Environmental Science and Pollution Research (2021) 28:1983–1991
https://doi.org/10.1007/s11356-020-10658-7

RESEARCH ARTICLE

The utilization of tryptophan and glycine amino acids as safe alternatives to chemical fertilizers in apple orchards

Walid F. A. Mosa[1] · Hayssam M. Ali[2,3] · Nader R. Abdelsalam[4]

Received: 20 April 2020 / Accepted: 26 August 2020 / Published online: 30 August 2020

Abstract

Our experiment was conducted during the seasons of 2018 and 2019 on 10-year-old "Anna" apple trees (*Malus domestica* L. Borkh) planted at 4 × 4 m apart in a clay soil under drench irrigation. Sixty uniform trees were selected and subjected to the same cultural practices during both seasons. Apple trees were sprayed three times as follows: before flowering, during full bloom, and 1 month later with the following treatments: control (water only); tryptophan at 25, 50, and 100 ppm; glycine at 25, 50, and 100 ppm; and their combinations, 25 ppm tryptophan + 25 ppm glycine, 50 ppm tryptophan + 50 ppm glycine, and 100 ppm tryptophan + 100 ppm glycine. The results demonstrated that the foliar spraying of "Anna" apple trees with glycine and tryptophan at 25, 50, and 100 ppm and their combinations significantly increased shoot length and diameter, leaf area, total chlorophyll, percentages of fruit set and yield, fruit physical and chemical characteristics, and leaf mineral composition of N, P, K, Ca, Fe, Zn, Mn, and B, whereas it reduced the fruit drop percentage in both seasons in comparison with control. Better results were obtained from the concentrations of 50 and 100 ppm which were more effective in both seasons in comparison with the concentration of 25 ppm. Moreover, the combination of 50 ppm glycine 50 ppm tryptophan was the best treatment and provided the highest results in both experimental seasons in comparison with the other applied treatments and control.

Keywords Apple · Amino acids · Crop improvement · Glycine · Fruit · Plant nutrition · Quality · Tryptophan · Yield

Introduction

Harvested apples in Egypt span across an area of 28,085 ha producing 70,4727 ton/ha (FAO and UNICEF 2018). The "Anna" apples (*Malus domestica* L. Borkh) belong to the family of Rosacea. In previous publications, it was suggested that amino acids enhance fresh and dry matter (Fawzy et al. 2012), increase growth behavior (Bahari et al. 2013; Romero et al. 2014; Rouphael et al. 2017), and stimulate plants' root growth, which may enhance both water and nutrient uptake capability, resulting in an increased yield productivity (Colla et al. 2017; Rouphael et al. 2017). When amino acids are applied through the root system or foliar feeding, the uptake and concentrations of leaf nutrients are improved (Mohammadipour and Souri 2019a, 2019b; Pranckietienė et al. 2015), including iron and zinc (Souri et al. 2018). The exogenous application of amino acids promotes growth, induces leaf pigmentation, stimulates chlorophyll biosynthesis, and reduces chlorophyll degradation in many crops (Fahimi et al. 2016; Kazem Souri et al. 2017; Mohammadipour and Souri 2019a, 2019b). Amino acids have several major biological functions in plant cells, such as detoxification (Bashir et al. 2018; Hussain et al. 2018; Rizwan et al. 2017), optimization of nutrient uptake, translocation and metabolism, vitamin biosynthesis, growth biostimulation, and increasing tolerance to environmental stresses (drought, salinity, and cold

Responsible Editor: Philippe Garrigues

Walid F. A. Mosa
walidbreeder@yahoo.com

Nader R. Abdelsalam
nader.wheat@alexu.edu.eg

Hayssam M. Ali
hayhassan@ksu.edu.sa

1 Plant Production Department, Faculty of Agriculture, Saba Basha, Alexandria University, Alexandria 21531, Egypt

2 Botany and Microbiology Department, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia

3 Timber Trees Research Department, Sabahia Horticulture Research Station, Horticulture Research Institute, Agricultural Research Center, Alexandria 21526, Egypt

4 Agricultural Botany Department, Faculty of Agriculture, Saba Basha, Alexandria University, Alexandria 21531, Egypt

conditions). Also, they play a vital role in the synthesis and production of amino chelate fertilizers (Souri and Hatamian 2019). The foliar application of amino acids was found to increase the biomass and crop productivity in plants (Basanth and Mahesh 2018; Kazem Souri et al. 2017). Radkowski et al. (2018) declared that the content of the investigated macro elements was increased by the foliar fertilization with the amino acid preparation. Khan et al. (2019) reported that amino acids are natural plant growth stimulators. As a result, they are utilized extensively for enhancing the yield and quality of crops. Noroozlo et al. (2019) stated that amino acids play various roles in plant metabolism; the exogenous application of amino acids may have benefits and stimulation effects on plant growth and quality. Souri and Hatamian (2019) declared that amino acids have an affinity for nutrient elements and some amino acids can make chelates with nutrients. They also mentioned that these properties assist them in enhancing the uptake and delivery of micronutrients, mainly Fe, in plants. These micronutrients are good sources of nitrogen for plant uptake and utilization as well as having stimulating effects on plant root and shoot growth. Mataffo et al. (2020) stated that foliar application of amino acid–enriched urea fertilizer is considered a promising environmentally friendly strategy for enhancing the yield and nutrient efficiency of plants. Matysiak et al. (2020) reported that the exogenous application of some amino acids has been proven to positively affect plant growth and development under stressful conditions. Sheng et al. (2020) examined the effects the amino acid-Fe compound fertilizer spraying has on leaf development in peaches (*Prunus persica* (L.) Batsch) at different developmental stages. The results demonstrated that the foliar spraying increased leaves' fresh weights considerably. Fe concentration, photosynthetic parameter, and Fe-S protein analyses were used to measure Fe accumulation, total chlorophyll content, net photosynthetic rate, and stomatal conductance. Furthermore, the same authors observed that amino acid-Fe compound fertilizer significantly increased the leaf content from nitrite reductase and succinate dehydrogenase's activities, over control. This study is aimed at utilizing some alternative fertilizers from amino acids, such as tryptophan and glycine, in apple orchards to decrease environmental hazards and increase apple production and quality.

