

RESEARCH

Open Access



Glycine mitigates fertilizer requirements of agricultural crops: case study with cucumber as a high fertilizer demanding crop

Fateme Zargar Shooshtari¹, Mohammad Kazem Souri^{2*}, Mohammad Reza Hasandokht³ and Sepideh Kalateh Jari¹

Abstract

Background: Different approaches have been used to improve mineral nutrient status of plants in absence of chemical fertilization and toward safer products and improved human health. Amino acids have been proposed with such roles in different recent studies. In this study, glycine was applied as foliar (250, 500 and 1000 ppm) or as soil (250 and 500 mg/plant) to cucumber plants compared to unfertilized control and NPK fertilization, under greenhouse conditions.

Results: The results showed that all glycine treatments increased leaf area and the economic life of plants compared to control. Soil application of glycine at higher concentration of 500 mg/plant was able to produce the same or better records than NPK fertilization, particularly regarding leaf mineral concentration, plant economic life and total yield. Leaf macro- micro-nutrients were most increased under 500 mg soil-glycine application. Foliar spray of 500 ppm glycine resulted in better records than the other two levels, as spray of 1000 ppm glycine showed adverse and toxic effects including leaf necrosis. Fruit firmness was increased only by NPK and soil application of 500 mg glycine, whereas fruit vitamin C was increased by NPK fertilization, soil application of glycine at both levels (250, 500 mg) and foliar application of glycine at 500 ppm compared to control plants.

Conclusion: The results indicate that soil application of 500 mg glycine/plant was able to improve leaf mineral and physiological characteristics towards higher yield and quality.

Keywords: Amino acid, Cucumber, Leaf protein, Nutrient uptake, Yield, Quality, Vegetable

Background

Cucumber represents one of the main vegetable crops and due to low calorie, it plays an important role in vegetarian diets [12]. The crop has a relatively short life period, but can produce high yields particularly in greenhouse cultivation. Application of new and suitable cultivars, intensive fertilization and optimization of the growth conditions are quite important to achieve high yield in cucumber culture [27]. High rates of chemical fertilizers are generally applied to cucumber culture in

the field and particularly under greenhouse cultivation. This has resulted in significant soil salinity in many parts of the world, particularly in arid regions with restricted water supply and low quality [32].

Different compounds and agronomic techniques have been proposed and used extensively to enhance mineral nutrition of plants under optimum or stress conditions [23, 28, 37]. The biostimulation effect of amino acids on plant growth and nutrient uptake enhancement has been frequently reported [2, 9, 46]. Due to their nature, low application rates and higher efficiency, amino acids are fully friendly to soil, environment and human health [32]. By far, glycine is one of the most widely used amino acid in plant nutrition. It is often used for production of a wide

*Correspondence: mk.souri@modares.ac.ir

² Dept. of Horticultural Sciences, Tarbiat Modares University, Tehran, Iran
Full list of author information is available at the end of the article

range of amino chelate fertilizers (amino acid-chelated nutrients). Exogenous application of amino acids can increase nitrogen status and concentration of mineral elements in plant tissues [14]. However, many factors govern the effectiveness of the applied amino acid (glycine), including plant species, growth stage, climatic conditions, number of foliar or soil applications and particularly the applied concentration [9, 32].

Nowadays, safe production of agricultural foods particularly fresh vegetables such as cucumber is vital regarding human health [24, 34, 37]. Application of amino acids such as glycine is a more sustainable approach toward safer production compared to chemical fertilization [32]. Moreover, adopting techniques to enhance the plant's mineral uptake, growth and fruiting characteristics is necessary when no chemical fertilization program is applied. In this regard, application of biostimulants such as organic acids and amino acids may be important. Exogenous application of amino acids in many crops has been shown to improve plant growth and productivity compared to unfertilized control [19, 20, 39, 45]; however, in high fertilizer demanding crops such as cucumber [25, 38], with multi-harvests and high-yield production (500–1000 t/ha), it is not clear whether glycine can benefit plant growth by maximizing the yield and quality or not. Nevertheless, application of amino acids such as glycine in cucumber production can better serve fruit quality than chemical fertilizers [32]. Therefore, in this study the effect of different concentrations and application methods of glycine on growth characteristics of cucumber was evaluated under greenhouse conditions.

Materials and methods

Experimental setup

This experiment was conducted during winter–spring of 2019 and under soil greenhouse cultivation. Cucumber seeds (*Cucumis sativus* var. Sultan) were sown in a mix of cocopeat/perlite in January, and the seedlings were transferred to soil medium at the middle of February under protected greenhouse conditions. The soil had a loamy-clay texture and a moderate lime content (8.7% as $\text{Ca}(\text{CO}_3)_2$ and 3.1% as $\text{Mg}(\text{CO}_3)_2$). The physico-chemical characteristics of the soil are presented in Table 1. The seedlings were transplanted on rows with 2 m distance, while the plants in rows were 1.5 m apart.

Application of treatments

Different concentrations of glycine (Goodchem Technology Co. Shanghai, China) were used as foliar or soil application including: (1) control (without any treatment application), (2) soil application of chemical NPK fertilizer, soil application of (3) 250 mg, and (4) 500 mg glycine per plant, and foliar application of (5) 250 ppm, (6) 500 ppm, and (7) 1000 ppm glycine. The treatments were arranged in a completely randomized design with four replications. Each replication was a plant that was randomized in the rows.

The foliar application of different glycine concentrations was done four times during the growth period. The first spray was applied 2 weeks after transplanting (at middle March). The other sprays were done in 2-week intervals. A total amount of 150–200 mL per plant was sprayed in all four foliar applications. The glycine solutions were prepared using distilled water, and sprayed on both sides of plant leaves, early in the morning using a portable sprayer. The soil application of glycine was done in three split applications of a final volume of 250 or 500 mg/plant, after dissolving the amounts in distilled water. For this purpose, the first application (83 mg or 167 mg per plant) was done 2 weeks after transplantation, and the next applications were done at 5 and 8 weeks after transplantation. Chemical NPK fertilizer (20:10:15) was used in three split applications of a final amount of 1500 mg/plant. The split applications were done at the same time as soil-glycine application and via irrigation water. The greenhouse temperature of days was in range of 29 ± 5 °C, and of nights was in range of 21 ± 3 °C, during the 4-month growth period. The air humidity was in the range of $73 \pm 5\%$. Plants were treated equally regarding the maintenance management, including irrigation and pruning. Plants were trained in a standard format of single stem with weekly remove of lateral shoots and leaves.

