributed worldwide today, approximately 90% of commercial goji berry (wolfberry) products appear in nutritional supplements, flavored teas, juices, jams, snacks, soups, and other foods (Yossa Nzeuwa et al., 2019).

Using amino acids for horticultural crops is a worldwide practice, and amino acids constitute organic fertilizers while acting as biostimulants (Colla et al., 2015). The effect of amino acids on plants depends on the type of amino acid supplied and the type of plant (Khan et al., 2019). Amino acids are biostimulants that promote plant growth, improve nutrient availability, and enhance plant performance (Khan et al., 2019). Amino acids also directly or indirectly affect performance (Abd El-Aal et al., 2010). Amino acids are a part of plant proteins that partake in biological and functional roles (Shetta & Zayed, 2016). They are the primary chains in protein structures and facilitate plant growth (Hashem et al., 2016). These protein subunits crucially function in enzymes, vitamins, alkaloids, terpenoids, etc., and other plant metabolites.

Phenylalanine is a precursor to many energetic secondary metabolites, such as phenylpropanoids, flavonoids, lignin, and anthocyanins. The phenylpropanoid metabolic structure has many features, especially for protecting against abiotic and biotic stresses (Tzin & Galili, 2010; Heydarnajad Giglou et al., 2024). Phenylalanine helps in producing fragrant compounds, antioxidants, lignin, anthocyanins, phenols, and cellulose as the primary tissue of the plant cell wall, as an ethanol carrier, and in the first segment of anthocyanin biosynthesis, phenylpropanoids, and flavonoids.

In metabolic tactics of plant cells, compared to previous techniques, iron is less expensive and helpful in disposing of anthocyanins (Edahiro et al., 2005). Research by Sarojnee et al. (2009) indicated that treating hot pepper (*Capsicum annum* L.) plants with amino acids sizably increased the plant canopy size, stem diameter, range of branches, and dry shoots, fruit length, fruit diameter, dry fruit percentage, and ascorbic acid content. Phenylalanine, tyrosine, and tryptophan are crucial components for plant protein synthesis, but they are precursors to various secondary metabolites essential for plant growth and human nutrition (Sarojnee et al., 2009). According to Watanabe et al. (2017), phenylalanine is a crucial element that can increase the folic acid content in spinach by two-fold. Amino acid administrations (20%) reportedly multiplied Chl and CLB contents. This increase contributed to leaf mineral enhancements, mainly by improving the photosynthetic mechanism (Garcia et al., 2011). Also, including amino acids in the nutrient solution improved the vegetative vigor of tomato plants while boosting the chlorophyll content and activity of antioxidant enzymes (Zhang et al., 2009).

Selenium (Se) is an essential component of selenoproteins and seleno-amino acids, contributing significantly to cellular growth and biological functions in both animals and humans. Due to its chemical similarity to sulfur, Se can substitute for S within biochemical systems, where it plays comparable roles. The absorption, transportation of simulated toxins, and replacement of sulfur with selenium can result in Se isotopes, which subsequently elevate Se concentrations within cells (Puccinelli et al., 2017). Abbas (2012) observed that low concentrations of selenate stimulated increases in chlorophylls, anthocyanins, sugars, proline, ascorbic acid, and enzymatic activities. Selenite also raised enzymatic antioxidant activities—such as ascorbic acid peroxidase and guaiacol peroxidase—and non-enzymatic antioxidants, including ascorbic acid and carotenoids, in sorghum seedlings. Moreover, Se enhanced carbohydrate and bioactive compound levels, including total flavonoid content, glutathione, and vitamins C and E in *Solanum lycopersicum* cv. Provence (Zhu et al., 2018). Overall, Se heightened antioxidant activity across plants, humans, animals, and microorganisms (Ramos et al., 2010). Sources of selenium, like selenate in low concentrations, have demonstrated a beneficial role in plant enhancement by acting as an antioxidant to promote growth; however, at elevated levels, Se adversely affects plant performance.

Biological fertilizers, comprising one or more beneficial soil organisms within a suitable carrier, play a crucial role in agriculture. Such fertilizers often include microorganisms capable of transforming nutrients from inaccessible to accessible forms through biological processes (Zahedyan et al., 2022). Zahedyan et al. (2022) confirmed that the application of nitroxine significantly influenced nutrient content, relative water content (RWC), and total antioxidant (TA) capacity. Additionally, plants treated with nitroxine showed notable increases in fresh and dry leaf and stem weights, as well as in chlorophyll a and b, carotenoids, and anthocyanin concentrations (Rahi, 2013).

Goji berry, specifically the GB1 variety, is widely cultivated in Iran and demonstrates compatibility with the country's diverse climates. Given the high economic value of goji berries compared to indigenous alternatives like blueberries and raspberries, cultivation efforts have intensified, particularly in Khorasan province. This study aims to assess the feasibility of goji berry cultivation in the region, optimizing growing conditions and exploring methods to enhance yield. Specifically, this research evaluates the effects of pre-harvest applications of the amino acid phenylalanine, selenium, and nitroxine biofertilizer on *Lycium barbarum* L.

MATERIALS AND METHODS

Plant materials

This experiment took place from 2021 to 2022 at a research farm at Ferdowsi University of Mashhad, Iran. In early May, we planted goji berry seedlings under field conditions to evaluate the effects of L-phenylalanine (Phe), sodium selenate (Se), and nitroxine on plant growth and yield. We applied these treatments through foliar spraying before harvest and throughout the growth period.

We planted two-year-old goji berry seedlings, cultivar GB1, sourced from Mashhad Seedling Company in Razavi Khorasan province, with 150 cm spacing between both rows and seedlings. We used a strip-drip irrigation system tailored to local climatic conditions, watering every two days, and performed weeding consistently throughout the growing season. We used a randomized complete block design (RCBD) with five replications to assess the effects of L-phenylalanine, selenium, and nitroxine. The experimental factors included three concentrations of L-phenylalanine (Phe: 0.5, 1, and 1.5 mM), sodium selenate (Se: 0.25, 0.5, and 1 mg L⁻¹), and nitroxine biological fertilizer (170, 330, and 500 µL L⁻¹), while distilled

water served as the control. Each row in the field represented one block for each repetition, and we established ten treatment groups, including the control. Following initial plant establishment, we applied foliar sprays every 15 days across three growth stages.

