DOI: 10.29050/harranziraat.1176239
Harran Tarım ve Gıda Bilimleri Derg. 2023, 27(1): 1-14

Research Article/Araştırma Makalesi

Effects of glutamic acid applications on the yield and growth parameters in garlic (*Allium sativum* L.) cultivation

Glutamik asit uygulamalarının sarımsak (Allium sativum L.) yetiştiriciliğinde verim ve büyüme parametreleri üzerine etkisi

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To cite this article:
Turfan, N. & Turan, B. (2023). Effects of glutamic acid applications on the yield and growth parameters in garlic (*Allium sativum* L.) cultivation. Harran Tarım ve Gıda Bilimleri Dergisi, 27(1): 1-14. DOI:10.29050/harranziraat.1176239

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Received Date:
16.09.2022
Accepted Date:
06.03.2023

ABSTRACT

Since exogenous amino acid applications to agricultural plants provide ready mineral nutrition for plants, they are widely used to enhance the yield and quality of crops as well as to strengthen resistance to environmental impacts. In the present study, the effects of L-Glutamic acid (GLU) (100, 200, 400, 800, and 1000 mg L^{-1}) treatments on the growth parameters, bulb yield, chemical contents, and weight loss of Taşköprü garlic were analyzed. Given the findings, 800 GLU dose was found to have the highest values in shoot length and width, fresh weight, width, and length of the bulb, length and of large and small cloves, and yield per plot. On the other hand, the lowest results in total free amino acid, nitrate, polyphenol, ascorbic acid, and % ash amounts were obtained with 400 GLU, while the maximum leaf length and width was obtained with 1000 GLU dose. The first and third weight losses were obtained in 100 GLU and control groups and the second weight loss value obtained in all groups were higher than in the control. Considering all the data, the doses of 800 GLU and 400 GLU yielded the most positive effect on growth parameters, bulb yield, and weight loss measurements in garlic, whereas the lowest level of effect was observed in the control and 100 GLU groups. In conclusion, it can be stated that the foliar application of L-Glutamic acid in garlic cultivation might increase the yield and quality.

Key Words: Garlic, Glutamic acid, Growth parameters, Yield

ÖZ

Tarım bitkilerine ekzojen amino asit uygulamaları bitkiler için hazır mineral beslenme kaynakları sağladığı için mahsullerin verimini ve kalitesini artırmanın yanı sıra çevresel etkilere karşı direnci artırmak için yaygın olarak kullanılmaktadır. Bu çalışmada L-Glutamik asit (100, 200, 400, 800 ve 1000 mg L^{-1}) uygulamalarının Taşköprü sarımsağında büyüme parametreleri, baş verimi, kimyasal içerik ve ağırlık kaybı üzerine etkileri araştırılmıştır. Bulgulara göre sürgün uzunluğu ve çapı, baş taze ağırlık, boy ve çapları, büyük ve küçük diş boy ve çapları, parsel verimi 800 GLU uygulamalarında en yüksek değerdedir. Buna karşın toplam serbest amino asit, nitrat, polifenol, askorbik asit ve % kül miktarları 400 GLU dozlarında ve yaprak boyu ve eni ise 1000 GLU uygulamalarında maksimum seviyededir. Örneklerde ilk ve üçüncü ağırlık kaybı 100 GLU ve kontrol grubunda yüksek iken ikinci ağırlık kaybı değerleri ise tüm uygulama gruplarında kontrole göre yüksektir. Tüm veriler göz önünde bulundurulduğunda 800 GLU ve 400 GLU dozları sarımsakta büyüme parametreleri, baş verimi ve ağırlık kaybı ölçümlerinde en olumlu etkiyi gösterirken, en düşük etki ise kontrol grup ve 100 GLU dozlarına aittir. Sonuç olarak, sarımsak yetiştiriciliğinde yapraktan L-Glutamik asit uygulamaları yapılarak verim ve kalitenin arttırılması sağlanabilir.

Anahtar Kelimeler: Sarımsak, Glutamik asit, Büyüme parametreleri, Verim

Introduction

Being one of the earliest cultivated crops, garlic (*Allium sativum* L.) having a specific aroma, taste, and high nutritional value has been used in kitchens for a long time. Moreover, besides being used in the treatment of diseases thanks to its antimicrobial and antioxidant properties, garlic also became popular as a food additive, as well as in cosmetics and agricultural chemicals. In the literature, it was reported that 1 bulb of garlic contained water by 84.09%, organic matter by 13.38%, and inorganic matter by 1.53% (Ulianych et al, 2020; Akan and Güneş, 2021). Its chemical composition includes sulfuric compounds, amino acids, phenolic compounds, vitamins, enzymes, and minerals. Besides being consumed fresh (green), it is consumed widely in dried form because of its ease of storage and long shelf-life and dry form has higher economic importance (Akan, 2019; Turfan, 2022).