Measurements

Plant height, leaf SPAD value and leaf area

Different parameters were recorded during plant growth period or at final harvest (end of June). The plant height was measured using a tape, and leaf SPAD value (The Soil and Plant Analysis Development) was measured using a portable SPAD meter (SPAD-502, Minolta, Japan). The average of 30 readings per plant of different leaves in the middle–upper part of shoots was recorded. The average plant leaf area for a single leaf was determined using leaf

Table 1 The physico-chemical characteristics of the soil used in the study

Soil texture	pH	EC (dS/m)	Total C (%)	CaCO_3 (%)	MgCO_3 (%)	Total N (%)	Extractable P (mg/kg)	Extractable K (mg/kg)
Loam-clay	7.6	5.4	0.68	8.7	3.1	0.14	14.1	286

area meter (Model CI 202, Germany), by recording the area of 3–4 randomly detached leaves from the middle part of plant.

Plant fresh and dry weight

Fresh and dry weights of plant tissues including fruits were determined using a digital scale. The cumulative amounts of pruned shoots (g) were also recorded. The dry matter of pruned shoots together with dry matter of plant at final was recorded as the plant shoot dry matter.

Mineral nutrient analysis

Fully expanded leaves from the upper half of plants were used for mineral nutrient analysis. Leaf mineral elements were determined with different methods. Nitrogen was determined using Kjeldahl method, Ca, Mg, Fe and Zn using atomic absorption spectroscopy and K using flame photometry.

Leaf protein and NR activity

Leaf protein concentration was determined following Bradford method [4]. The activity of leaf nitrate reductase enzyme (NR) was determined after grinding and homogenizing the leaf materials in a mortar containing liquid nitrogen. Nitrate reductase was extracted in a buffer consisting of 100 mM HEPES (pH 7.5) (Sigma-Aldrich), 1 mM EDTA (MERCK), 7 mM cysteine (MERCK), 3% polyvinyl polypyrrolidone (PVPP) (Sigma-Aldrich), 10 mM leupeptin, and 1 mM phenyl methyl sulfonyl fluoride (PMSF) (Merck). After preparation of extracts, sulfanilamide (0.5%) (Merck) and *N*-(1-naphthyl)-ethylenediamine dihydrochloride (0.01%) (Sigma-Aldrich) in 1.5 M hydrochloric acid (HCl) (Merck) were used for color development. The amount of NO_2^- was determined spectrophotometrically at 540 nm and then nitrate reductase activity was calculated accordingly.

Plant economic life

The economic life of plants was considered as the time of active flowering and fruiting period, and it was determined from first fruit set to when more than 50% of plant leaves were chlorotic/defoliated and with significant reduction in fruiting and fruit appearance quality.

Fruit yield and quality traits

Fruits were regularly harvested when the size of fruits reached a nearly 18 cm and a weight of about 80 g. The cumulative fruit numbers and their associated weight, as total plant yield, have been recorded. The harvested fruits at the first week of May (9 weeks after transplanting) were used for fruit biochemical characterization. Fruit firmness was measured by a portable penetrometer (Model FT327, Wagner, USA) with two applications for each

fruit, and the records were presented as kg/cm^2 in the results. Fruit total soluble solids (TSS%) were determined using portable refractometer (Atago, Tokyo, Japan) and under room temperature of 25 °C. Fruit L-ascorbic acid content was determined using di-chlorophenol indophenol reagent. Briefly, 10 g of fresh fruit tissues including skin were crushed in a mortar in presence of 10 mL metaphosphoric acid 6% (Merck), thereafter the solution was centrifuged at 4000g for 5 min at 4 °C. Five mL of the supernatant transferred into an Erlenmeyer flask, and received 20 mL of metaphosphoric acid 3%. Then titration of the extract was done by di-chloro phenol indophenols (Sigma-Aldrich) until the appearance of a rose color, from which the amount of vitamin C (mg/100 g FW) was calculated accordingly and based on a standard curve of L-ascorbic acid (Merck) concentrations.

Statistical analysis

Data were subjected to analysis of variances by SPSS software and comparison of means was done at 5% level of LSD test.

Results and discussion

The results showed that the most of cucumber growth traits were influenced by treatments under the lime soil condition with 8.7% calcium carbonate and 3.1% magnesium carbonate. Despite cucumber being a relatively tolerant species to moderate lime, its yield and quality may have been negatively influenced by soil lime conditions. The results of ANOVA showed that the effects of treatments on plant height, leaf SPAD value and leaf area were significant at $P=0.01$. Plant height and leaf SPAD value were significantly increased by NPK and soil application of glycine at both levels (Table 2). Foliar applications of glycine also resulted in insignificant increase in plant height and leaf SPAD value; however, spray of 1000 ppm glycine resulted in lowest leaf SPAD value (Table 2), probably due to chlorotic and necrotic effects on plant leaves. Cucumber leaf area was also increased by all levels and methods of glycine application as well as by NPK compared to control plants. The highest leaf area was observed in NPK treatment that showed no significant difference to soil application of 500 mg/plant glycine (Table 2).