Materials and methods

The present experiment was performed during the two successive seasons of 2018 and 2019 on 10-year-old "Anna" apple trees (*Malus domestica* L. Borkh), planted 4 × 4 m apart in a clay soil under flood irrigation in a private orchard located at Kafr El-Dawar, Beheira governorate, Egypt. Table 1 presents the physiochemical analysis of experimental soil, which was conducted as per Cottenie and Verlo (1982). Sixty uniform trees were chosen for this study and were subjected to the same cultural practices in both seasons. They were sprayed three times as follows: before flowering, during the full bloom, and 1 month later with the following treatments: control (water only); tryptophan ((S)-2-amino-3-(3-indoly) propionic acid—$C_{11}H_{12}N_2O_2$—ROTH Besteffen Sie Zum Nufftarif—China) at 25, 50, and 100 ppm; glycine (aminoethanoic; glycocoll $C_2H_5NO_2$—Anhui BBCA Pharmaceutical Company Ltd.—China) at 25, 50, and 100 ppm; and their combinations, tryptophan at 25 ppm + glycine at 25 ppm, tryptophan at 50 ppm + glycine at 50 ppm, and tryptophan at 100 ppm + glycine at 100 ppm.

The previously applied treatments were arranged in a randomized complete block design where each treatment consisted of sex trees/sex replicate. The following parameters were utilized to investigate the impact of the treatments mentioned above.

Vegetative parameters

At the beginning of the vegetative growth, eight shoots from each side of each replicate/tree were selected and labeled, whereas at the end of each growing season, the shoot length and diameter were measured in centimeters and the leaf area was measured in centimeters squared. Total chlorophyll was also measured by Spad 502 (μ Mol/m^2).

Fruit set and fruit drop percentage

Four branches from each side from each tree/replicate were selected carefully and labeled in March from 2018 and 2019, and the number of flowers on each branch was calculated. The fruit set percentage was also calculated as per Westwood (1988) equation:

$$\text{Fruit set\%} = (\text{number of set fruitlets}/\text{number of opened flowers}) \times 100.$$

Estimation of preharvest fruit drop was done by calculating the number of dropping fruit after fruit setting until the harvesting date in each season (June). Then, the fruit drop was calculated as a percentage as follows:

$$\text{Fruit drop\%} = (\text{number of dropped fruitlets}/\text{number of set fruitlets}) \times 100.$$

Fruit yield

Estimation of yield was conducted in kilograms per tree and ton per hectare at harvest time (June).

Leaves chemical composition

A random selection of samples of thirty leaves was made from the middle part of the shoots according to Abdelsalam et al.

Table 1 Physiochemical properties of the experimental soil

Depth	Texture	pH	EC (ds/cm)	N (Mg/kg)	P (Mg/kg)	K (Mg/kg)	Fe (mg/l)	Zn (mg/l)	Mn (mg/l)
0–60	Clay	8.8	0.3	50.23	41.52	362	0.28	0.1	0.01

(2018) and Chuntanaparb and Cummings (1980) from each replicate following harvest time in June to determine their content of N%, P%, K%, and Ca%, and Fe, Zn, Mn, and B at ppm. The leaf samples were washed first with tap water, then with distilled water, and dried at a temperature of 70 °C until it maintained a constant weight; finally, they were ground and acid digested using H_2SO_4 and H_2O_2 until the solution became clear (Wilde et al. 1985). The digested solution was used for determining each of the following: nitrogen using micro Kjeldahl method, phosphorus using vanadomolybdo method, and potassium using flame photometer following the method presented by Chapman and Pratt (1961). Zinc, manganese, calcium, and iron were determined using an atomic absorption spectrophotometer (2-8200 Series Polarized Zeeman, Hitachi, Tokyo, Japan) through specific lamps for specific nutrients. By dry ashing, boron was estimated using the method described by Chapman and Pratt (1961), and, subsequently, it was measured through colorimetry, Azomethine-H, as indicated by Bingham (1983).

Fruit quality

At harvest time, samples of thirty fruit were randomly selected from each replicate/tree to determine the physical and chemical characteristics of fruit.

Fruit physical characteristics

Fruit weight (g), fruit length (cm), fruit diameter (cm), and fruit firmness (Ib/inch^2) were determined via a Magness and Taylor pressure tester with a $^7/_{18}$-inch plunger.

Fruit chemical characteristics

Total soluble solids were estimated by using a hand refractometer. The percentage of titratable acidity in fruit juice was determined according to Resio et al. (2005). Total sugars, reducing and nonreducing sugars, were estimated as per Malik and Singh (1980). Anthocyanin was determined at the stage of coloration (mg/100 g fresh weight peel) according to Rabino (1977).

Statistical analysis

The obtained data were subjected to one-way ANOVA as per Snedecor and Cochran (1980), and least significant difference (LSD) instances at 0.05% were utilized for comparing the means of treatments.

Results

Vegetative growth parameters

Data listed in Table 2 indicated that a significant improvement was observed in shoot length and diameter by the foliar spraying of glycine and tryptophan at 50 and 100 ppm and their combinations of 50 ppm glycine + 50 ppm tryptophan and 100 ppm glycine + 100 ppm tryptophan in comparison with the usage of 25 ppm and control in both seasons. Furthermore, 50 ppm glycine + 50 ppm tryptophan was shown to be the best treatment which gave the best results in the two seasons in comparison with control and the other applied treatments. Regarding leaf area, it was considerably increased by 100 ppm tryptophan sprays and by the combinations of tryptophan at 50 ppm + glycine at 50 ppm as well as tryptophan at 100 ppm + glycine at 100 ppm compared with control in both seasons. Moreover, remarkable improvement is observed in the leaf area by spraying glycine at 50 and 100 ppm and tryptophan at 50 ppm in both seasons compared with control. Total chlorophyll was notably enhanced by foliar spraying of glycine and tryptophan at 25, 50, and 100 ppm and their combinations compared with control in the two seasons. Spraying the combination of tryptophan + glycine at 50 ppm and glycine at 100 ppm and tryptophan at 50 ppm showed the highest increment in comparison with control in the two experimental seasons.