Garlic is an economically important vegetable in Turkey. In the year 2020, the area of garlic growing in Turkey was reported to be 12.700 ha and the production of dried garlic has increased by 13.5% in comparison to the previous year. The provinces having the largest cultivation areas were Kastamonu (25.6 thousand da), Gaziantep (20.4 thousand da), and Kahramanmaraş (13.7 thousand da) (TUIK, 2021). Garlic production in Turkey is generally based on dry garlic production. Regarding dried garlic production, Kastamonu has the highest amount of production (23.000 tons), followed by Gaziantep (22.000 tons) and Kahramanmaraş (14.000 tons) (TUIK, 2021). Turkey playing a role as both importer and exporter is the 14^{th}-largest garlic producer in the world (TUIK, 2021). For this reason, garlic is an important source of revenue for both farmers and agricultural businesses in the regions, where the level of garlic growing is high. However, in recent years, the demand for Chinese garlic increased because of the low prices and it causes a problem for the local farmers and lowers their competitive advantage, besides causing problems such as abandonment the domestic gene sources that have adapted to the region. Moreover, the problems related with the climatic and soil conditions in the garlic growing regions decrease the yield and quality of garlic (Turan et al., 2013; Turfan, 2021). From this aspect, the studies on the use of amino acid-based compounds on leaves or roots in garlic growing draw attention (Hegazi et al., 2016). Plants intake the nitrogen from the soil in inorganic (NH_4^+: ammonium; NO_3^-: nitrate) form and, while NH_4^+ is directly involved in the structure of organic compounds, NO_3 is reduced into ammonium form first (Kuşvuran et al., 2019). Researchers reported that, in comparison to NH_4^+, three times more energy is consumed during the assimilation of NO_3 (Cheng et al., 2016; Zhang et al., 2019). Hence, exogenous amino acid treatments might offer advantages in increasing the plant yield and stimulating the resistance to stress (Hildebrandt, 2018; Haghighia et al., 2020; Bakır et al., 2022). Since glutamic acid is an important ammonium recipient, it transforms into other amino acids such as proline, glycine, and arginine, as well as playing important roles in chlorophyll synthesis, activation of vegetal hormones, cell division, and phytochelatin activities (Sun et al., 2019; Ren et al., 2022). In previous studies carried out on the solutions for problems affecting the quality and yield in garlic cultivation in Turkey, besides the chemical or mineral treatments and organic-inorganic fertilizer applications in soil, also the practices such as planting depth/distance and planting season are widely employed (Turan et al., 2013; İkinci et al, 2021; Bakır et al., 2022; Kibar, 2022). However, there is no comprehensive study examining the effects of amino acid derivatives on the yield, chemical content, and shelf-life of garlic bulbs. The main objectives of this study were to reveal, firstly, the effects of foliar glutamic acid applications on growth parameters and bulb yield, secondly, the chemical compounds of cloves, and finally, the weight changes in the garlic bulbs during the storage period.

Material and Methods

The present study was carried out in an agricultural area in Yunuslu Village (Ereğli district of Zonguldak province) between 21st March 2021 and 16th July 2021 by using samples of Taşköprü garlic. Having an altitude of 1.043 m^2, this village is located at the altitude of 41.238934 and the longitude of 31.564613. The region is dominated by the typical Black Sea climate (warm and mild). Having a high level of precipitation, the annual mean precipitation in the district is 1.163 kg.

Table 1. Mean monthly climatic parameters between garlic planting and harvesting in the study area

Months	°C			mm	%
	Mean	Maximum	Minimum	Mean precipitation	Humidity
February	6.2	9.0	3.4	87	78
March	8.3	11.2	5.1	92	75
April	11.8	14.7	8.3	63	76
May	16.2	18.9	12.7	59	79
June	20.3	22.8	16.9	67	79
July	22.7	25.3	19.3	52	79

Table 2. Soil characteristics of the study area

		dS m^{-1}	%	mg kg^{-1}						
	pH	EC	Organic matter	K	Ca	Mg	Fe	Mn	Zn	Cu
Soil	6.55	0.452	3.14	34748	23755	2118	62.501	159,875	35.865	23.372

The annual mean temperature is 14.1°C; the lowest value is 6 °C (January-February) and the highest one is 21 °C (July). Also, the highest temperature was recorded as 26 °C (July) and the lowest temperature was recorded as 3.4 °C (February). The climatic data of the study area are presented in Table 1 (Anonymous,2021). The soil characteristics determined in the study area are shown in Table 2. Given the results, the soil was found to have a pH of 6.55, an electrical conductivity of 0.452, organic matter content of 3.14 (%), whereas the mineral composition was found to be as follows; K: 34748 mg kg^{-1}, Ca: 23755 mg kg^{-1}, Mg: 2118 mg kg^{-1}, Fe: 62.501 mg kg^{-1}, Mn: 159. 875 mg kg^{-1}, Zn: 35. 865 mg kg^{-1}, and Cu: 23.372 mg kg^{-1}.

Material

In this study, Taşköprü garlic was used as plant material and its bulbs were obtained from Taşköprü district of Kastamonu province in northern Turkey.