Plant height, leaf area and leaf SPAD value are among the most important morphological traits of plant growth that are influenced by many factors including mineral fertilization [2, 18]. These traits, in cucumber as a high resource demanding crop, are more critically influenced by fertilization than other crops [34]. Nitrogen, phosphorus and potassium are the most needed minerals for plant growth, and in this study with NPK fertilization in three splits, significant improvement in plant growth

Table 2 Comparison of means for some growth traits of cucumber plants under different glycine treatments compared to NPK and control plants

Treatments	Plant height (cm)	Leaf SPAD value	Leaf area (cm ²)	Pruned shoots weight (g/FW)	Total shoot DW (g)	Plant economic life ^a (day)
Control	1.7 ± 0.2d	29 ± 5c	117 ± 18e	510 ± 140b	90 ± 15d	41.25 ± 4.6d
NPK fertilizer	2.7 ± 0.1ab	42 ± 2a	280 ± 24a	1409 ± 420a	304 ± 31a	77 ± 7.8b
Soil-glycine (250 mg/plant)	2.4 ± 0.4abc	36 ± 4ab	204 ± 29c	743 ± 216b	224 ± 35b	76 ± 5.8b
Soil-glycine (500 mg/plant)	2.8 ± 0.3a	40 ± 4a	271 ± 32ab	1379 ± 241a	322 ± 49a	93 ± 9.5a
Foliar-glycine (250 ppm)	2.2 ± 0.4bcd	34 ± 3bc	234 ± 31bc	675 ± 121b	167 ± 27c	58.25 ± 9.6c
Foliar-glycine (500 ppm)	2.2 ± 0.5bcd	33 ± 2bc	205 ± 30c	589 ± 119b	180 ± 27bc	65.25 ± 8.3bc
Foliar-glycine (1000 ppm)	2 ± 0.3 cd	25 ± 4d	162 ± 22d	390 ± 79b	104 ± 16d	55.25 ± 11.5c
ANOVA**	<i>P</i> -value = 0.01	<i>P</i> -value = 0.01	<i>P</i> -value = 0.01	<i>P</i> -value = 0.01	<i>P</i> -value = 0.01	<i>P</i> -value = 0.01

Data are average of four replications ± SD, and in each column means with similar letter are not significantly different at 5% level of LSD test

***P*-value levels of 0.01, 0.05 and ns mean significant effect at 1%, 5% and no significant effect, respectively

^a Plant economic life indicates the active period of flowering and fruiting

was observed compared to unfertilized control plants. Glycine particularly in soil applications also improved these morphological traits, probably due to biostimulation effect as well as enhancing mineral bioavailability and uptake under the soil limed conditions [20]. The biostimulation of amino acids on plant vegetative growth traits including leaf SPAD value and leaf area has been also reported in other studies [21, 41]. In a study with foliar application of glycine or glutamine in concentrations of 250, 500 and 1000 ppm, it was shown that only glycine at 250 ppm and glutamine at 1000 ppm significantly increased leaf chlorophyll compared to control plants; however, amino acids had no effect on lettuce plant height [2]. Foliar treatment of glycine at 1000 ppm adversely influenced many growth traits of cucumber. It seems that cucumber is relatively sensitive to foliar spray of some nitrogenous chemicals such as ammonium and glycine amino acid [32] particularly at higher application rates.

The ANOVA for economic life of plant was significant at *P* = 0.01. The NPK treatment as well as all soil and foliar-glycine treatments significantly increased the economic life of cucumber plants (the period of active flowering and fruiting). The plants treated with 500 mg soil application of glycine had the longest economic life compared to other treatments (Table 2). For crops such as cucumber with several harvests, a prolonged economic life is desirable particularly when cultivated under greenhouse conditions. In some situations, such as soil salinity, alkalinity and calcareous conditions, mineral deficiency, pathogen infections, water stress and extreme weather, the economic life of crops is generally shortened that can affect the profitability of cultivation practice [18, 32]. Application of mineral nutrients, particularly under

lime soils can significantly improve plants economic life [18, 40]. In coriander (*Coriandrum sativum*) application of glycine at 300 and 600 mg/kg soil reduced the number of flowered plants and resulted in longer growth period and higher yield and biomass production [20]. Glycine with different mechanisms may increase cucumber economic life including, optimization of plant growth, nutrient uptake and photosynthetic activity that are associated with biostimulation, signaling and protective effects of glycine on plants [14, 30]. Glycine also has semi-hormonal effects on plant growth [34] and sometimes is regarded as a plant growth regulator [16]. It has been shown that soil application of glycine can also positively influence the soil microbial activity resulting in enhanced soil fertility status particularly under limed, drought or salinity soil conditions [32, 45].

The results of ANOVA showed that the effects of treatments on the fresh weight of pruned shoots as well as on plant shoot dry weight was significant at *P* = 0.01. The measured traits were significantly higher in NPK and soil application of 500 mg glycine than other treatments (Table 2). The lowest dry weight was in control plants that showed no difference with 1000 ppm foliar-glycine treatment (Table 2). Mineral fertilization can increase plant biomass production particularly under limited soil nutrients availability [18]. In the present study, soil and foliar applications of glycine were able to improve cucumber plant growth and biomass production almost to the same level of chemical NPK fertilization. Higher biomass production due to application of glycine ([20, 21, 39, 42]) or other amino acids [5, 6, 8, 15] has been also reported. Optimization of photosynthesis processes and leaf metabolic reactions, as well as prolongation of plant economic life probably play roles in this regard [16, 19,

Table 3 Comparison of means for leaf mineral nutrients of cucumber plants under different glycine treatments compared to NPK fertilization and control plants

Treatments	Leaf N (%)	Leaf K (%)	Leaf Ca (%)	Leaf Mg (%)	Leaf Fe (mg/kg DW)	Leaf Zn (mg/kg DW)
Control	2.2 ± 0.3c	2.4 ± 0.47c	2.8 ± 0.52b	0.30 ± 0.05c	42 ± 8.8c	29 ± 4.0c
NPK fertilizer	3.5 ± 0.5a	4.0 ± 0.45a	2.9 ± 0.41b	0.32 ± 0.07bc	46 ± 7.5c	32 ± 4.9bc
Soil-glycine (250 mg/plant)	2.6 ± 0.4bc	3.0 ± 0.52bc	3.3 ± 0.62b	0.43 ± 0.06ab	61 ± 10.5ab	39 ± 4.8ab
Soil-glycine (500 mg/plant)	3.2 ± 0.5ab	3.4 ± 0.42b	4.3 ± 0.46a	0.46 ± 0.09a	73 ± 11.3a	43 ± 5.2a
Foliar-glycine (250 ppm)	2.5 ± 0.3bc	2.6 ± 0.28c	3.1 ± 0.52b	0.34 ± 0.05bc	49 ± 5.4bc	35 ± 5.6abc
Foliar-glycine (500 ppm)	2.9 ± 0.5ab	3.0 ± 0.36bc	3.2 ± 0.42b	0.41 ± 0.09abc	51 ± 8.1bc	40 ± 7.1ab
Foliar-glycine (1000 ppm)	2.8 ± 0.4bc	2.7 ± 0.31c	3.1 ± 0.40b	0.34 ± 0.08bc	45 ± 5.3c	35 ± 6.6abc
ANOVA*	<i>P</i> -value = 0.05	<i>P</i> -value = 0.01	<i>P</i> -value = 0.01	<i>P</i> -value = 0.05	<i>P</i> -value = 0.01	<i>P</i> -value = 0.05