Fruit set percentage and fruit yield

The data in Table 3 showed that spraying “Anna” apple trees by tryptophan or glycine and their combinations at the different concentrations of 25, 50, and 100 ppm significantly improved the fruit set percentage and the fruit yield per tree and per hectare, whereas they reduced the fruit drop percentage in comparison with control in both seasons. The foliar spraying of glycine or tryptophan with a concentration of 50 or 100 ppm gave better results than that with a concentration of 25 ppm in the two seasons. Furthermore, the combination of tryptophan + glycine at 50 ppm was the best treatment, yielding the highest fruit set and fruit yield percentages and the lowest fruit drop percentage in comparison with the other applied treatments in both seasons.

Table 2 The effect of the foliar application of tryptophan and glycine on length and diameter of shoots, leaf area, and total chlorophyll during 2018 and 2019 seasons

Treatment	Shoot length (cm)		Shoot diameter (cm)		Leaf area (cm^2)		Total chlorophyll Spad (μ Molm^{-2})	
	2018	2019	2018	2019	2018	2019	2018	2019
Control	37.49^{e}	39.92^{e}	0.67^{e}	0.69^{e}	27.81b	28.09^{c}	38.12^{d}	37.67^{c}
Tryptophan at 25 ppm	39.84^{de}	39.65^{e}	0.74^{d}	0.73d	28.99^{b}	29.76^{bc}	50.82c	51.92^{b}
Tryptophan 50 ppm	42.06^{cd}	43.55^{cd}	0.83^{b}	0.83^{c}	31.16^{ab}	32.26^{b}	54.35^{a}	54.27^{a}
Tryptophan 100 ppm	44.32^{bc}	43.64^{cd}	0.86^{b}	0.89^{b}	33.27^{a}	32.62^{b}	52.92^{abc}	53.5^{ab}
Glycine 25 ppm	39.90^{de}	41.01^{de}	0.73^{d}	0.76^{d}	30.15^{ab}	30.15^{bc}	53.07^{abc}	53^{ab}
Glycine 50 ppm	42.58^{bcd}	44.85^{bc}	0.84^{b}	0.84^{c}	31.66^{ab}	31.58^{b}	53.75^{a}	54.07^{ab}
Glycine 100 ppm	45.80^{b}	47.14^{b}	0.91^{a}	0.94^{a}	30.91^{ab}	32.14^{b}	53.57^{a}	54.55^{a}
Tryptophan at 25 ppm + glycine 25 ppm	40.03^{de}	41.80^{cde}	0.78^{c}	0.82^{c}	28.48^{b}	30.88^{bc}	51.05^{bc}	54^{ab}
Tryptophan at 50 ppm + glycine 50 ppm	51.17^{a}	54.28^{a}	0.93^{a}	0.94^{a}	33.46^{a}	36.44^{a}	54^{a}	54.75^{a}
Tryptophan at 100 ppm + glycine 100 ppm	43.52^{bc}	47.35^{b}	0.85^{b}	0.89^{b}	33.26^{a}	32.10^{b}	53.25^{ab}	54.07^{ab}
$LSD_{0.05}$	3.41	3.08	0.03	0.03	3.91	3.40	2.26	2.19

Means not sharing the same letter(s) within each column are significantly different at 0.05 level of probability

Fruit quality

The results in Table 4 indicated that spraying tryptophan and glycine at 25, 50, and 100 ppm, whether each one alone or in combination with the other, exhibited great improvement in weight, size, length, and diameter of the fruit of "Anna" apple tree in the two experimental seasons in comparison with control, and the foliar spraying of 100 ppm tryptophan was superior in these terms. Besides, the foliar application of tryptophan with glycine combinations at 50 and 100 ppm provided the highest increase in the fruit weight and size, whereas the foliar spray of tryptophan and glycine at 50 and 100 ppm gave the highest increment in fruit length and diameter in the two seasons compared with control.

Data in Table 5 demonstrated that fruit content of "Anna" apple trees from TSS percentage, fruit firmness, total sugars, and reduced sugars was noticeably increased by spraying glycine and tryptophan amino acids at 25, 50, and 100 ppm and their combinations in the two seasons. The highest increment in TSS percentage was obtained by spraying tryptophan at 50 ppm and glycine at 50 and 100 ppm in both seasons in comparison with the other applied treatments and control. The foliar spray of glycine and tryptophan and their combinations at 50 and 100 ppm provided the best increase in fruit firmness

Table 3 The effect of the foliar application of tryptophan and glycine on fruit set %, fruit drop %, and fruit yield during 2018 and 2019 seasons

Treatments	Fruit set (%)		Fruit drop (%)		Fruit yield (kg/tree)		Fruit yield (ton/hectare)	
	2018	2019	2018	2019	2018	2019	2018	2019
Control	11.86^{d}	12.14^{g}	83.04^{a}	83.96^{a}	20.28^{f}	19.44^{e}	12.67^{f}	12.15^{e}
Tryptophan at 25 ppm	22.12^{bc}	24.30^{e}	65.45^{b}	66.96^{b}	36.25^{de}	35.13^{d}	22.66^{de}	21.95^{d}
Tryptophan 50 ppm	24.01^{b}	25.51^{de}	64.53^{b}	65.81^{bc}	38.86^{cd}	44.75^{c}	24.29^{cd}	27.97^{c}
Tryptophan 100 ppm	23.39^{b}	25.63^{cde}	62.25^{bc}	63.72^{bcd}	47.04^{a}	47.18^{bc}	29.4^{a}	29.48^{bc}
Glycine 25 ppm	20.54^{c}	21.49^{f}	66.48^{b}	63.20^{cd}	35.52^{e}	36.26^{d}	22.20^{e}	22.66^{d}
Glycine 50 ppm	23.33^{b}	27.31^{cd}	62.29^{bc}	62.69^{cde}	43.96^{b}	45.19^{c}	27.48^{b}	28.24^{c}
Glycine 100 ppm	26.34^{a}	27.96^{c}	62.27bc	61.29^{de}	43.86^{b}	49.31^{ab}	27.41^{b}	30.82^{ab}
Tryptophan at 25 ppm + glycine 25 ppm	22.17^{bc}	26.70^{cde}	62.73^{bc}	65.64^{bc}	40.82^{c}	45.44^{c}	25.51^{c}	28.40^{c}
Tryptophan at 50 ppm + glycine 50 ppm	28.51^{a}	36.18^{a}	57.58^{d}	59.88^{e}	49.19^{a}	50.95^{a}	30.74^{a}	31.84^{a}
Tryptophan at 100 ppm + glycine 100 ppm	27.80^{a}	33.28^{b}	59.14^{bc}	59.44^{e}	47.01^{a}	47.56^{abc}	29.38^{a}	29.72^{abc}
$LSD_{0.05}$	2.22	2.40	4.53	3.30	2.98	3.72	1.86	2.32