Method

Preparation of the experiment area

The experiments were established in a field that had not been cultivated and fertilized before. The study area was hoed manually and cleaned using a rake. No fertilizer was applied before the planting or during the harvesting season and the cultivation was conducted organically. The experiment area was established with three repeats by using randomized blocks experimental design (RBD). Firstly, the plots were assigned to the groups as control (0), 100 GLU (100 mg L^{-1} L-glutamic acid), 200 GLU (200 mg L^{-1} L-glutamic acid), 400 GLU (400 mg L^{-1} L-glutamic acid), 800 GLU (800 mg L^{-1} L-glutamic acid), and 1000 GLU (1000 mg L^{-1} L-glutamic acid), respectively. Before planting the garlic cloves in the soil, soil samples were taken from the top soil layer (20 cm) in different parts of the land and some physical and chemical properties of the soil were determined by soil analysis. For each application group, plots were arranged as 1 m^2 (1 m x 1 m), and and a space of 50 cm (1 m) has been left between the plots. Then, uniform-sized garlic cloves were planted in 2 rows with 30 cm spacing between rows and 10 cm spacing between rows. The sowing of cloves was done in such a way that the ends of the cloves were visible on the soil surface (Vural et al., 2000). After planting, the parcels were given the first irrigation using a springer irrigation system. L-GLU were dissolved in pure water at determined concentrations. L-glutamic acid treatments were given after the 4th or 5th leaves (24 April) on leaves. Treatments were repeated twice a week for 4 weeks. L-Glutamic acid (GLU: Sigma-Aldrich, 128430) was applied by dissolving in pure water, whereas the control group plants were irrigated wetted by a sprayer using only pure water. Plants were irrigated three times until the harvest by using a springer irrigation system. Manual weeding was performed three times to clear weeds during the growing period. Moreover, no plant protection product was used during the experiment. Shoot height and width and leaf (width, length, and number per plant) measurements were performed after the treatments.

Harvesting

The mature garlic bulbs were harvested when the leaves turn completely yellow and dry (16th July 2021). The plants taken off of the soil were left to dry for 2 weeks on cardboard in the shade (Francois, 1994). Then, garlic bulbs were transported to the laboratory in bags. These samples were used to measure garlic bulb yield (g bulb^{-1}), chemical analysis, and weight loss.

Sample preparation and storage conditions

In the present study, different doses of L-glutamic acid applications (C, 100-GLU, 200-GLU, 400-GLU, 800-GLU and 1000-GLU), storage temperatures (5 ±2 °C - 23±2 °C), and storage durations (1 Agu 2020-03 Feb 2021) were compared. In determining the weight changes, the first weights (**I**: 1 Agu 2020) of 15 samples taken from each group were measured, and the garlic samples were then put into a paper bag according to the determined concentrations.

Table 3. The monthly average temperature and humidity amounts recorded during the storage period of the garlic samples

	2020					2021	
	August	September	October	November	December	January	February
Mean Temperature (°C)	20-23	20-22	18-20	14-16	10-12	6-10	5-10
Humidity (%)	53	56	62	67	73	74	70

The samples were then kept at a temperature between 5 and 23 °C without direct sunlight. The monthly average temperature and humidity (%) values determined (by the 4IN1 brand device) during the storage period of the garlic samples are given in Table 3. The second weight (**II**) measurements were performed on 01 Nov 2020 and the third measurements (**III**) were performed on 03 Feb 2021. Weight loss was calculated using Eq. (1) given below (Hosseini et al., 2018).

$$\text{Weight loss} = \frac{\text{weight at the beginning of storage} - \text{weight at the time of storage}}{\text{weight at the beginning of storage}} \times 100 \qquad (1)$$

Determination of the growth rate parameters of leaf

Regarding the plant height (cm), the heights of garlic from the ground surface to the top point of the plants were measured using a measurement stick, whereas the stem width (mm) was measured at a point slightly above the ground by using a caliper. The number of leaves was determined by counting the number of leaves per plant, whereas length (cm) was measured as the distance from the leaf node to the tip of the leaf, and width (mm) was measured as the width of garlic leaves.

Determination of the yield parameters of bulbs

The weights of bulbs and cloves were determined using a precision scale, whereas width and height measurements were performed using a digital caliper. The number of cloves per bulb (number/bulb) was determined by counting all the cloves in a bulb after peeling them, whereas clove weight was determined by individually weighing the cloves using a precision scale. Bulb length and width (mm) of bulbs separated from harvested plants were determined using a caliper. Using a caliper, the clove length (mm) was determined by measuring longitudinally and the width (mm) was measured at the midpoint. Morphological measurements were carried out with 20 garlic seedlings, while bulb yield was carried out using 20 garlic bulbs.

Determination of the ash content of bulbs

Dry matter (%) was determined by drying the garlic samples in a drying oven at 65 °C until reaching a stable weight. Dried samples (~25-30 g) were put into a tared crucible and combusted in an ash furnace at 525±25 °C until becoming white ash. The %ash was calculated using the % ash formula (Marshall, 2010).

pH measurements and elemental analysis of soil

pH values of samples were determined using the method introduced by Gülçur (1974). The samples were kept in 1/2.5 pure water for 24 hours and the pH was measured by using a digital pH-meter. Soil samples were dried in the laboratory in the shade. Then, it was used in the elemental analysis at Kastamonu University Central Research Laboratory by using the SPECTRO brand XEPOS model XRF device.