Soil preparation included treatment adjustments based on pre-planting soil nitrogen levels, as determined through soil analysis (Table 1). To enhance soil fertility, we applied vermicompost at a rate of 7.8 tons per hectare. After ensuring initial plant establishment, we administered the first foliar spray containing amino acid phenylalanine, selenium, and nitroxine, with subsequent applications at 15-day intervals across three stages. We sprayed all treatments at the same times and used distilled water for the control plants' foliar applications. After completing three stages of foliar applications, we conducted analyses to determine chlorophyll, carotenoid, carbohydrate, phenol, and flavonoid content. We also measured antioxidant activity, antioxidant enzyme activities (phenylalanine ammonia-lyase [PAL], superoxide dismutase [SOD], and catalase [CAT]), and anthocyanin content across all seedlings. Table 1 presents the soil analysis results.

Leaf area and specific leaf area

Leaf surface measurements involved using a leaf surface measuring device (CID-CI2002 made in America) to calculate the average leaf surface in square centimeters (Gong et al., 2013). The specific goji berry leaf area was calculated by the following equation (1):

$$SLA = LA / LDW \quad (1)$$

Where LA: leaf area and LDW: leaf dry weight.

Photosynthetic pigments

To evaluate photosynthetic pigment contents, we collected three leaves from three plants per replicate in each treatment. We extracted photosynthetic pigments from 0.5 g of fresh leaves using 80% acetone. Then, we measured the absorbance of the extracts at 663 and 645 nm for chlorophyll and at 480 and 510 nm for carotenoids, using a spectrophotometer (ND One, Thermo Fisher, USA). We expressed concentrations of total chlorophyll and carotenoids as mg g^{-1} fresh weight and calculated them using the formula (2 & 3) reported by Arnon (1967):

$$\text{Chlorophyll contents (mg g}^{-1} \text{ FW)} = [20.2 (A_{645}) + 8.02 (A_{663})] \times V / (W \times 1000) \quad (2)$$

$$\text{Carotenoids (mg g}^{-1} \text{ FW)} = [7.6 (A_{480}) - 1.49 (A_{510})] \times V / (W \times 1000) \quad (3)$$

Soluble sugars

We measured total sugar content following the modified method of Liu et al. (2015). For this analysis, we mixed 0.1 mg of powdered sample with 4 mL of 80% (v/v) ethanol and incubated it in a water bath at 80°C for 20 minutes. After incubation, we centrifuged the mixture at 5000 rpm for 10 minutes, and then added 1 mL of 5% (v/v) phenol solution to 1 mL of the resulting extract. We further added 5 mL of sulfuric acid to this solution, thoroughly mixed it, and maintained the solution at room temperature for 30 minutes to cool. We then measured absorbance at 490 nm using a spectrophotometer (ND One, Thermo Fisher, USA).

Table 1. The physical and chemical properties of soil on the site of experimental field.

Depth	Soil Texture	Sand	Clay	Loam	pH	EC (dS m ⁻¹)	N (mg kg ⁻¹)	P (mg kg ⁻¹)	K (mg kg ⁻¹)
0-30	Sandy loam	40	33	27	7.5	1.3	610	60.6	625.1

Total phenolic content (TPC) and total flavonoid content (TFC)

To determine the TPC, we combined each extract with 80% methanol, and then added 100 µL of this mixture to 2.8 mL of distilled water. Following this, we added 2 mL of 2% sodium carbonate (Na₂CO₃) and 100 µL of 50% Folin-Ciocalteu reagent, allowing the mixture to incubate for 30 minutes. We measured the absorbance at 720 nm relative to the control, expressing the total phenolic content of extracts as mg gallic acid equivalents per gram of plant weight (mg GAE/g) (Namvar et al., 2018). For TFC determination, we followed the colorimetric method of Kaijv et al. (2006) and measured absorbance at 510 nm, reporting results in millimoles of quercetin equivalents per 100 g fresh weight (mM QE/100 g FW).

DPPH radical scavenging activity

We evaluated DPPH radical scavenging activity following the modified protocol of Sanchez-Moreno et al. (1998). To start, we added 100 µL of extract or control (methanol instead of extract) to 100 µL of a prepared 0.2 mM DPPH solution in methanol. We recorded absorbance at 517 nm after a 15-minute reaction period. Results for DPPH radical scavenging activity are presented as percentages (Sanchez-Moreno et al., 1999) (4).

$$\text{Radical scavenging activity (\%)} = [(A \text{ blank} - A \text{ sample}) / A \text{ blank}] \times 100 \quad (4)$$

A: absorbance.

Antioxidant enzymes activity

Phenylalanine ammonia-lyase changed into extracted by way of 5 ml, 100 mM sodium borate buffer (pH 8.8, containing 1mM EDTA, five mM β-mercaptoethanol, and 0.1% polyvinylpyrrolidone), and its activity changed into measured in line with the approach of Kovács et al. (2014). Soluble protein content material turned into results as described by Bradford (1976). Catalase pastime changed into measured data as by Aebi (1984), with slight modifications. We monitored the destruction of H₂O₂ at 240 nm absorbance while using a spectrophotometer (ND One, Thermo Fisher, USA) for 1 min. The response changed into 3 ml reaction aggregate containing 2.78 ml phosphate buffer (pH 7), 0.1 mL enzyme extract, and 30µl of 15 mM H₂O₂ (Aebi, 1984). Enzyme pastime was calculable via $\epsilon = 0.28 \text{ mM}^{-1} \text{ cm}^{-1}$ and expressed. We measured SOD by the nitro blue tetrazolium technique (Giannopolitis and Ries, 1977). We mixed the enzyme extract (0.1 mL) of one hundred mM phosphate buffer (pH7.6) with 1.5 mM Na₂CO₃, 2.25 mM NBT, two hundred mM methionine, three mM ethylene diamine tetraacetic acid (EDTA), 0.06 mM riboflavin, and distilled water. The response combos without illumination were usable as blank. The absorbance of the response combination became measurable at 560 nm with a UV spectrophotometer (ND One, Thermo Fisher, United States of America). Enzymes were expressable as units consistent with milligrams of protein (U mg⁻¹ protein).