Data are average of four replications ± SD, and in each column means with similar letter are not significantly different at 5% level of LSD test

**P*-value levels of 0.01, 0.05 and ns mean significant effect at 1%, 5% and no significant effect, respectively

32]. It has been shown that treatment with glycine significantly improved the biomass and chlorophyll content of rice compared to ammonium and nitrate-treated plants under cold stress [39]. Similarly, improvement in leaf characteristics including water content and photosynthesis due to applied glycine concentrations has been reported [39, 40].

Leaf nutrients concentration was positively influenced by NPK and glycine treatments (Table 3). The results of ANOVA on leaf concentration of potassium (K), calcium (Ca), and iron (Fe) were significant at $P=0.01$, and on leaf concentration of nitrogen (N), magnesium (Mg), and zinc (Zn) were significant at $P=0.05$. Leaf N and K concentrations showed an increasing trend with glycine treatments. Leaf N was increased by NPK, soil application of 500 mg glycine and foliar spray of 500 ppm glycine, while leaf K was increased by NPK and soil application of 500 mg glycine compared to control plants. Soil application of 500 mg glycine significantly increased leaf calcium compared to other treatments. Leaf magnesium and iron concentrations showed a similar response in which an increasing trend was observed with treatments; however, only soil application of glycine at the both levels (250 and 500 mg/plant) significantly increased leaf Mg and Fe compared to control plants. Similarly, leaf zinc concentration was increased by soil application of glycine at both levels as well as by foliar application of 500 ppm glycine compared to control plants (Table 3).

Application of NPK increased leaf N and K concentrations and it has also positive effects on the uptake of other mineral nutrients (Table 3). Under limed soil conditions, low N and K may reduce plant root growth resulting in restricted mineral uptake [18]. Application of N, K and P, alone or in combination, can benefit other minerals availability and uptake [32, 44]. In this regard, nitrogen application is more important particularly when a specific form of nitrogen is applied [18].

In the present study, all mineral nutrients were influenced by glycine, particularly in soil application. The enhancement in leaf mineral nutrients due to application of glycine [20, 21, 41] or even other amino acids [3, 9, 14, 17] has been reported. In a study with lettuce plants only leaf Zn, but not other minerals, was increased by foliar application of 500 ppm glycine [2]. Plant genotype, growth conditions and soil fertility levels are important factors in this regard. Glycine, as a reduced form of nitrogen, can enhance nutrient bioavailability and translocation, particularly under soil limed conditions [17, 18, 39]. In maize, foliar application of a biostimulant containing amino acids, obtained by enzymatic hydrolysis from chicken feathers, significantly increased the leaf concentrations of macro- micro-nutrients, grain yield (14%) and protein content (26%) [37].

It has been shown that amino acids have a high ability to form complexes or chelates with metal minerals [10, 46]. Amino acids are ligands with different dentations that can produce chelate of minerals under specific conditions [31]. However, following application in soil, their chelate formation with native soil minerals has not been assayed and accepted yet, despite their complex formation is quite possible [32]. This effect can significantly increase bioavailability of mineral nutrients particularly metal trace elements [1, 26]. In addition, application of Fe(II)-amino acids chelate has been shown to improve the uptake of other mineral elements such as Zn beside Fe, by stimulation plant (root) growth and higher soil nutrient mobility [32]. Similarly, in coriander, leaf nutrient concentrations of N, Ca, K, P, Fe, and Zn, but not Mg and Mn, were significantly increased by soil application of glycine (at 300 and 600 mg/kg soil), whereas soil-applied NPK significantly increased only P and Ca of leaves compared to unfertilized control plants [20]. Application of amino acids in the nutrient solution resulted in higher Fe uptake by tomato roots [28]. In a nutrient solution

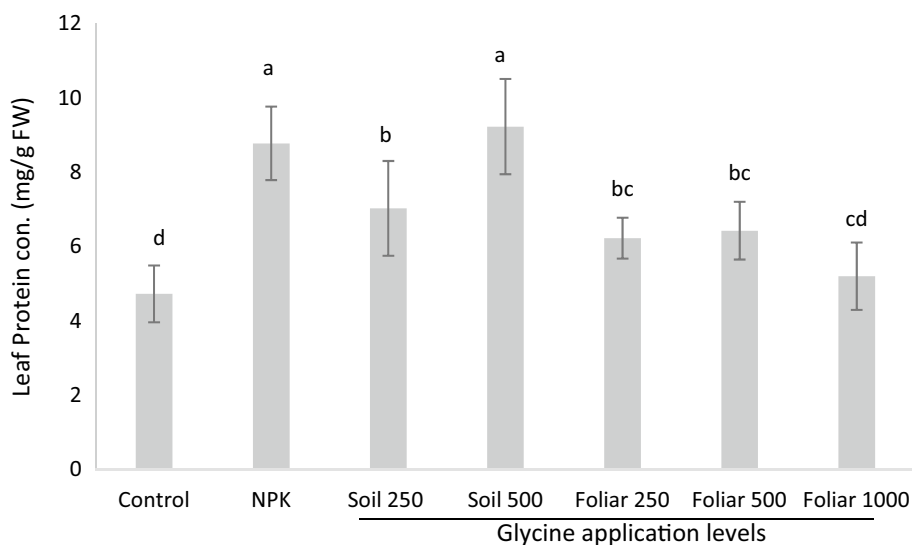


Fig. 1 Leaf protein concentration in cucumber plants under different treatments. Data are average of four replications \pm SD ($P=0.01$). Comparison of means was done at 5% level of LSD test