Chemical analyses

The total free amino acid content of samples was measured following the Spies (1957) method, while the ascorbic acid content of the samples was measured using ascorbic acid as standard, with some modifications. The samples (1 g) were homogenized with 4 ml oxalic acid (1%) and filtered. Then, polyvinylpolypyrrolidone (PVPP) (100 g) was added to 2.5 ml of the filtered, and 2-3 drops of H_2SO_4 (25% sulfuric acid) were dropped to reduce the pH to below 1. The absorbance of the samples was recorded at 254 nm. Results were expressed as mg ascorbic acid 100 g^{-1} fresh weight (Pantelidis et al., 2007). The total polyphenol levels of garlic samples were performed according to the method introduced by Folin and Denis (1915). Total polyphenols were calculated with the help of std. the curve of 0.1mg mL^{-1} tannic acid and expressed as g 100g^{-1} dry weight. Nitrate content was measured colorimetrically (Cataldo et al., 1975). Fresh frozen tissue (2.0 g) was soaked in a tube with 10 mL distilled water and placed in boiling water for 30 min. The extract was filtered into a 25 mL volumetric flask and distilled water was added. Then, 9.5 mL NaOH (8% Sodium hydroxide) and 0.4 mL salicylic and sulfuric acid (5%) were added into 0.1 mL extracting solution. The absorbance of the mixture was detected at 410 nm by a UV spectrophotometer.

Statistical analysis

One-way ANOVA (Analysis of variance) was conducted to analyze the differences in the growth parameters, bulb yield, chemical constituents of cloves, and weight loss of bulb samples of garlic grown in an open field. The statistical analysis was performed using the SPSS program (Version 11 for Windows). Following the results of ANOVAs, Tukey's honestly significant difference (HSD) test (α = 0.05 was used for significance.

Results and Discussion

Effects of L-GLU treatments on the growth parameters of garlic

The data about the effects of L-Glutamic acid (GLU) treatments on garlic's shoot length (the plant height: the heights of garlic from the ground surface to the top point of the plants), stem width (for shoots), leaf length, width, and the number of leaves per plant are presented in Table 4. Given the data, the parameters being examined varied significantly by the GLU doses ($p<0.05$). Shoot length was found to be at the highest level with GLU doses of 800 and 400 (50.80 cm, 49.74 cm), whereas it was at the lowest level in the control group (37.34 cm). On the other hand, there was an increase in stem width at high GLU doses compared to other applications. Leaf lengths of seedlings treated with GLU were longer than the control, but the change was not at a very high level. Leaf width was large in all the GLU doses; however, the values found especially in 800 GLU and 400 GLU doses were higher than the control by 13.28% and 14.06% (Table 4). Leaf number per plant was found to be high in all the treatment doses in comparison to the control group but the highest levels of difference were found in 800 GLU (50.50%), 400 GLU (42.83%), and 200 GLU (42.26%) (Table 4). Exogenous amino acids and their combinations were reported to have positive effects of on many plant species.

Table 4. Effects of L-GLU treatments on the growth parameters of garlic seedling (100 GLU: 100 mg L^{-1} L-Glutamic acid, 200 GLU: 200 mg L^{-1} L-Glutamic acid; 400 GLU: 400 mg L^{-1} L-Glutamic acid; 800 GLU: 800 mg L^{-1} L-Glutamic acid; 1000 GLU: 1000 mg L^{-1} L-Glutamic acid; ± Standard errors experiments)

Groups	**Shoot (a whole plant)**		**Leaf**		
	Length (cm)	**Width (mm)**	**Length (cm)**	**Width (mm)**	**Number/Plant**
Control	37.34±1.10d*	8.27±0.39c	9.20±0.15c	38.4±0.84b	12.14±0.66c
100 GLU	40.80±1.11c	11.60±0.54bc	10.14±0.24b	39.05±1.10b	15.74±0.52b
200 GLU	44.47±1.23b	12.34±0.53b	11.34±0.42b	41.87±1.14bc	17.2±0.89a
400 GLU	49.74±1.10a	12.67±0.59b	10.67±0.31b	42.6±1.28a	17.34±0.64a
800 GLU	50.80±1.20a	13.74±0.57a	10.80±0.28b	43.54±1.30a	18.27±0.58a
1000 GLU	42.14±0.90bc	12.74±0.57b	11.40±0.44a	43.80±1.39a	17.27±0.84
F	22.361	12.934	6.651	3.738	9.961
Sig.	<0.001	<0.001	<0.001	0.004	<0.001

*: Means indicated with different letters within same column are significantly different (P < 0.05)

Previous studies examining glutamic acid for chive (Cao et al., 2010), arginine and glutamine for onion (Shafeek et al., 2012), glutamine for rice (Kan et al., 2017), several amino acid-based bio-stimulants for garlic (Shalaby and El-Ramady, 2014; İkinci et al. 2021), amino-chelated fertilized for squash, tomato, and bean (Souri et al., 2017), arginine and glutamine for mallow (Greenwell and Ruter, 2018), glycine and glutamine for lettuce (Aghaye Noroozlo et al., 2019), and urea for garlic (Sitaula et al., 2020**)** reported increases in shoot length, leaf length and width, leaf number per plant, and dry/fresh weights of shoots and leaves and that the reactions varied depending on the dose of treatment and the species of plant being treated (Sun et al., 2019). Researchers also reported that, directly being involved in nitrogen assimilation thanks to their ammonium (NH_4^+) contents, amino acids and their derivatives stimulated the synthesis of chlorophyll, protein, and phytohormones, as well as stimulating the cell division (Hildebrandt et al., 2015; Aghaye Noroozlo et al., 2019).