Anthocyanin

Anthocyanin changed into quantified data via the Wagner et al. (1979) method. To determine the concentration of anthocyanins, we extracted 0.25 g sparkling leaves in 15 ml glass centrifuge tubes containing 3 ml of acidified methanol (methanol: HCl 99: 1 v:v) and

maintained them overnight in the darkish. We delivered the samples and measured the absorbance at 550 nm. Anthocyanin concentration was calculable via an extinction coefficient of $33000 \text{ mol}^{-1} \text{ cm}^{-1}$ (Krizek et al., 1998).

Statistical analysis

The experiments operated on a Randomized Complete Block design (RCBD). We applied each treatment to three replications on one plant. We analyzed variance (ANOVA) via SAS. V.9.1 Statistical Program (SAS Institute Inc., Cary, NC, USA). Differences between mean values were determined by Duncan's test, with differences considered significant at $P \leq 0.05$.

RESULTS

Morphological characteristics

The study of annual effects (2021-2022) on leaf area (LA) and specific leaf area (SLA) revealed that both indicators reached their highest values in 2022, with LA and SLA increasing by 10.10% and 11.01%, respectively, compared to 2021 (Table 2). The results showed that treatments with nitroxine, phenylalanine, and selenium significantly influenced both indicators compared to control conditions. Specifically, goji berry plants treated with nitroxine at a concentration of $500 \mu\text{L}$ exhibited the highest LA and SLA, representing increases of 19.58% and 21.93%, respectively, relative to control values (Table 2).

Chlorophyll and carotenoid contents

Figure 1 presents the total chlorophyll content of goji berry plants treated with varying concentrations of Phe, Se, and nitroxine across both years. In 2022, total chlorophyll levels were significantly higher than in 2021. The nitroxine treatment positively influenced chlorophyll content across all concentrations, with the highest content ($1.84 \text{ mg g}^{-1} \text{ FW}$) observed in plants treated with $333 \mu\text{L L}^{-1}$ nitroxine in 2022 (Fig. 1A & B). Maximum carotenoid content resulted from Se at 1 mg L^{-1} , and data indicated that plants grown in 2021 contained significantly higher carotenoid levels than those in 2022.

Table 2. Changes in the amount of LA and LSA during the 2021-2022 and the treatments used in goji berry leaves.

Year	leaf area (LA)	specific leaf area (SLA)
2021	13.37 ^b	7.11 ^b
2022	15.022 ^a	7.99 ^a
Treat		
Control	13.001 ^d	7.01 ^{cd}
Phe0.5mM	14.022 ^{cd}	7.40 ^{cd}
Phe1mM	14.48 ^{bc}	7.51 ^c
Phe1.5mM	13.23 ^{cd}	7.17 ^{cd}
Se 0.25mg.L ⁻¹	13.19 ^{cd}	6.91 ^{cd}
Se 0.5mg.L ⁻¹	14.13 ^{cd}	6.70 ^d
Se 1mg.L ⁻¹	13.48 ^{cd}	7.39 ^{cd}
N 167 μL	13.92 ^{cd}	7.46 ^{cd}
N 333 μL	15.61 ^b	8.30 ^b