Means not sharing the same letter(s) within each column are significantly different at 0.05 level of probability

Table 4 The effect of the foliar application of tryptophan and glycine on fruit weight, fruit size, fruit length, and fruit diameter during 2018 and 2019 seasons

Treatment	Fruit weight (g)		Fruit size (cm^3)		Fruit length (mm)		Fruit diameter (mm)	
	2018	2019	2018	2019	2019	2018	2018	2019
Control	127.67^{d}	129.67^{d}	138d	140.67^{e}	38.27^{g}	39.97^{g}	36.4^{f}	37.97^{f}
Tryptophan at 25 ppm	148.33^{c}	151.67^{c}	161.67^{bc}	163.67^{cd}	57.13^{cd}	59.73^{cd}	53.64^{bcd}	56.75^{bc}
Tryptophan 50 ppm	152.67^{b}	155.33^{b}	166^{a}	170^{a}	61^{ab}	64.05^{ab}	58.09^{ab}	60.11^{ab}
Tryptophan 100 ppm	156.67^{a}	161.33^{a}	167^{a}	170a	62.9^{a}	65.78^{a}	60.56^{a}	63.56^{a}
Glycine 25 ppm	147.33^{c}	150.67^{c}	161^{c}	166^{bc}	53.53^{de}	56.12^{de}	50.56^{cd}	53.82^{cd}
Glycine 50 ppm	153.33^{b}	156.33^{b}	166a	169.67^{ab}	57.7^{bc}	60.07^{c}	53.07^{bcd}	54.08^{cd}
Glycine 100 ppm	152.67^{b}	156^{b}	165.33^{ab}	168^{ab}	57.3^{c}	60.57^{bc}	54.58^{bc}	56.78^{bc}
Tryptophan at 25 ppm + glycine 25 ppm	145.67^{c}	149^{c}	158.33^{c}	161d	46.67^{f}	50.87^{f}	45.2^{e}	48.75^{e}
Tryptophan at 50 ppm + glycine 50 ppm	154.67^{ab}	157.67^{b}	167.33^{a}	169.67^{ab}	51.47^{e}	54.83^{e}	48.57^{de}	51.77^{de}
Tryptophan at 100 ppm + glycine 100 ppm	154.30^{ab}	157.25^{b}	166.33^{a}	169^{ab}	52.83^{e}	54.27^{ef}	48.93^{de}	51.16^{de}
$LSD_{0.05}$	3.25	3.30	3.72	3.74	3.61	3.75	5.31	4.67

Means not sharing the same letter(s) within each column are significantly different at 0.05 level of probability

compared with the other applied treatments and control in both seasons. The foliar application of glycine at 100 ppm and the combination of 100 ppm glycine + 100 ppm tryptophan gave the highest increase in total and reduced sugar percentages compared with the used concentrations and control in both experimental seasons. A significant increase in nonreduced sugar percentage was indicated through applying glycine at 100 ppm and the combinations of glycine and tryptophan at 50 and 100 ppm compared with the other applied treatments and control in the two seasons. The foliar spraying of tryptophan or glycine at 25, 50, and 100 ppm individually or in combinations exhibited a significant reduction in fruit acidity in comparison with control in both seasons. Spraying tryptophan or glycine and their combination at 50 ppm showed the lowest values compared with the other applied treatments and control in the two seasons.

Leaves chemical composition of macro and micronutrients

The results in Table 6 indicated that obvious improvement was observed in leaf composition of N, P, K, and Ca after spraying "Anna" apple trees with glycine or tryptophan at 25, 50, and 100 pm and by their combinations compared with

Table 5 The effect of the foliar application of tryptophan and glycine on TSS, acidity, total sugar, reduced sugar, nonreduced sugar percentages, and firmness of fruit during 2018 and 2019 seasons

Treatment	TSS (%)		Firmness (Ib/ inch2)		Acidity (%)		Total sugar (%)		Reduced sugar (%)		Nonreduced sugar (%)	
	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
Control	10.8^{b}	10.87^{b}	13^{c}	15^{d}	0.88^{a}	0.88^{a}	6.54^{e}	7.21^{f}	4.02^{g}	4.58^{g}	2.52^{cd}	2.63^{d}
Tryptophan at 25 ppm	12.67^{a}	12.47^{a}	21.67^{a}	18.33^{c}	0.72^{b}	0.72^{b}	7.11^{d}	7.7^{e}	4.74^{e}	5.34^{e}	$2.37d^{ef}$	2.36^{de}
Tryptophan 50 ppm	14.07a	13.8a	22.33a	23.67^{ab}	0.66^{cd}	0.64^{de}	7.61c	7.87^{e}	4.9^{e}	5.45^{e}	2.71^{cd}	2.41^{de}
Tryptophan 100 ppm	12.93^{a}	12.53^{a}	22.67^{a}	24^{ab}	0.66^{cd}	0.68^{bc}	7.84^{c}	7.93^{e}	5.95^{a}	5.32^{e}	2.13^{f}	2.61^{d}
Glycine 25 ppm	13.50^{a}	13.00^{a}	16.67^{b}	18.33^{c}	$0.68b^{cd}$	$0.67c^{d}$	6.56^{e}	7.20^{f}	4.41^{f}	4.96^{f}	2.14^{ef}	2.24^{e}
Glycine 50 ppm	14^{a}	13.6^{a}	22.33^{a}	22.33^{b}	0.63d	0.63e	7.87^{c}	8.38^{d}	5.37^{c}	5.96^{c}	2.50^{cde}	2.42^{de}
Glycine 100 ppm	14^{a}	13.2a	24^{a}	23^{ab}	0.68^{bcd}	0.68^{bc}	9.53^{a}	10.23^{a}	5.95^{a}	6.52^{a}	3.58^{ab}	3.72^{b}
Tryptophan at 25 ppm + glycine 25 ppm	12.9^{a}	13.13^{a}	16^{bc}	17.67^{c}	0.69^{bc}	0.66^{cde}	8.46^{b}	9.34^{c}	5.62^{b}	6.18^{b}	2.85^{c}	3.16^{c}
Tryptophan at 50 ppm + glycine 50 ppm	13.93^{a}	13.6^{a}	25^{a}	25^{a}	0.65^{cd}	0.66^{cde}	8.33^{b}	9.71^{b}	5.08^{d}	5.67^{d}	3.26^{b}	4.04^{a}
Tryptophan at 100 ppm + glycine 100 ppm	13.33^{a}	13.43^{a}	23.33^{a}	22.33^{b}	0.66^{cd}	0.66^{cde}	9.44^{a}	10.16^{a}	5.70^{b}	6.23^{b}	3.74^{a}	3.93^{ab}
$LSD_{0.05}$	1.44	1.49	3.53	2.58	0.05	0.04	0.3	0.27	0.17	0.19	0.36	0.32