Effects of L-GLU treatments on bulb yield in garlic

The effects of GLU treatments on bulb yield in garlic were found to be statistically significant (p<0.05). As seen in Table 5, exogenous GLU applications increased the fresh weight, width, and length values of bulbs. When compared to the control group, the highest bulb weight, length, and width were found in 800 GLU (2.88 folds) and 400 (2.53 folds) GLU doses, whereas the lowest weight was found in the control group. Bulb lengths at 800 GLU and 200 GLU dozes were found to be higher than the control group by 10.75% and 10.72%, respectively. Bulb widths in 800, 400, and 200 GLU (34.08%, 30.37%, and 23.29%) treatments reached the maximum. The number of cloves (cloves/bulb) increased in all the treatment groups in comparison to the control group (9), but the highest level was found at 100 GLU (11.00) (Table 5). Clove characteristics of samples (large-small) also varied by the GLU doses. The highest clove length and width values were found at 800 GLU dose. However, the clove width was found to be the lowest in the control group among large cloves, whereas it was found at the highest GLU for the small cloves (Table 5). In comparison to the control group, the highest parcel yield was obtained from 800 GLU (0.461 kg) and 400 GLU (0.428 kg) doses. Effects of GLU doses on % ash contents were found to vary by concentration (Table 5). Ash content of samples ranged between 16.29 % (control) and 49.15% and the highest difference in comparison to the control group was found at 400 GLU by 49.15% and 800 GLU dose by 43.17%. At these concentrations, ash (%) content was found to be higher than the control group by 3.02 times at 400 GLU and 2.65 times at 800 GLU. GLU treatments showed positive effects in general on bulb yield, % ash content, and parcel yield (Table 5). Considering the parameters examined here, the highest yields were found at 400 and 800 GLU doses, whereas the lowest values were found in control (Table 5). The parameters examined here were found to be consistent with the literature. In previous studies examining garlic and onion (Shalaby and El-Ramady, 2014; Sitaula et al., 2020; Kibar, 2022), potato (Röder et al., 2018), apricot (Bakır et al., 2022), and vegetable, the leaves of which are consumed such as mellow, lettuce, and spinach (Chen et al., 2016; Greenwell and Ruter, 2018), amino acid-based compounds applied exogenously caused increases in morphological characteristics such as length and width and yield/quality parameters such as fresh/dry weight, dry matter content, and % ash content. Shafeek et al. (2012) examining the effects of a low and moderate dose of arginine and glutamine treatments on bulb weight, width, length, dry weight, and parcel yield, Shalaby and El-Ramady (2014) examining the effects of foliar amino acid treatments on bulb yield and shelf-life of garlic, and Hegazi et al. (2016) analyzing the effects of Moringa leaf extract, glutamine, and cysteine treatments on growth, yield, and quality of garlic reported that low dose of glutamine and cysteine caused an increases in growth parameters such as shoot length, number of leaf per plant, and leaf surface area. Majkowska-Gadomska et al. (2019), in their study on the effects of exogenously applied amino acid-containing compounds on winter garlic varieties, determined that those treatments had positive effects on garlic's bulb weight, length, and width, as well as the number of cloves per bulb, % ash content, and dry matter content.

Table 5. Effects of L-GLU treatments on bulb yield and ash (%) contents of garlic samples (100 GLU: 100 mg L^{-1} L-Glutamic acid, 200 GLU: 200 mg L^{-1} L-Glutamic acid; 400 GLU: 400 mg L^{-1} L-Glutamic acid; 800 GLU: 800 mg L^{-1} L-Glutamic acid; 1000 GLU: 1000 mg L^{-1} L-Glutamic acid; ± Standard errors of three repeat experiments)