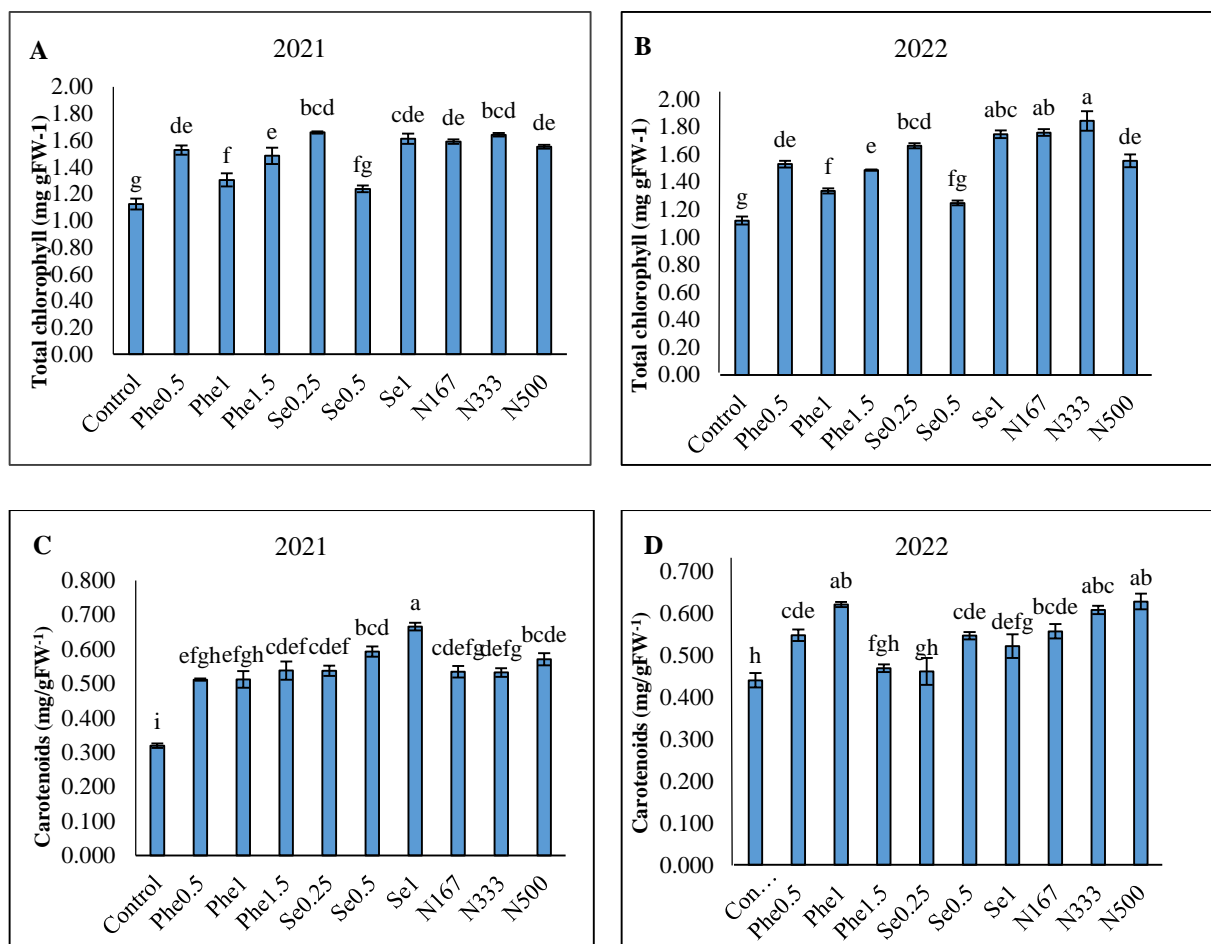


Fig. 1. Effect of phenylalanine (Phe), sodium selenate (Se) and nitroxin (N) foliar spray on total chlorophyll (A and B) and carotenoids (C and D) contents of Goji berry in two years (2021 and 2022). Values were the means of three replicates and bars represent the standard errors ($n = 3$). Values with the same letters are not significantly difference at $p \leq 0.05$

Carbohydrate content

Figure 2 illustrates the carbohydrate content in plants treated with different concentrations of Phe, Se, and nitroxine over the two study years. The carbohydrate content in 2021 significantly exceeded that of 2022 (Fig. 2A & B). Our findings confirmed that foliar applications of Phe, Se, and nitroxine increased carbohydrate levels compared to the control, with the highest carbohydrate values recorded in plants treated with 0.5 mg L^{-1} Se and 1 mM Phe in 2021.

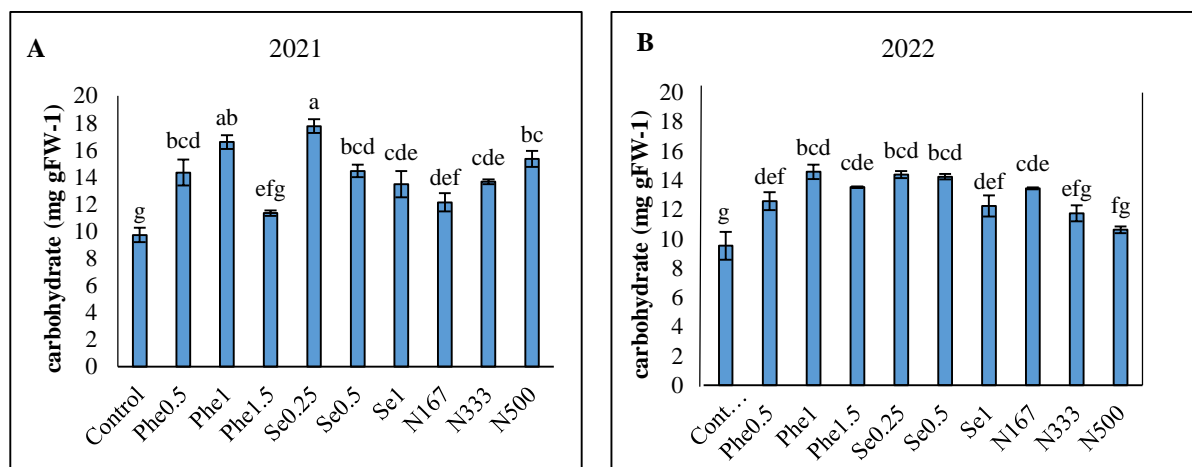


Fig. 2. Effect of phenylalanine (Phe), sodium selenate (Se) and nitroxin (N) foliar spray on carbohydrate (A and B) of Goji berry in two years (2021 and 2022). Values were the means of three replicates and bars represent the standard errors (n = 3). Values with the same letters are not significantly difference at $p \leq 0.05$.