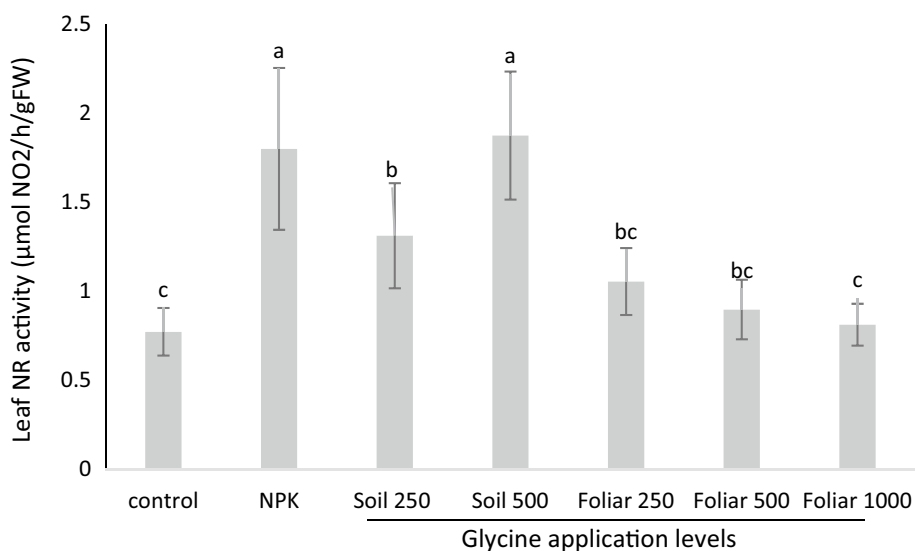


Fig. 2 Changes in the activity of leaf nitrate reductase enzyme under treatments. Data are average of four replications \pm SD ($P=0.01$). Comparison of means was done at 5% level of LSD test

experiment with coriander plants it has been shown that application of glycine increased leaf concentrations of N and K (at 10 mg L glycine), Mg (at 5 mg L glycine), and Zn (at all glycine levels of 5, 10, 20 and 40 mg/L) compared to the control plants [21]. Amino acids have diverse roles in plant metabolism and their exogenous application may influence plant growth in different aspects. The positive effect of amino acids application on root uptake and

root-to-shoot translocation of Fe and Zn in tomato seedlings exposed to Cd stress has also been reported [43].

The ANOVA for leaf protein concentration (Fig. 1) and leaf nitrate reductase (NR) activity (Fig. 2) was significant at $P=0.01$. Leaf protein was significantly increased by NPK and all soil and foliar-glycine treatments except foliar application of 1000 ppm (Fig. 1). Leaf protein was significantly higher in NPK and soil application of 500 mg glycine than other treatments. Similarly, leaf

nitrate reductase was significantly increased by NPK and soil application of glycine at both levels, while foliar applications of glycine showed no difference in leaf NR compared to control plants (Fig. 2). Higher protein biosynthesis due to application of amino acids and glycine has been shown in other studies [17, 37, 26, 39]. In coriander, application of different glycine levels of 5, 10, 20 and 40 mg/L significantly increased leaf antioxidant activity, while leaf protein was increased only by 10 or 20 mg/L glycine in nutrient solution [21]. In soybean, significant increase in leaf total amino acids occurred following seed or foliar application of amino acids [35]. From nutritional point of view, higher leaf content of protein is desirable regarding metabolic reaction and optimum photosynthesis activity [18, 32]. In this study, it seems that foliar and soil applications of glycine were effective to increase leaf protein probably due to enhancement in soil minerals availability, uptake, translocation and distribution in plant tissues [1, 9, 39]. In addition, glycine amino acid is a reduced form of N that in leaves can directly be assimilated to and accelerate protein biosynthesis [18]. Moreover, higher leaf NR activity in soil applied glycine treatment, but not in foliar applications could be due to better adaptive mechanisms of roots for glycine uptake, assimilation and its translocation in plants. Glycine at high concentration of 0.01% has been shown to induce phytotoxicity on leaves of some plant species [32]. Similar results of toxicity and leaf necrosis were also observed in this study under 1000 ppm spray. Based on plant species and physiological status, glycine probably acts as a proton donor resulting in cell hyper-acidification, quite similar to ammonium spray toxicity [32]. Similarly, exogenous application of glycine at 0, 5, 10 and 20 mM in *Chorispora bungeana* showed that 10 mM was best to improve plant growth trait of leaf biochemical reactions, while application of 20 mM glycine showed an opposite effect [40]. Lettuce growth was also improved by applying

L-methionine at the lowest concentrations of 0.2 mg/L rather than higher concentrations [14].

The ANOVA for number of fruits and total yield in plant was significant at $P=0.01$ (Table 4). The plant fruit number was significantly increased by NPK, soil application of glycine at both levels and foliar spray of glycine at 500 ppm compared to control plants. The plant fruit yield was significantly increased by NPK as well as all glycine treatments except foliar application of 1000 ppm glycine, compared to control plants. The highest and lowest plant fruit number or fruit yield was in soil application of 500 mg glycine and in control plants, respectively. Application of glycine and/or other amino acids increased the plant yield in different studies [5, 11, 14, 22, 24, 37]. In our study, both soil and foliar application of glycine were able to increase cucumber plant yield under the limed soil conditions. Optimization of leaf photosynthesis efficiency probably was the key behind higher cucumber yield under glycine application [39, 40], as higher leaf area, leaf SPAD value and leaf water content (data not shown) were observed under glycine application. Extended leaf area, higher chlorophyll, enzymes activity and vitamin C content that were observed in this study are good indicator for improved leaf photosynthesis [18]. Higher soil mineral bioavailability, enhanced root uptake, translocation and distribution, higher leaf protein, osmolite and antioxidant contents, and prolonged economic life of plants play key roles in optimization of photosynthesis and higher yield production in this study [32].