Group	Bulb			Clove	Big bulb		Small bulb		Yield	Ash (%)
	FW (g)	Length (mm)	Width (mm)	Number	Length of large clove (mm)	Length of small clove (mm)	Large clove (mm)	Small clove (mm)	Parcel kg	
Control	15.99±1.01e*	30.14±0.73	34.01±0.66c	9.00±0.24	27.10±.42c	17.16±0.65c	18.73±1.22c	10.96±0.64	0.160±0.0005f	16.29±0.40e
100 GLU	22.50±1.04d	31.11±1.04	40.69±1.58b	11.00±0.37	28.04±0.69b	17.82±0.77c	19.17±1.25c	11.76±1.24	0.225±0.0003e	19.28±0.30d
200 GLU	30.80±1.40c	33.37±0.95	41.93±1.67b	10.34±1.02	29.60±0.79b	24.08±0.83a	22.12±0.49b	10.32a±0.46	0.308±0.0004d	23.18±0.48c
400 GLU	42.70±1.97b	32.53±0.97	44.34±1.79a	10.00±0.42	31.13±0.65ab	23.28±0.72b	23.93±0.55ab	11.27±0.48	0.428±0.0003b	49.15±0.51a
800 GLU	46.0±1.97a	33.38±0.77	45.60±1.11a	9.94±0.44	33.79±0.48a	25.80±0.56a	25.11±0.40a	12.30±0.31	0.461±0.0003a	43.18±0.40b
1000 GLU	40.48±1.33b	31.83±1.11	39.67±1.20b	9.67±0.52	28.78±0.63b	22.95±0.67b	21.39±0.47b	9.81±0.31	0.405±0.0005c	19.80±0.72d
F	64.288	1.907	8.711	1.432	15.103	25.270	9.770	1.974	103741.56	3611.971
Sig.	<0.001	0.102	<0.001	0.221	<0.001	<0.001	<0.001	0.091	<0.001	<0.001

*: Means indicated with different letters within same column are significantly different ($P < 0.05$)

Effects of L-GLU treatments on total soluble amino acid, total nitrate, total polyphenol, and ascorbic acid contents of garlic

The effects of L-GLU treatments on total free amino acid, nitrate, polyphenol, and Vitamin C contents of garlic cloves were found to be statistically significant (P<0.05). The results achieved in this regard are presented in Figures 1, 2. The foliar application of ammonia compounds might stimulate plant growth and development more since their assimilation are faster than the compounds containing NO_3 (Hildebrandt, 2018; Cheng et al., 2016). In previous studies, it was reported that the accumulation of NO_3 in mature organs and reserve organs was higher than in young organs. Total nitrate content was found to be high in 200 GLU and 400 GLU doses and low in others (Figure 1). The total nitrate content of garlic samples was found to be high only at GLU doses of 200 and 400 mg GLU (Figure 1) and it might be because of the difference between garlic's NH_4^+ and NO_3^- assimilations or because the bulb is a mature organ (Hildebrandt, 2018; Aghaye Noroozlo et al., 2020). The amino acid content ranged between 27.74 mg and 42.74 mg and GLU doses caused increases in total amino acid contents in comparison to the control group. The highest value was found at 400 GLU, whereas the lowest one was found in the control group (Figure 2).

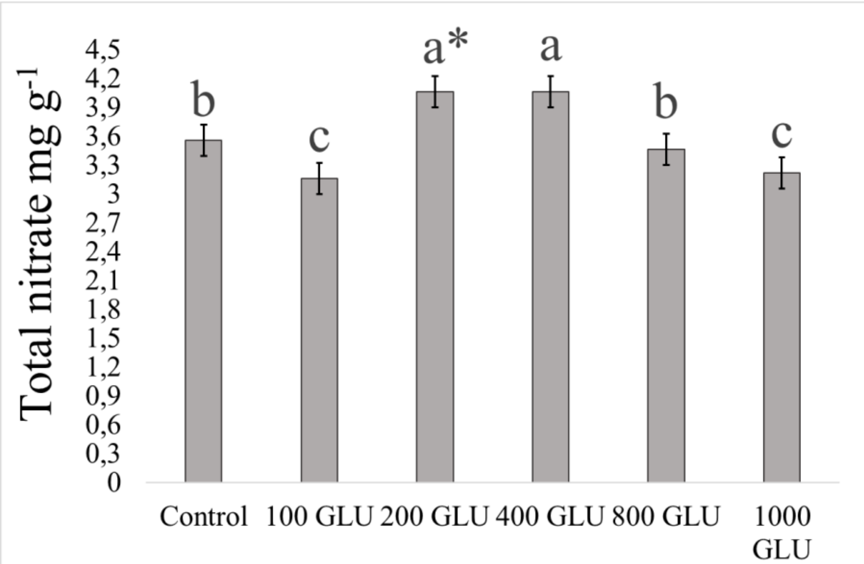

Figure 1. Effects of L-GLU treatments the total nitrate contents of garlic. 100 GLU: 100 mg L^{-1} L-Glutamic acid, 200 GLU: 200 mg L^{-1} L-Glutamic acid; 400 GLU: 400 mg L^{-1} L-Glutamic acid; 800 GLU: 800 mg L^{-1} L-Glutamic acid; 1000 GLU: 1000 mg L^{-1} L-Glutamic acid *: Means indicated with different letters within the bars are significantly different (P < 0.001)

Amino acid data have similarities with the results reported in previous studies. Similar results were reported in studies carried out on onion (Shaheen et al. 2010), garlic (Shalaby and El-Ramady, 2014), okra (Greenwell and Ruter, 2018), lettuce (Aghaye Noroozlo et al., 2019), guar seedling (Kuşvuran et al., 2019), purslane (Tavallali et al., 2019), and basil (Aghaye Noroozlo et al., 2020). Similarly, Majkowska-Gadomska et al. (2019) determined that exogenous amino acid treatments increased the nitrate content of winter garlic varieties' cloves. As reported by researchers, amino acids and their combinations increase the bulb yield and quality of garlic by stimulating the synthesis of protein, amino acids, enzymes, photosynthetic pigments, and several vitamins, activating phytohormones, and accelerating the cell cycle.