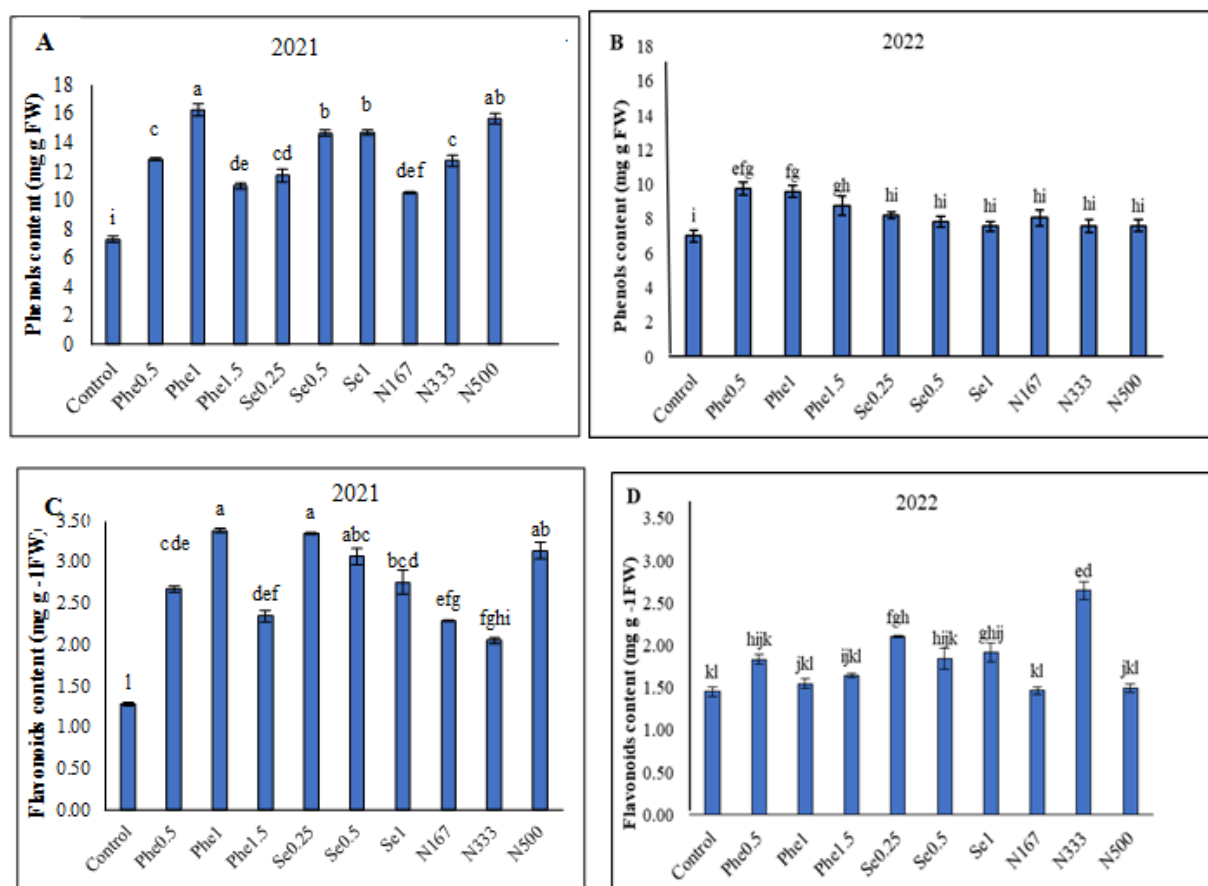


Fig. 3. Effect of phenylalanine (Phe), sodium selenate (Se) and nitroxin (N) foliar spray on Phenols content (A and B) and Flavonoids content (C and D) of Goji berry in two years (2021 and 2022). Values were the means of three replicates and bars represent the standard errors (n = 3). Values with the same letters are not significantly difference at $p \leq 0.05$.

Flavonoid and phenol content

Figure 3 indicated significant treatment effects on flavonoid and phenol content in goji berry. The highest flavonoid content occurred in the 1 mM Phe treatment (Fig. 3C & d). Foliar applications of Phe, Se, and nitroxine notably increased flavonoid content, with levels in 2021 being significantly higher than in 2022. The maximum phenol content resulted from nitroxine at 500 $\mu\text{L L}^{-1}$, with values in 2021 significantly exceeding those of 2022 (Fig 3A & B).

Antioxidant capacity

As shown in Figure 4, nitroxine and phenylalanine applications substantially influenced antioxidant capacity throughout the growth period. The highest antioxidant capacity occurred in 2021, with 2022 showing the lowest values. The nitroxine treatment at 500 $\mu\text{L L}^{-1}$ had a significant impact on antioxidant capacity. However, values showed no significant difference when compared to 1 mg L^{-1} selenium and 1.5 mM Phe. Higher treatment concentrations generally proved more effective than lower concentrations in enhancing antioxidant capacity.

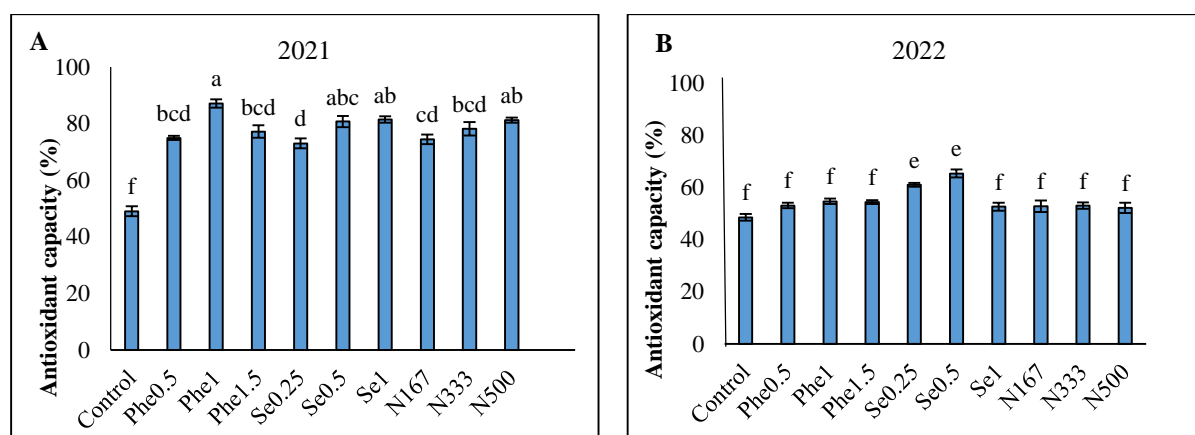


Fig. 4. Effect of phenylalanine (Phe), sodium selenate (Se) and nitroxin (N) foliar spray on antioxidant capacity (A and B) of Goji berry in two years (2021 and 2022). Values were the means of three replicates and bars represent the standard errors ($n = 3$). Values with the same letters are not significantly difference at $p \leq 0.05$.

Enzymatic antioxidant activity (PAL, SOD, CAT)

The exogenous application of Phe, Se, and nitroxine significantly enhanced phenylalanine ammonia-lyase (PAL) enzyme activity in goji berry plants (Fig. 5). Notably, plants grown in 2022 exhibited significantly higher PAL enzyme activity than those in 2021 ($p \leq 0.01$), though no significant variation appeared between the years in specific treatment responses. Treatments with 1.5 mM Phe in 2022 produced the maximum PAL content, with no substantial difference between the 1.5 mM Phe treatments in the two years (Fig. 5A & B). Similarly, Phe, Se, and nitroxine applications considerably impacted superoxide dismutase (SOD) activity. The SOD enzyme levels increased with Phe application (Table 1). Foliar application of 1 mM Phe in 2021 notably boosted SOD activity, with the highest recorded values of 16.46 and 15.90 protein units in plants treated with 1 mM Phe and 500 $\mu\text{L L}^{-1}$ nitroxine, respectively (Fig. 5C & D).