The ANOVA for fruit firmness was significant at $P=0.05$, and for fruit vitamin C concentration was significant at $P=0.01$, and it was not significant for fruit TSS (Table 4). Fruit vitamin C was increased by all treatments; however, the increase by two foliar treatments of 250 and 1000 ppm was not statistically significant. On the other hand, fruit firmness was significantly increased

Table 4 Cucumber plant yield and some quality traits of fruit under different treatments

Treatments	Number of fruits/plant	Total plant yield (kg)	Fruit firmness (kg/cm ²)	Fruit vitamin C (mg/100 g FW)	Fruit TSS (%)
Control	32 ± 7d	2.6 ± 0.08e	12.3 ± 1.0bc	3.5 ± 0.5c	7.0 ± 0.3a
NPK fertilizer	70 ± 11ab	4.9 ± 0.61ab	15.6 ± 1.5a	5.9 ± 1.1a	7.6 ± 0.4a
Soil-glycine (250 mg/plant)	64 ± 9ab	4.6 ± 0.59ab	14.1 ± 1.3abc	5.3 ± 0.9ab	7.3 ± 0.5a
Soil-glycine (500 mg/plant)	74 ± 10a	5.3 ± 0.53a	15.1 ± 1.1a	6.0 ± 1.0a	7.2 ± 0.3a
Foliar-glycine (250 ppm)	45 ± 7 cd	3.5 ± 0.45cd	13.6 ± 1.4abc	4.2 ± 0.8bc	7.2 ± 0.4a
Foliar-glycine (500 ppm)	58 ± 8bc	4.2 ± 0.60bc	14.2 ± 2.1abc	5.8 ± 0.9a	7.3 ± 0.6a
Foliar-glycine (1000 ppm)	41 ± 7d	3.0 ± 0.51de	12.0 ± 1.4c	4.3 ± 0.7bc	7.1 ± 0.3a
ANOVA*	P -value = 0.01	P -value = 0.01	P -value = 0.05	P -value = 0.01	P -value = ns

Data are average of four replications ± SD, and in each column means with similar letter are not significantly different at 5% level of LSD test

* P -value levels of 0.01, 0.05 and ns mean significant effect at 1%, 5% and no significant effect, respectively

only by NPK and 500 mg/plant glycine treatments compared to control (Table 4). This effect might be due to in general improvement in plant physiological status including photosynthesis optimization and better plant water status induced by soil (at both levels) and foliar (at 500 ppm) application of glycine [32], while other treatments (foliar 250 ppm) were not fully effective in this regard or were stressful at all (foliar 1000 ppm). Vitamin C despite being a secondary metabolite and involved in defensive reactions of plants; however, under the major environmental constrains such as drought and salinity its concentration in plant tissues is diminished [16]. Similarly, soil application of glycine at 300 and 600 mg/kg soil increased soluble solids (TSS) and vitamin C of coriander leaves than control plants [20]. Nutrient bioavailability has been shown to affect fruit quality in many horticultural crops [12, 18]. The improvement in crop quality due to application of amino acids has been shown [5, 6, 29]. Leaf vitamin C in coriander plants was increased by foliar and especially by soil application of glycine [20]. Soil application of an amino chelate resulted in significantly higher fruit or pod vitamin C and TSS content in three vegetables of cucumber, tomato and green bean versus their control plants [34]. Increase in fruit firmness could be a result of amino acid enrichment, higher mineral, protein and sugar concentrations [13, 34].

In the present study, soil application of glycine was more effective to stimulate the growth, yield and quality of cucumber plants rather than foliar spray. In a study, foliar application of glycine and glutamine had no significant increase in leaf mineral concentrations except for iron, in which 1000 mg/L glycine spray resulted in significantly higher leaf Fe concentration compared with the control plants [2]. Amino acids have various roles in plant metabolism, and exogenous application of amino acids can have beneficial and stimulatory effects on plant growth and quality [10, 30, 33]. It has been shown that amino acids such as glutamate, cysteine, phenylalanine, and glycine can do a signaling job, since small doses are enough to increase the activity of the antioxidant enzymes [36]. By far, glycine is the most and widely used amino acid in plant nutrition (as biostimulant) or in the production of amino chelate fertilizers [32]. Plants can simply absorb glycine by their roots or their leaves, despite their uptake kinetics and efficiency is influenced by various factors including plant N status, stress conditions and light intensity [17, 32]. Moreover, application of reduced form of nitrogen such as amino acids may serve as an excellent energy source for soil microorganisms, and could play role for improving soil life and microorganisms activity.

Amino acids are well-known biostimulants that can have positive effects on plant growth and yield, and

significantly mitigate the injuries caused by abiotic stresses [26]. It has been shown that exogenous spray of glycine betaine reduced membrane permeability, lipid peroxidation, leaf SPAD value and H_2O_2 content in salt-stressed lettuce plants. Glycine betaine reduced Na accumulation, but significantly increased other element contents under salinity conditions [41]. Foliar application of zinc lysine under Cd treatment, significantly increased the photosynthesis, grain yield, enzyme activities and Zn contents in different plant tissues of wheat [24]. In addition, in a nutrient solution experiment, foliar application of methionine-complexed manganese 4 days before inoculation significantly reduced powdery mildew severity on cucumber plants compared to control and mineral Mn application. The foliar application of Mn–amino acid complexes before inoculation resulted in significant increase of leaf cell wall lignin content [7].

Conclusion

In the present study, besides chemical NPK fertilization, the soil and foliar applications of glycine significantly increased cucumber economic life, leaf area, leaf protein, shoot dry weigh, and plant yield and quality compared to control plants. However, the vegetative, yield and quality responses of cucumber were much better to soil rather than foliar application of glycine. Moreover, soil application of 500 mg/plant glycine was superior to application of 250 mg, as the soil application of 500 mg glycine was able to record the same or better than NPK fertilization regarding many traits. This could have significant implications for crop production and in the reduction of fertilization and the associated salinity pressures on agricultural soil particularly in greenhouses. Nowadays cucumber genotypes for greenhouse cultivation have generally high mineral and fertilization demand to produce optimum growth and high-quality yields. On the other hand, safe production of cucumbers is quite important with regard to consumer's health, and application of biostimulants such as glycine amino acid, as a full or partial replacement of chemical fertilization as shown in this study, can benefit the soil, plant and human health issues.