Means not sharing the same letter(s) within each column are significantly different at 0.05 level of probability

Table 6 The effect of the foliar application of tryptophan and glycine on leaf mineral composition of N, P, K, and Ca during 2018 and 2019 seasons

Treatment	N (%)		P (%)		K (%)		Ca (%)	
	2018	2019	2018	2019	2018	2019	2018	2019
Control	1.61^{d}	1.79^{e}	0.33^{d}	0.35c	1.92^{f}	1.98^{d}	1.29^{d}	1.29^{d}
Tryptophan at 25 ppm	1.84^{c}	1.99^{d}	0.37^{abcd}	0.39^{abc}	1.98^{ef}	1.99^{d}	1.37^{cd}	1.40^{bc}
Tryptophan 50 ppm	2.10^{a}	2.11^{b}	0.41^{ab}	0.41^{ab}	2.08^{bcd}	2.11^{c}	1.49^{ab}	1.49^{a}
Tryptophan 100 ppm	2.08^{a}	2.12^{b}	0.38^{abcd}	0.40^{abc}	2.17ab	2.37^{a}	1.53^{a}	1.52^{a}
Glycine 25 ppm	1.92^{bc}	1.99^{d}	0.36^{bcd}	0.37^{abc}	1.97^{ef}	2.00^{d}	1.37^{cd}	1.36^{c}
Glycine 50 ppm	2.08^{a}	2.11^{b}	0.39^{abc}	0.40^{abc}	2.06^{cde}	2.06^{cd}	1.40^{bc}	1.46^{a}
Glycine 100 ppm	2.11^{a}	2.23^{a}	0.42^{a}	0.42^{a}	2.25^{a}	2.33^{ab}	1.49^{a}	1.49^{a}
Tryptophan at 25 ppm + glycine 25 ppm	2.04^{a}	2.08^{bc}	0.35^{cd}	0.37^{abc}	1.98^{def}	2.23^{b}	1.37^{cd}	1.38^{c}
Tryptophan at 50 ppm + glycine 50 ppm	2.06^{a}	2.10^{bc}	0.40^{abc}	0.41^{ab}	2.12^{bc}	2.25^{b}	1.49^{a}	1.52^{a}
Tryptophan at 100 ppm + glycine 100 ppm	1.95^{b}	2.01^{cd}	0.36^{bcd}	0.36^{bc}	2.10^{bc}	2.11^{c}	1.45^{abc}	1.48^{a}
$LSD_{0.05}$	0.09	0.08	0.05	0.05	0.10	0.10	0.09	0.06

Means not sharing the same letter(s) within each column are significantly different at 0.05 level of probability

control in both seasons. Spraying tryptophan or glycine at 50 and 100 ppm and their combinations at 50 ppm exhibited the highest increment in comparison with the other applied concentrations in the two experimental seasons. Additionally, the combination of 100 ppm glycine + 100 ppm tryptophan raised also the leaf mineral composition of K and Ca over control.

Data in Table 7 demonstrated that leaf mineral composition of Fe, Zn, Mn, and B was significantly increased by spraying "Anna" apple trees with glycine or tryptophan at 25 and 50 compared with control in both seasons. Spraying glycine and tryptophan at 50 or 100 ppm and their combination of 50 ppm glycine + 50 ppm tryptophan gave the best results in comparison with the other applied treatments in both seasons. Additionally, the combination of 100 ppm glycine + 100 ppm tryptophan provided good results in enhancing the leaf mineral composition of Fe and B over control.

Discussion

The obtained results indicated that foliar spraying of glycine and tryptophan and their combinations had positive effects on shoot length, shoot diameter, total chlorophyll, yield, and fruit quality of "Anna" apple cultivar. Our obtained results were previously explained by Ahmed et al. (2012) and Talaat et al. (2014) who observed that foliar spraying of "Valencia" orange trees with 25, 50, and 100 mg/l of tryptophan increased shoot length, shoot thickness, leaves number, leaves area,

Table 7 The effect of the foliar application of tryptophan and glycine on leaf mineral composition of Fe, Zn, Mn, and B during 2018 and 2019 seasons