Phe, Se, and nitroxine applications enhanced catalase (CAT) activity in 2021 and 2022 (Table 1 & Fig. 5). CAT activity was significantly higher ($P \leq 0.01$) in 2021 compared to 2022. The highest CAT activity values of 2.72, 2.42, and 2.40 protein units appeared in treatments with 1 mg L^{-1} Se, 1.5 mM Phe, and 167 $\mu\text{L L}^{-1}$ nitroxine in 2021 and 2022, respectively (Fig. 5E & F).

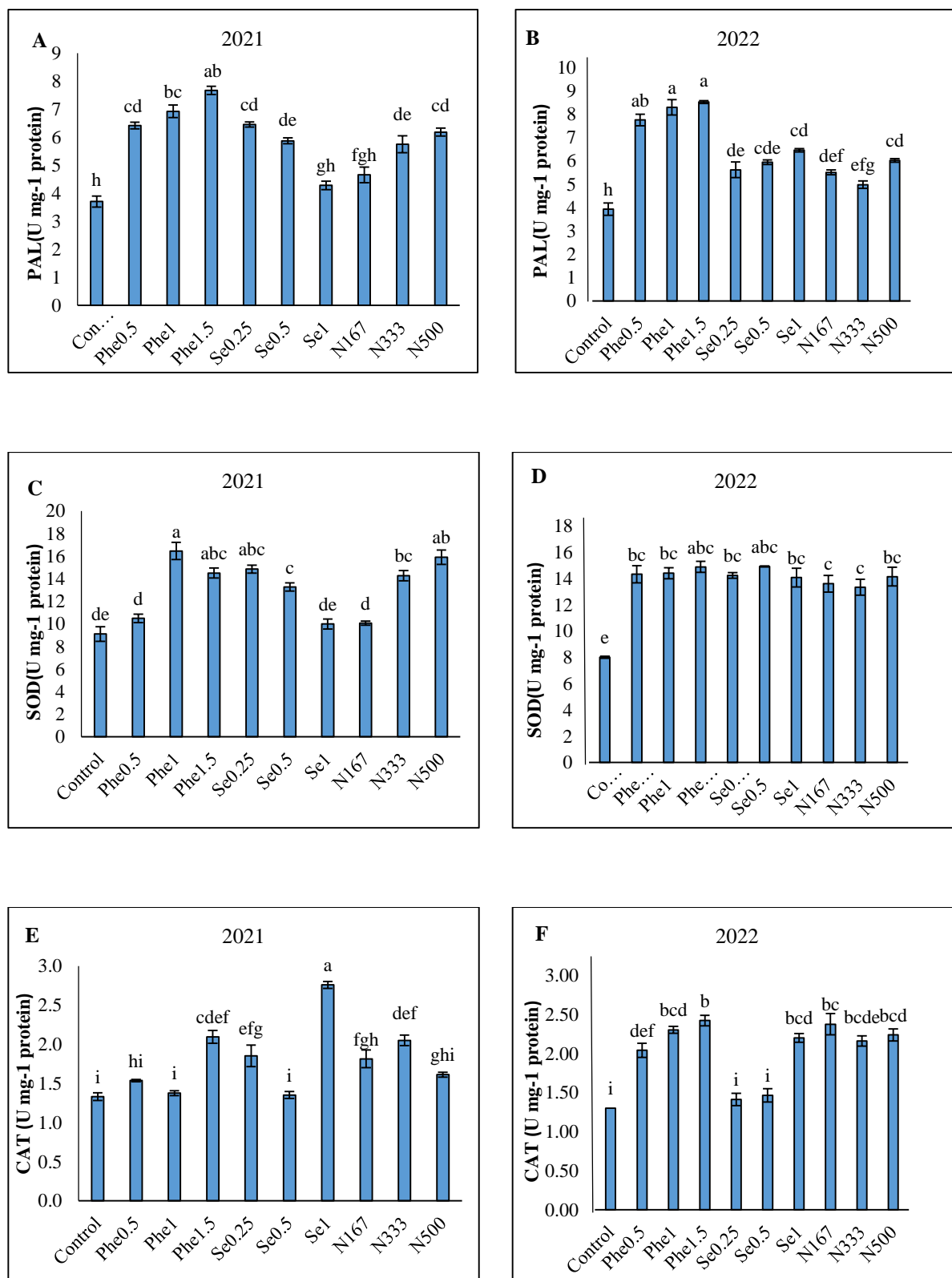


Fig. 5. Effect of phenylalanine (Phe), sodium selenate (Se) and nitroxin (N) foliar spray on phenylalanine ammonia-lyase (PAL) enzyme (A and B), Superoxide dismutase (SOD) enzyme (C and D) and Catalaz (CAT) enzyme (E and F) of Goji berry in two years (2021 and 2022). Values were the means of three replicates and bars represent the standard errors (n = 3). Values with the same letters are not significantly difference at $p \leq 0.05$.