Abbreviations

NPK: Nitrogen, phosphorus, potassium; SPAD: The soil and plant analysis development; ppm: Parts per million; TSS: Total soluble solids; NR: Nitrate reductase.

Acknowledgements

We give our thanks to M. Moghadamyar for his technical assistance during this research.

Authors' contributions

MKS, MRH and SKJ designed the experiment; FZS conducted the experiment and did the laboratory measurements; MKS and FZS analyzed the data; FZS wrote the paper. All authors read and approved the final manuscript.

Funding

Not applicable.

Availability of data and materials

Not applicable.

Ethics approval and consent to participate

All authors listed have contributed significantly to the work and agree to be in the author list.

Consent for publication

All authors agree to publish the work.

Competing interests

The authors declare no competing interest.

Author details

¹ Dept. of Horticulture, Science and Research Branch, Islamic Azad University, Tehran, Iran. ² Dept. of Horticultural Sciences, Tarbiat Modares University, Tehran, Iran. ³ Dept. of Horticultural Sciences, University of Tehran, Karaj, Iran.

Received: 26 November 2019 Accepted: 25 April 2020

Published online: 15 October 2020

References

- Abdul-Qados AMS. Effect of arginine on growth, yield and chemical constituents of wheat grown under salinity condition. *Acad J Plant Sci.* 2009;2:267–78.
- Aghaye Noroozlo A, Souri MK, Delshad M. Stimulation effects of foliar applied glycine and glutamine amino acids on lettuce growth. *Open Agric.* 2019;4(1):164–72.
- Amin AA, Gharib FAE, El-Awadia M, Rashad ESM. Physiological response of onion plants to foliar application of putrescine and glutamine. *Scientia Hortic.* 2011;129:353–60.
- Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem.* 1976;72(1–2):248–54.
- Cao JX, Peng ZP, Huang JC, Yu JH, Li WN, Yang LX, Lin ZJ. Effect of foliar application of amino acid on yield and quality of flowering Chinese cabbage. *Chin Agric Sci Bull.* 2010;26:162–5.
- El Sayed OM, El Gammal OHM, Salama ASM. Effect of proline and tryptophan amino acids on yield and fruit quality of Manfalouty pomegranate variety. *Scientia Hortic.* 2014;169:1–5.
- Eskandari S, Khoshgoftarmansh AH, Sharifnabi B. The effect of foliar-applied manganese in mineral and complex forms with amino acids on certain defense mechanisms of cucumber (*Cucumis sativus* L.) against powdery mildew. *Plant Growth Regul.* 2018;37(2):481–90.
- Faten SA, Shaheen AM, Ahmed AA, Mahmoud AR. Effect of foliar application of amino acids as antioxidants on growth, yield and characteristics of Squash. *Res J Agric Biol Sci.* 2010;6(5):583–8.
- García AL, Madrid R, Gimeno V, Rodríguez-Ortega WM, Nicolas M, García-Sánchez F. The effects of amino acids fertilization incorporated to the nutrient solution on mineral composition and growth in tomato seedlings. *Spanish J Agric Res.* 2011;9(3):852–61.
- Ghasemi S, Khoshgoftarmansh AH, Afyuni M, Hadadzadeh H. Iron (II)-amino acid chelates alleviate salt-stress induced oxidative damages on tomato grown in nutrient solution culture. *Scientia Hortic.* 2014;165:91–8.
- Ghonaime AA, El-Bassiouny AM, Abdel-Mawgoud AMR, El-Tohamy WA, Gruda N. Growth, yield and blossom-end rot incidence in bell pepper as affected by phosphorus level and amino acid applications. *Gesunde Pflanzen.* 2012;64:29–37.
- Huang Y, Tang R, Cao Q, Bie Z. Improving the fruit yield and quality of cucumber by grafting onto the salt tolerant rootstock under NaCl stress. *Scientia Hortic.* 2009;122(1):26–31.
- Keutgen A, Pawelzik E. Contribution of amino acids to strawberry fruit quality and their relevance as stress indicators under NaCl salinity. *Food Chem.* 2008;111:642–7.
- Khan S, Yu H, Li Q, Gao Y, Sallam BN, Wang H, Liu P, Jiang W. Exogenous application of amino acids improves the growth and yield of lettuce by enhancing photosynthetic assimilation and nutrient availability. *Agronomy.* 2019;9(5):266.
- Koukounaras A, Tsouvaltzis P, Siomos AS. Effect of root and foliar application of amino acids on the growth and yield of greenhouse tomato in different fertilization levels. *J Food Agric Environ.* 2013;11(2):644–8.
- Kurepin LV, Ivanov AG, Zaman M, Pharis RP, Hurry V, Hüner NP. Interaction of glycine betaine and plant hormones: Protection of the photosynthetic apparatus during abiotic stress. *Photosynthesis: Structures, mechanisms, and applications.* Springer: Cham; 2017. p. 185–202.
- Ma Q, Cao X, Wu L, Mi W, Feng Y. Light intensity affects the uptake and metabolism of glycine by pakchoi (*Brassica chinensis* L.). *Sci Rep.* 2016;6:21200.
- Marschner P. Marschner's mineral nutrition of higher plants. 3rd ed. London: Academic Press, Elsevier; 2011.
- Mohammadi P, Khoshgoftarmansh AH. The effectiveness of synthetic zinc (Zn)-amino chelates in supplying Zn and alleviating salt-induced damages on hydroponically grown lettuce. *Scientia Hortic.* 2014;172:117–23.
- Mohammadipour N, Souri MK. Beneficial effects of glycine on growth and leaf nutrient concentrations of coriander (*Coriandrum sativum*) plants. *J Plant Nutr.* 2019;42:1–8.
- Mohammadipour N, Souri MK. Effects of different levels of glycine in the nutrient solution on the growth, nutrient composition, and antioxidant activity of coriander (*Coriandrum sativum* L.). *Acta Agrobotanica.* 2019. <https://doi.org/10.5586/aa.1759>.
- Osman HS. Enhancing antioxidant–yield relationship of pea plant under drought at different growth stages by exogenously applied glycine betaine and proline. *Ann Agric Sci.* 2015;60(2):389–402.
- Pérez-Jiménez M, Pazos-Navarro M, López-Marín J, Gálvez A, Varó P, del Amor FM. Foliar application of plant growth regulators changes the nutrient composition of sweet pepper (*Capsicum annum* L.). *Scientia Hortic.* 2015;194:188–93.
- Rizwan M, Ali S, Hussain A, Ali Q, Shakoor MB, Zia-ur-Rehman M, Farid M, Asma M. Effect of zinc-lysine on growth, yield and cadmium uptake in wheat (*Triticum aestivum* L.) and health risk assessment. *Chemosphere.* 2017;187:35–42.
- Ruiz JM, Romero L. Commercial yield and quality of fruits of cucumber plants cultivated under greenhouse conditions: response to increases in nitrogen fertilization. *J. Agric Food Chem.* 1998;46(10):4171–3.
- Sadak M, Abdoelhamid MT, Schmidhalter U. Effect of foliar application of amino acids on plant yield and some physiological parameters in bean plants irrigated with sea water. *Acta Biol Colomb.* 2015;20(1):141–52.
- Saeedi R, Etemadi N, Nikbakht A. Calcium chelated with amino acids improves quality and postharvest life of lisanthus (*Eustoma grandiflorum* cv. Cinderella Lime). *HortScience.* 2015;50:1394–8.
- Sánchez AS, Juárez M, Sánchez-Andreu J, Jordá J, Bermúdez D. Use of humic substances and amino acids to enhance iron availability for tomato plants from applications of the chelate FeEDDHA. *J Plant Nutr.* 2005;28:1877–86.
- Shaheen AM, Rizk FA, Habib HAM, Abdel Baky M. Nitrogen soil dressing and foliar spraying by sugar and amino acids as affected the growth, yield and its quality of onion plant. *J Am Sci.* 2010;6(8):420–7.
- Sharma SS, Dietz KJ. The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. *J Exp Bot.* 2006;57(4):711–26.
- Souri MK. Amino chelate fertilizers: the new approach to the old problem; a review. *Open Agric* 2016;1:118–123.
- Souri MK, Hatamian M. Amino chelates in plant nutrition: a review. *J Plant Nutr.* 2019;42(1):67–78.
- Souri MK, Najji M, Aslani M. Effect of Fe-glycine amino chelate on pod quality and iron concentrations of bean (*Phaseolus vulgaris* L.) under lime soil conditions. *Commun Soil Sci Plant Anal.* 2018;49(2):215–24.
- Souri MK, Yaghoobi Sooraki F, Moghadamyar M. Growth and quality of cucumber, tomato, and green bean under foliar and soil applications of an amino chelate fertilizer. *Hortic Environ Biotechnol.* 2017;58(6):530–6.