Treatment	Fe (mg/l)		Zn (mg/l)		Mn (mg/l)		B (mg/l)	
	2018	2019	2018	2019	2018	2019	2018	2019
Control	105^{f}	107.67^{f}	23.33^{e}	27.67^{d}	43.67^{e}	47.33^{e}	66.67^{e}	71.67^{e}
Tryptophan at 25 ppm	122^{de}	119.33^{e}	32.67^{cd}	32.33^{c}	45.33^{de}	49.33^{de}	73.33^{d}	74^{de}
Tryptophan 50 ppm	126.67^{bc}	128.33^{abc}	33 cd	33.67^{bc}	47.33^{cde}	52.33^{cd}	75.67^{d}	79^{c}
Tryptophan 100 ppm	126.33^{bcd}	125^{bcd}	35.67^{bc}	37^{b}	49.67^{bcd}	58.33^{ab}	76.33^{c}	75^{de}
Glycine 25 ppm	119.67^{e}	120.33^{de}	30.67^{d}	32^{cd}	45^{de}	50.33^{de}	75^{d}	76^{cd}
Glycine 50 ppm	123^{cde}	123.33^{cde}	40.00^{a}	37.33^{a}	53.67^{ab}	58^{ab}	83.33^{c}	92.33^{b}
Glycine 100 ppm	126.67^{bc}	129.67^{ab}	40.33^{a}	43.67^{a}	56^{a}	58.33^{ab}	92.67^{a}	97.33^{a}
Tryptophan at 25 ppm + glycine 25 ppm	128.67^{b}	128.33^{abc}	33^{cd}	37.33^{b}	49^{bcd}	52^{cd}	85^{bc}	90.67^{b}
Tryptophan at 50 ppm + glycine 50 ppm	135.33^{a}	132.67^{a}	40.67^{a}	44.67^{a}	57.33^{a}	59.33^{a}	88.33^{b}	90.33^{b}
Tryptophan at 100 ppm + glycine 100 ppm	133.33^{a}	129.67^{ab}	38.33^{ab}	37^{b}	50.67^{bc}	54.67^{bc}	88.33^{b}	90^{b}
$LSD_{0.05}$	4.48	5.19	4.04	4.45	4.89	4.33	3.64	3.60

Means not sharing the same letter (s) within each column are significantly different at 0.05 level of probability yield, fruit quality, leaf mineral composition of N, P, K, Mg, Zn, Cu, Fe, and Mn leaf content of cytokines, gibberellins, auxins, and chemical elements. On the other hand, it reduced the concentration of abscisic acid. Tryptophan amino acid foliar application increased the yield and its components of chickpea (Abbas et al. 2013), lupine (Amin et al. 2014), and onion (Abd El-wahed et al. 2016; Mustafa et al. 2016). Furthermore, El Sayed et al. (2014) and Ghasemi et al. (2013) illustrated that glycine amino acid plays a vital role in forming the total chlorophyll and vegetative growth and in increasing the viability of Fe, Zn, Mn, and Cu to the plants. Spraying "Manfalouty" pomegranate cultivar with 50, 75, and 100 mg/l tryptophan at full bloom and 4 weeks later increased the vegetative growth, yield, and fruit quality. Meanwhile, the best results were provided by the concentration of 100 mg/l compared with control (El Sayed et al. 2014). Glycine has been incorporated into nutrient fertilizer formulation in the form of a chelate or a simple complex, to improve how the plant efficiently uses applied fertilizers and facilitate nutrient uptake and translocation in plants (Fahimi et al. 2016; Souri and Hatamian 2019). It was noticed that spraying apple trees with tryptophan amino acid at the stages of pre- and post-bloom increased the yield greatly in comparison with control (Sharma 2016). Recent studies by Khan et al. (2019), Souri and Hatamian (2019), and Weiland et al. (2016) showed that amino acids are widely utilized for improving the yield and quality of crops and the better root development supported by the addition of amino acids which can boost nitrogen fixation and induce the root-enhancing surface for nutrient uptake. The highest values of fruit set percentage, yield, and vegetative growth characteristics such as shoot length, shoot diameter, number of leaves, and leaf area of "Washington" navel orange were recorded with 50 ppm tryptophan treatment, followed by 25 ppm tryptophan treatment in comparison with control (Ahmed et al. 2017). Furthermore, Teixeira et al. (2017) stated that L-tryptophan and L-glycine are signal-transducing molecules and make the nutrients ready-made by the plants. Additionally, Khedr (2018) reported that spraying tryptophan at 50 and 100 ppm twice, at full bloom stage and the beginning of fruit set stage (21 days after full bloom stage), exhibited positive results in terms of Le-Conte pear's productivity and fruit quality. Moreover, 100 ppm tryptophan greatly increased the percentage of fruit set, yield, fruit weight, TSS, and total sugars of Le-Conte pear fruit when crosschecked against the use of water as a control. Application of different concentrations of glycine at 0, 5, 10, 20, or 40 mgL^{-1} to coriander plants (*Coriandrum sativum* L.) showed that leaf SPAD value, fresh and dry weights of shoots, roots, and leaf concentrations of N, K, Mg, and Zn were significantly increased by 10 mgL^{-1} glycine in comparison with the control plants (Mohammadipour and Souri 2019a; Noroozlo et al. 2019). Noroozlo et al. (2019) investigated the effect of foliar spraying of Romaine lettuce (*Lactuca sativa subvar Sahara*) with glycine at 0, 250, 500, and 1000 mgL^{-1}. The obtained results indicated that significant increase in leaf total chlorophyll content and root fresh weight was observed by 250 mgL^{-1} concentration compared with control. Besides, the highest shoot fresh and dry weights and leaf vitamin C were indicated with a concentration of 500 mgL^{-1}. Besides, 1000 mgL^{-1} glycine spray significantly increased leaf Fe concentration compared with control plants. Moreover, Souri and Hatamian (2019) demonstrated that in many plants, especially horticultural crops, glycine plays a vital role in controlling nutrition. Elkhatib et al. (2020) reported that foliar spraying of common bean cv. Nebraska with tryptophan amino acid at 0.5 and 1 mgL^{-1} concentrations increased vegetative growth, leaf chlorophyll, and N, P, and K contents, yield, and its components. Hancı and Tuncer (2020) investigated the effects of exogenous L-tryptophan at 125, 250, and 375 ppm on some growth parameters of lettuce plants grown under salt stress. They noticed that foliar spray of high concentrations gave the announced impact on the leaf number, salinity, weight of fresh leaf and fresh root, and surface area of lettuce plants under salinity at 200 mM.