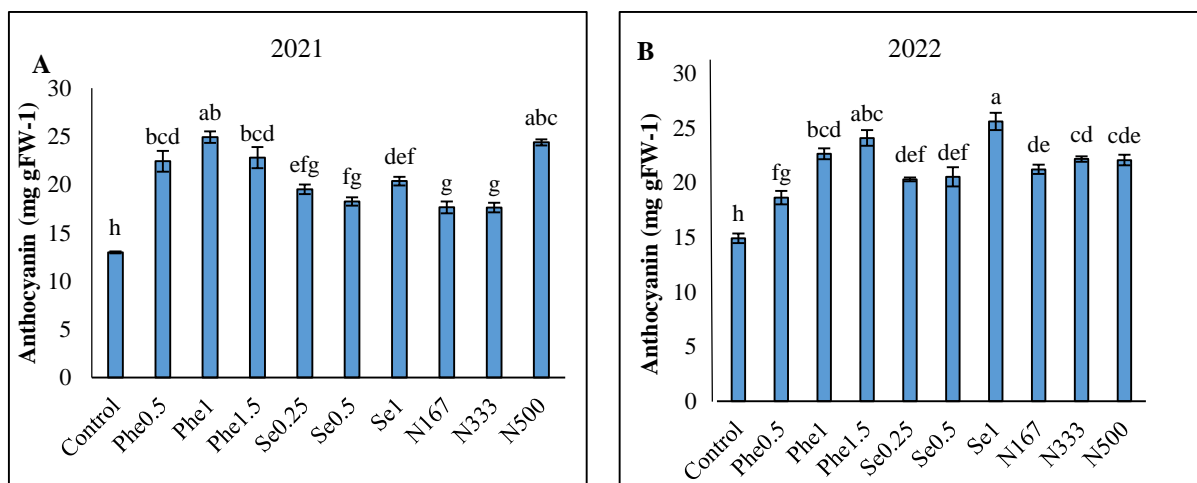


Fig. 6. Effect of phenylalanine (Phe), sodium selenate (Se) and nitroxin (N) foliar spray on anthocyanin (A and B) of Goji berry in two years (2021 and 2022). Values were the means of three replicates and bars represent the standard errors ($n = 3$). Values with the same letters are not significantly difference at $p \leq 0.05$.

Anthocyanin content

Anthocyanin levels in goji berry plants varied across different concentrations of Phe, Se, and nitroxine over the two years (Fig. 6A & B). The anthocyanin content in control plants was significantly lower ($P \leq 0.01$) than in all treated plants. Treatments with Phe, Se, and nitroxine markedly increased anthocyanin content compared to the control, demonstrating the beneficial effects of these applications.

DISCUSSION

Increasing antioxidant system function enhances total antioxidant capacity, governed by the content of low-molecular antioxidants and the activity of antioxidant enzymes. Key low-molecular antioxidants, including ascorbic acid, glutathione, tocopherol, carotenoids, anthocyanins, endogenous metal chelators, TPC, TFC, and alkaloids, play essential roles in this capacity (Radyuk et al., 2009; Giglou et al., 2023). Nitroxine, which binds atmospheric nitrogen, aids in nutrient balance within plants by promoting the secretion of amino acids, antibiotics, hydrogen cyanide, and siderophores, which stimulate root and shoot growth and protect roots from pathogens, thereby increasing yield (Shoaei et al., 2012; Fahramand et al., 2013). Research by Zahedian et al. (2022) identified the highest chlorophyll content ($1.84 \text{ mg g}^{-1} \text{ FW}$) following the nitroxine treatment.

Anthocyanins, beneficial secondary metabolites and natural pigments, demonstrate medicinal properties. L-phenylalanine, a pivotal precursor in anthocyanin biosynthesis, activates the phenylpropanoid pathway, promoting TFC accumulation. Due to its relative affordability and high efficiency, Phe is valuable for inducing pigment production (Edahiro et al., 2005). Endogenous Phe, derived from the shikimic acid pathway, or exogenously supplied Phe stimulates the phenylpropanoid pathway and PAL activity, crucial for accumulating phenols, flavonoids, and anthocyanins. This process also underscores the necessity of ROS accumulation, an essential plant response (Akkad et al., 2019). Anthocyanins, a subset of flavonoids in plant vacuoles, epidermal, and mesophyll cells, shield chlorophyll from light-induced oxidation, making them reliable indicators of plant oxidative stress (Abbas, 2012; Hatier & Gould, 2008).

Supporting these findings, Garavand et al. (2019) observed that Se treatment increased phenolic acids and anthocyanins in red and green lettuce. Application of Se on sweet basil

leaves enhanced anthocyanin pigments by 73% compared to the control, while selenate application significantly increased anthocyanins (Abbas, 2012). Nitroxine also promoted chlorophyll and carotenoid production in celery grown in Iraq and Iran, leading to linear improvements in photosynthetic pigments (Dahham, 2021; Rahi, 2013).

Increased carbohydrate content in sprayed plants may relate to higher atmospheric CO₂ levels due to improved stomatal permeability or activation of enzymes integral to CO₂ absorption (Hajiboland et al., 2015). Turakainen et al. (2015) highlighted carbohydrate accumulation and senescence processes in potato roots and tubers, attributing the rise in soluble sugar in selenium-treated alfalfa to fructose 1,6-bisphosphatase, a key enzyme in carbohydrate metabolism. Simojoki et al. (2003) noted that selenium supplementation enhances root efficiency, positively impacting carbohydrate production and accumulation.

Amino acids, essential components of plant proteins, contribute to numerous biochemical and physiological processes (Kandil et al., 2017). Phe, as a precursor for secondary metabolites, supports phenylpropanoid, flavonoid, and other essential metabolic pathways (Tzin and Galili, 2010). Port et al. (2015) found that foliar application of L-phenylalanine increased anthocyanin and phenolic compound levels in grapefruit, aligning with Pakkish and Mohammadrezakhani (2021) research, which demonstrated that amino acid sprays on mango trees elevated anthocyanin, carotenoid, and phenolic contents. Additionally, bio-nitroxide fertilizer application increased total phenolic content in cantaloupe fruits (Zahedian et al., 2022), and Jalil Shishbehra et al. (2022) confirmed that nitroxine treatment significantly elevated phenolic yields in purple conifer plants.

Applying nitroxine enhances antioxidant enzyme activity and facilitates the accumulation of compatible osmolytes in black cumin, ultimately boosting biological performance. Similarly, biological fertilizers have been shown to increase antioxidant activity in sweet sorghum (Wang et al., 2019). Treatments with biofertilizers also increased total phenolic content (TPC), total flavonoid content (TFC), and DPPH activity in *Cephalaria syriaca* (L.). Foliar applications of nitroxine and iron (Fe) significantly raised the antioxidant capacity in goji berry plants, with high flavonoid and phenolic compound levels correlating strongly with antioxidant activity, as previously established between TFC and antioxidant capacity (Ghasemzadeh et al., 2012). Amino acids, such as phenylalanine, significantly influence plant antioxidant activity (Khaki et al., 2020).