Polyphenols are glucosides playing roles in plants' characteristics such as taste, odor, and color and they can stimulate the plant's resistance to stress factors such as UV, pathogen attacks, light, and temperature (Kałużewicz et al., 2017).

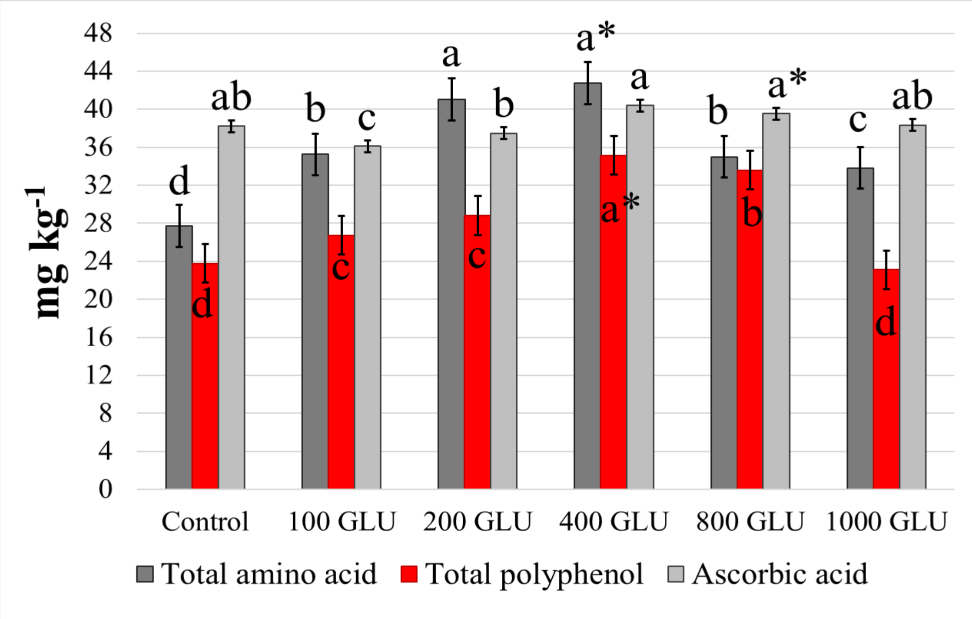

Figure 2. Effects of L-GLU treatments the total free amino acid, polyphenol and free amino acid of garlic cloves. 100 GLU: 100 mg L^{-1} L-Glutamic acid, 200 GLU: 200 mg L^{-1} L-Glutamic acid; 400 GLU: 400 mg L^{-1} L-Glutamic acid; 800 GLU: 800 mg L^{-1} L-Glutamic acid; 1000 GLU: 1000 mg L^{-1} L-Glutamic acid: *: Means indicated with different letters within the bars are significantly different ($P < 0.001$)

Total polyphenol content was found to range between 23.10 mg and 31.15 mg. The highest level of polyphenol content was found at the GLU dose of 400 (35.15 mg), followed by 800 GLU (33.60 mg), whereas the lowest polyphenol contents were found in 1000 GLU (23.1 mg) treatment and in the control group (23.78 mg) samples (Figure 2). The changes in polyphenol contents of garlic samples were related to the stimulation of the polyphenol synthesis pathway by GLU treatment. Many researchers reported that foliar application of amino acid derivatives stimulated the phenolic compound synthesis in plants (Jin et al., 2019). Musbah and Ibrahim (2019) examining coleus and Arslan et al. (2022) examining cauliflower and broccoli reported that foliar melatonin and phenylalanine applications stimulated the amino acid and phenolic compounds depending on the concentration. Kałużewicz et al. (2017) observed that amino acid-containing stimulants applied in combination with *Ascophyllum nodosum* filtrate during the growth period of broccoli heads stimulated the total phenolic accumulation and increased the cold storage period. Researchers claimed that these results might be related with the treatments' stimulating effect on endogenous hormone synthesis and antioxidant activity. Similarly, it was also reported that foliar amino acid applications caused increases in total phenolic content in mints grown in open lands (Tarasevìciene et al., 2021).

Ascorbic acid is one of the most abundant vitamins in plants. Its concentration is very high in the apoplast, meristems, flowers, immature fruits, tuberous roots, roots, and especially in photosynthetic organelles (Pantelidis et a., 2007; Chang et al., 2013). As shown in Figure 2, applied GLU doses caused an increase in ascorbic acid content. When compared to the control group, the highest value was found to be 40.40 mg in the group given 400 GLU, followed by the group given 800 GLU. Besides that, a slight decrease in ascorbic acid content in comparison to the control group was observed in the group given 100 GLU (Figure 2). In literature, it was reported that ascorbic acid promoted the growth and development of plants by regulating the metabolic reactions such as cell division and differentiation, signal transmission, and detoxification of ROSs (Gorecka et al., 2014; Aghaye Noroozlo et al., 2019). Majkowska-Gadomska et al. (2019) determined that the exogenous application of amino acids increased the ascorbic acid content of the winter garlic varieties' cloves.