Conclusions

- The foliar spraying of "Anna" apple trees with glycine and tryptophan at 25, 50, and 100 ppm and their combinations increased significantly shoot length, shoot diameter, leaf area, total chlorophyll, percentages of fruit set and fruit yield, fruit physical and chemical characteristics, and leaf mineral composition of N, P, K, Ca, Fe, Zn, Mn, and B, whereas it reduced the fruit drop percentage in the two seasons in comparison with control.
- The concentrations of 50 and 100 ppm gave better results and were more effective than 25 ppm in both seasons.
- Spraying glycine or tryptophan at a concentration of 50 or 100 ppm provided good results. However, better effects and higher increment were displayed when both glycine and tryptophan were combined at the same mentioned concentrations in the two seasons.
- The best treatment was the combination of 50 ppm glycine + 50 ppm tryptophan, providing the highest results in comparison with the other applied treatments and control in both experimental seasons.
- The obtained results indicated that amino acids, such as tryptophan and glycine, may be good alternatives to chemical fertilizers for reducing the environmental hazards and pollution.

Acknowledgments The authors gratefully acknowledge the Researchers Supporting Project number RSP-2020/123, King Saud University, Riyadh, Saudi Arabia.

Authors' contributions The authors contributed to the present study as follows: data curation: Walid F.A. Mosa; formal analysis: Nader R. Abdelsalam; funding acquisition: Hayssam M. Ali; investigation: Walid F.A. Mosa; resources: Walid F.A. Mosa and Nader R. Abdelsalam; software: Hayssam M. Ali; visualization: Walid F.A. Mosa; writing—original draft: Walid F.A. Mosa and Nader R. Abdelsalam; writing—review and editing: Hayssam M. Ali and Ibrahim A. Alaraidh Nader R. Abdelsalam.

Funding This research was funded by King Saud University, Researchers Supporting Project number RSP-2020/123, King Saud University, Riyadh, Saudi Arabia.

Data availability The data used to support the findings of this study are included within the article and the coding of the data is available from the corresponding author upon reasonable request.

Compliance with ethical standards

Competing interests The authors declare that they have no conflicts of interest.

Ethics approval and consent to participate This current study does not include any animals. "Not applicable."

Consent for publication All the authors agreeing for Consent for publication and current article does not contain data from any individual person

References

Abbas SH, Sohail M, Saleem M, Mahmood T, Aziz I, Qamar M, Majeed A, Arif M (2013) Effect of L-tryptophan on plant weight and pod weight in chickpea under rainfed conditions. Sci Technol Dev 32: 277–280

Abd El-wahed M, El-Awadi M, Salama D, Haggag W (2016) Application of nitrogen, tryptophan and their relation on growth, yield and some chemical constituents in green onion. J Chem Pharm Res 8:694–701

Abdelsalam NR, Ali HM, Salem MZ, Ibrahem EG, Elshikh MS (2018) Genetic and morphological characterization of Mangifera indica L. growing in Egypt. HortScience 53:1266–1270

Ahmed A, Khalil M, Abd El-Rahman A, Nadia AH (2012) Effect of zinc, tryptophan and indole acetic acid on growth, yield and chemical composition of Valencia orange trees. J Appl Sci Res 901–914

Ahmed FK, Hamed NA, Magdy A, ELazazy AM (2017) Effect of tryptophan and some nutrient elements foliar application on yield and fruit quality of Washington Navel orange. J Hortic Sci Ornamental Plants 9:86–97

Amin A, Awadi M, Dawood M, Gharib F, Hassan E (2014) Kinetin and tryptophan enhance yield and production efficiency of lupine (Lupinus termis L.) plants. World Rural Observations 6:50–56

Bahari A, Pirdashti H, Yaghubi M (2013) The effects of amino acid fertilizers spraying on photosynthetic pigments and antioxidant enzymes of wheat (Triticum aestivum L.) under salinity stress. Int J Agron Plant Prod 4:787–793

Basanth N, Mahesh G (2018) Bioefficacy of Nova Nutri Boost for yield and yield components in paddy (Oryza sativa L.). Int J Curr Microbiol App Sci 7:2250–2253

Bashir A, Rizwan M, Ali S, ur Rehman MZ, Ishaque W, Riaz MA, Maqbool A (2018) Effect of foliar-applied iron complexed with lysine on growth and cadmium (Cd) uptake in rice under Cd stress. Environ Sci Pollut Res 25:20691–20699

Bingham FT (1983) Boron. Methods of soil analysis: part 2. Chem Microbiol Properties 9:431–447

Chapman H, Pratt P (1961) Methods of analysis for soils. Plants Waters: 169–176

Chuntanaparb N, Cummings G (1980) Seasonal trends in concentration of nitrogen, phosphorus, potassium, calcium, and magnesium in leaf portions of apple, blueberry, grape, and peach. J Am Soc Hortic Sci 105:933–935

Colla G, Cardarelli M, Bonini P, Rouphael Y (2017) Foliar applications of protein hydrolysate, plant and seaweed extracts increase yield but differentially modulate fruit quality of greenhouse tomato. HortScience 52:1214–1220

Cottenie A, Verlo L (1982) Kjekens and Camerlynch, R.(1982): Chemical analysis of plant and soil. Laboratory of Analytical Agrochemistry. State Univ. Gent, Belgium, pp 280–284

El Sayed OM, El Gammal O, Salama A (2014) Effect of proline and tryptophan amino acids on yield and fruit quality of Manfalouty pomegranate variety. Sci Hortic 169:1–5

Elkhatib HA, Gabr SM, Roshdy AH, Kasi RS (2020) Effects of different nitrogen fertilization rates and foliar application of humic acid, fulvic acid and tryptophan on growth, productivity and chemical composition of common bean plants (Phaseolus vulgaris L.). Alexandria Sci Exch J 41:191–204

Fahimi F, Souri M, Yaghobi F (2016) Growth and development of greenhouse cucumber under foliar application of Biomin and Humifolin fertilizers in comparison to their soil application and NPK. J Sci Technol Greenh Cult 7

FAO I, UNICEF (2018) WFP and WHO (2017) The state of food security and nutrition in the world 2017: building resilience for peace and food security. Food and Agriculture Organization of the United Nations (FAO), Rome

Fawzy Z, El-Shal Z, Li Y, Zhu O, Sawan OM (2012) Response of garlic (Allium sativum, L.) plants to foliar spraying of some bio-stimulants under sandy soil condition. J Appl Sci Res 770–776