Phenylalanine is essential in synthesizing aromatic compounds, antioxidants, lignin, anthocyanins, and other phenolic compounds, playing a role in cellulosic ethanol production as well (Edahiro et al., 2005). Studies indicate that applying amino acids, including phenylalanine, positively impacts antioxidant metabolism in soybean plants, benefiting both seeds and leaves (Teixeira et al., 2017). In goji berry plants, antioxidant enzymes such as phenylalanine ammonia-lyase (PAL), superoxide dismutase (SOD), and catalase (CAT) increased in response to Phe, Se, and nitroxine treatments. Plants naturally produce an antioxidant system comprising enzymes like SOD, CAT, and PAL and metabolites such as ascorbic acid, glutathione, α -tocopherol, carotenoids, and flavonoids (El-Desouky et al., 2011). Various stimulants and environmental conditions can enhance these antioxidant components in plants.

Phenylalanine, an aromatic amino acid, is vital for synthesizing phenolic compounds via the phenylpropanoid pathway. PAL, the initial enzyme in this pathway, catalyzes phenylalanine's conversion to flavonoids, phenols, and anthocyanins. This pathway activation is often a response to biotic and abiotic stress, resulting in the accumulation of bioactive compounds (Aghdam et al., 2019). Studies have consistently demonstrated that phenylalanine effectively boosts PAL activity and phenolic compound accumulation (Aghdam et al., 2019; Sogvar et al., 2020). Foliar and seed treatments with phenylalanine enhanced PAL activity in

soybean plants (Teixeira et al., 2017; Wu et al., 2011). Increased SOD activity, induced by environmental stress, can result in H_2O_2 accumulation, a signaling molecule that further stimulates the phenylpropanoid pathway and promotes phenol production, enhancing the plant's oxygen radical absorbance capacity (Jacobo-Velázquez et al., 2011).

Overall, phenylalanine remains one of the most effective treatments for increasing phenolic compound buildup (Aghdam et al., 2019; Sogvar et al., 2020). Additionally, it plays a critical role in anthocyanin biosynthesis by activating PAL, leading to heightened anthocyanin accumulation (Wu et al., 2011; Heydarnajad Giglou & Torabi Giglou, 2023).

In the available literature, amino acid application on soybean, both during processing and as a foliar spray, increased the activity of superoxide dismutase (SOD) and catalase (CAT) enzymes, respectively (Teixeira et al., 2017). Similarly, Ulianych et al. (2020) reported an upward trend in SOD and CAT activities in garlic plants following amino acid treatments. Our findings align with those of Aghdam et al. (2019), who observed that tomato fruits treated with 0.5 mM phenylalanine (Phe) exhibited higher total phenolic content (TPC), total flavonoid content (TFC), and phenylalanine ammonia-lyase (PAL) activity compared to untreated fruits. The treated fruits also showed elevated CAT and SOD levels, underscoring the role of amino acid intake in enhancing both enzymatic and non-enzymatic antioxidant activity. For instance, exogenous amino acids were shown to significantly increase SOD levels in tomato leaves (Liu et al., 2015).

Adequate selenium (Se) concentrations are also essential for optimal plant growth, antioxidant function, photosynthesis, and osmoregulation. Selenium adjusts the antioxidant system primarily through three mechanisms: it promotes the conversion of superoxide (O_2^-) to H_2O_2 independent of SOD enzymatic catalysis, it quenches reactive oxygen species (O_2^- and OH^-) via selenium compounds, and it directly regulates the activity of antioxidant enzymes (Chongping et al., 2022). These functions highlight Se's antioxidative effects on plants (Huang et al., 2018). Research shows that Se can boost plant growth in challenging environments, such as by enhancing root development in chili plants (Mozafariyan et al., 2014). Low Se concentrations have also been associated with increased antioxidant enzyme activity and non-enzymatic antioxidants in lettuce plants (Ríos et al., 2009). Additionally, Rady et al. (2020) found that semi-cured tomatoes exhibit increased activity of several antioxidant enzymes, including SOD and CAT, confirming Se's role in enhancing antioxidant defenses. El-Ramady et al. (2016) demonstrated that Se impacts the growth of groundnut cultivars by modulating photosynthetic pigments, catalase activity, phenolic content, and total flavonoid levels.

Nitroxine, a biological fertilizer rich in microorganisms, further stimulates antioxidant enzyme activity. Studies show that treatments with Phe, Se, and nitroxine in goji berries elevate PAL, SOD, and CAT activities. Biofertilizers, in particular, have been found to significantly increase CAT activity, enhancing cellular stability (Hashem et al., 2016). Numerous studies have indicated that biofertilizers promote CAT and SOD activity, providing superior protection to the photosynthetic apparatus and, subsequently, improving plant growth (Weng et al., 2015). The highest CAT levels occurred in plants subjected to foliar applications of nitroxine, which likely contributed to increased CAT activity in the leaves. This effect may be linked to the nitrogen supply facilitated by nitrogen-fixing bacteria like *Azotobacter* and *Azospirillum* (Najafi et al., 2021).

CONCLUSION

Our findings indicated that phenylalanine and selenium treatments enhanced quality traits in goji berry seedlings, resulting in increased anthocyanins, carbohydrates, chlorophyll, and antioxidant compounds. Foliar applications of phenylalanine, selenium, and nitroxine stimulated the biosynthesis of photosynthetic pigments and total phenolic content, which elevated antioxidant and phytoactive compound levels. Over two years, these treatments demonstrated positive effects on various traits, particularly by promoting the biosynthetic pathways for active substances and bolstering plant defenses. Specifically, applying Phe (0.5 mM) and Se (0.5 mg L⁻¹) substantially improved both enzymatic and non-enzymatic antioxidant mechanisms.

Conflict of interest

The authors have reviewed the journal's policies and declare no conflicts of interest. This manuscript is original, has not been published, and is not under consideration elsewhere.

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