35. Teixeira WF, Fagan EB, Soares LH, Soares JN, Reichardt K, Neto DD. Seed and foliar application of amino acids improve variables of nitrogen metabolism and productivity in soybean crop. *Front Plant Sci.* 2018;9:396.
36. Teixeira WF, Fagan EB, Soares LH, Umburanas RC, Reichardt K, Neto DD. Foliar and seed application of amino acids affects the antioxidant metabolism of the soybean crop. *Front Plant Sci.* 2017;8:327.
37. Tejada M, Rodríguez-Morgado B, Paneque P, Parrado J. Effects of foliar fertilization of a biostimulant obtained from chicken feathers on maize yield. *Eur J Agron.* 2018;96:54–9.
38. Tian Y, Wang Q, Zhang W, Gao L. Reducing environmental risk of excessively fertilized soils and improving cucumber growth by *Caragana microphylla*-straw compost application in long-term continuous cropping systems. *Sci Total Environ.* 2016;544:251–61.
39. Xiaochuang C, Chu Z, Lianfeng Z, Junhua Z, Hussain S, Lianghuan W, Qianyu J. Glycine increases cold tolerance in rice via the regulation of N uptake, physiological characteristics, and photosynthesis. *Plant Physiol Biochem.* 2017;112:251–60.
40. Yang N, Wang CL, He WP, Qu YZ, Li YS. Photosynthetic characteristics and effects of exogenous glycine of *Chorispora bungeana* under drought stress. *Photosynthetica.* 2016;54(3):459–67.
41. Yildirim E, Ekinci M, Turan M, Dursun A, Kul R, Parlakova F. Roles of glycine betaine in mitigating deleterious effect of salt stress on lettuce (*Lactuca sativa* Lzhau). *Arch Agron Soil Sci.* 2015;61(12):1673–89.
42. Zeid IM. Effect of arginine and urea on polyamines content and growth of bean under salinity stress. *Acta Physiologiae Plantarum* 2009;31(1):65–70. <https://doi.org/10.1007/s11738-008-0201-3>.
43. Zhang S, Hu F, Li H. Effects of earthworm mucus and amino acids on cadmium subcellular distribution and chemical forms in tomato seedlings. *Bioresour Technol.* 2009;100:4041–6. <https://doi.org/10.1007/s11104-007-9206-8>.
44. Zhang HX, Chi DC, Qun W, Jun F, Fang XY. Yield and quality response of cucumber to irrigation and nitrogen fertilization under subsurface drip irrigation in solar greenhouse. *Agric Sci China.* 2011;10(6):921–30.
45. Zhang J, Wang J, Zhong W, Cai Z. Organic nitrogen stimulates the heterotrophic nitrification rate in an acidic forest soil. *Soil Biol Biochem.* 2015;80:293–5.
46. Zhou Z, Zhou J, Li R, Wang H, Wang J. Effect of exogenous amino acids on Cu uptake and translocation in maize seedlings. *Plant Soil.* 2007;292:105–17.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.