Effects of GLU treatments on weight loss of garlic samples

Effects of GLU applications on the weight changes of garlic samples differed by concentration and duration (Table 6). Given the first weight (%) measurements, the loss of weight in comparison to the control group was found to be very low at doses other than 100 GLU. Weight loss was remarkably lower than in the control group, especially in 800 GLU, 1000 GLU, and 400 GLU treatments (43.24%, 38.68%, and 24.9%, respectively). In the second measurement, the weight loss increased in all groups when compared to the control group. The highest level of weight loss was found in 100 (2.78 folds), 1000 (2.19 folds), and 800 (2.18 folds) doses of GLU application, whereas the lowest level of weight loss was found in the control group (3.77%).

Table 6. Effect of GLU application (ppm) on the bulb weight loss (%) during storage, days after harvest (samples (100 GLU: 100 mg L^{-1} L-Glutamic acid, 200 GLU: 200 mg L^{-1} L-Glutamic acid; 400 GLU: 400 mg L^{-1} L-Glutamic acid; 800 GLU: 800 mg L^{-1} L-Glutamic acid; 1000 GLU: 1000 mg L^{-1} L-Glutamic acid; ± Standard errors of three repeat experiments)

Groups	Weight loss during the storage period (%)		
	I	II	III
Control	12.28±0.43a	3.77±0.22d	15.58±0.28b
100 GLU	13.02±0.06a	10.49±0.31a	22.14±0.26a
200 GLU	9.22±0.75b	6.62±0.49c	15.24±0.37b
400 GLU	8.08±0.37b	5.69±0.08c	13.30±0.39c
800 GLU	6.97±0.20c	8.20±0.21b	14.60±0.27b
1000 GLU	7.53±0.43c	8.26±0.39b	15.17±0.45b
F	35.430	56.718	83.818
Sig.	0.000	0.000	0.000

*: Means indicated with different letters within the same column are significantly different (P < 0.05)

In the third weight measurement, in comparison to the control, the weight loss in the 100 GLU treatment was found to be higher by 42.10%. In other groups, the weight loss was lower than in the control groups and the lowest levels of weight loss were found to be 14.64% in 400 GLU and 6.29% in 800 GLU (Table 6). Garlic maintains its vitality and keeps breathing after the harvest. The most important factors affecting the weight loss of garlic are the temperature and the treatments applied before and after harvesting (Martins et al., 2016; Akan and Güneş, 2021). In literature, there are studies examining the effects of ascorbic acid, gibberellic acid, and other chemicals on the weight loss and storage duration of garlic (Woldayes et al., 2017; Sharma et al., 2020) but the number of studies utilizing the amino acid and its derivatives is limited. Shalaby and El-Ramady (2014) observed the effects of bio-stimulant and ascorbic acid treatments on the weight loss of garlic stored under room conditions for 9 months and the authors determined that the weight loss increased with prolonging storage duration and the lowest level of weight loss was obtained with ascorbic acid. Differing from the present study, researchers found that the weight losses observed in amino acid and other treatments were higher than in the control group. Ulianych et al. (2020), in their study examining the effects of amino acids on yield, chemical content, and storage duration of garlic, reported that the highest level of weight loss in garlic cloves was observed in the first month of the storage period (14.8-18.2%) and that the weight loss was higher in comparison to the control group. The authors thought that the differences between weight loss values might be because of the storage temperature of garlic. During the storage, the room temperature was kept between +5 °C and +23 °C (Table 3). At these temperatures, the respiration of garlic might be slower and it might have reduced the loss of weight and water (Pöhnl et al., 2019; Turfan, 2022).

Conclusion

In the present study examining the effects of GLU treatments on the yield, quality, and storage period of garlic, which is of significant economic importance for our country, it was determined that GLU treatments had significant effects depending on the concentration. GLU doses generally had positive effects on yield criteria of garlic bulbs in terms of fresh weight, length and width of shoot and leaf in green parts, fresh weight, length, width, number of cloves, clove weight, yield per parcel, dry weight, and % ash content. Examined parameters generally reached their peak values at 400, 800, and 200 GLU doses. Similarly, chemical contents increased at 400 GLU, 800 GLU, and 200 GLU doses when compared to the control group. The effects of GLU treatments on the weight loss of garlic varied depending on the dose. In conclusion, it can be stated that L-Glutamic acid showed positive effects on the development, yield, and storage period of garlic, that amino acid treatments would benefit the producer in marketing the garlic, and that it would be useful to investigate the effects of different amino acid derivatives on the yield and storage period of garlic.

Conflict of interest: The authors declare no conflict of interest, financial or otherwise.

Author contributions: Nezahat TURFAN was responsible for the selection of the study topic, performing the chemical analyzes, writing and submitting the manuscript.
Buse TURAN: was responsible for the cultivation and morphological measurements of garlic samples. All authors read and approved the final manuscript

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