Ghasemi S, Khoshgoftarmanesh AH, Hadadzadeh H, Afyuni M (2013) Synthesis, characterization, and theoretical and experimental investigations of zinc (II)–amino acid complexes as ecofriendly plant growth promoters and highly bioavailable sources of zinc. J Plant Growth Regul 32:315–323

Hancı F, Tuncer G (2020) How do foliar application of melatonin and L-tryptophan affect lettuce growth parameters under salt stress? Turk J Agric Food Sci Technol 8:960–964

Hussain A, Ali S, Rizwan M, ur Rehman MZ, Hameed A, Hafeez F, Alamri SA, Alyemeni MN, Wijaya L (2018) Role of zinc–lysine on growth and chromium uptake in rice plants under Cr stress. J Plant Growth Regul 37:1413–1422

Kazem Souri M, Sooraki FY, Moghadamyar M (2017) Growth and quality of cucumber, tomato, and green bean under foliar and soil applications of an aminochelate fertilizer. Hortic Environ Biotechnol 58: 530–536

Khan S, Yu H, Li Q, Gao Y, Sallam BN, Wang H, Liu P, Jiang W (2019) Exogenous application of amino acids improves the growth and yield of lettuce by enhancing photosynthetic assimilation and nutrient availability. Agronomy 9:266

Khedr E (2018) Improving productivity, quality and antioxidant capacity of Le-Conte pear fruits using foliar tryptophan, arginine and salicylic applications. Egypt J Hortic 45:93–103

Malik C, Singh M (1980) Extraction and estimation of amino acids and keto acids. In: Plant Enzymology and Histo-Enzymology. Kalyani Publishers, New Delhi, p 286

Mataffo A, Scognamiglio P, Dente A, Strollo D, Colla G, Rouphael Y, Basile B (2020) Foliar application of an amino acid-enriched urea fertilizer on 'Greco'grapevines at full veraison increases berry yeast-assimilable nitrogen content. Plants 9:619

Matysiak K, Kierzek R, Siatkowski I, Kowalska J, Krawczyk R (2020) Effect of exogenous application of amino acids L-arginine and glycine on maize under temperature stress. Agronomy 10:769

Mohammadipour N, Souri MK (2019a): 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 72

Mohammadipour N, Souri MK (2019b) Beneficial effects of glycine on growth and leaf nutrient concentrations of coriander (Coriandrum sativum) plants. J Plant Nutr 42:1637–1644

Mustafa A, Hussain A, Naveed M, Ditta A, Nazli Z-e-H, Sattar A (2016) Response of okra (Abelmoschus esculentus L.) to soil and foliar applied L-tryptophan. Soil Environ 35

Noroozlo YA, Souri MK, Delshad M (2019) Stimulation effects of foliar applied glycine and glutamine amino acids on lettuce growth. Open Agric 4:164–172

Pranckietienė I, Mažuolytė-Miškinė E, Pranckietis V, Dromantienė R, Šidlauskas G, Vaisvalavicius R (2015) The effect of amino acids on nitrogen, phosphorus and potassium changes in spring barley under the conditions of water deficit. Zemdirbyste-Agriculture 102:265–272

Rabino L (1977) Alberto and MK Monrad (1977). Photocontrol of synthesis. J Plant Physiol 59:569–573

Radkowski A, Radkowska I, Godyn D (2018) Effects of fertilization with an amino acid preparation on the dry matter yield and chemical composition of meadow plants. J Elem 23

Resio ANC, Aguerre RJ, Suarez C (2005) Analysis of simultaneous water absorption and water–starch reaction during soaking of amaranth grain. J Food Eng 68:265–270

Rizwan M, Ali S, Hussain A, Ali Q, Shakoor MB, Zia-ur-Rehman M, Farid M, Asma M (2017) Effect of zinc-lysine on growth, yield and cadmium uptake in wheat (Triticum aestivum L.) and health risk assessment. Chemosphere 187:35–42

Romero I, Téllez J, Yamanaka LE, Steindel M, Romanha AJ, Grisard EC (2014) Transsulfuration is an active pathway for cysteine biosynthesis in Trypanosoma rangeli. Parasit Vectors 7:1–11

Rouphael Y, Colla G, Giordano M, El-Nakhel C, Kyriacou MC, De Pascale S (2017) Foliar applications of a legume-derived protein hydrolysate elicit dose-dependent increases of growth, leaf mineral composition, yield and fruit quality in two greenhouse tomato cultivars. Sci Hortic 226:353–360

Sharma L (2016) Effect of nutrients sprays on growth, yield and fruit quality of apple under cold desert condition of Himachal Pradesh. J Appl Nat Sci 8:297–300

Sheng Y, Cheng H, Wang L, Shen J, Tang M, Liang M, Zhang K, Zhang H, Kong Q, Yu M (2020) Foliar spraying with compound amino acid-iron fertilizer increases leaf fresh weight, photosynthesis, and Fe-S cluster gene expression in peach (Prunus persica (L.) Batsch). BioMed Res Int 2020

Snedecor GW, Cochran WG (1980) Statistical methods. 7th edn. Iowa State University USA, pp 80–86

Souri MK, Hatamian M (2019) Aminochelates in plant nutrition: a review. J Plant Nutr 42:67–78

Souri MK, Naiji M, Aslani M (2018) Effect of Fe-glycine aminochelate on pod quality and iron concentrations of bean (Phaseolus vulgaris L.) under lime soil conditions. Commun Soil Sci Plant Anal 49:215–224

Talaat IM, Khattab HI, Ahmed AM (2014) Changes in growth, hormones levels and essential oil content of Ammi visnaga L. plants treated with some bioregulators. Saudi J Biol Sci 21:355–365

Teixeira WF, Fagan EB, Soares LH, Umburanas RC, Reichardt K, Neto DD (2017) Foliar and seed application of amino acids affects the antioxidant metabolism of the soybean crop. Front Plant Sci 8:327

Weiland M, Mancuso S, Baluska F (2016) Signalling via glutamate and GLRs in Arabidopsis thaliana. Funct Plant Biol 43:1–25

Westwood MN (1988) Temperate-zone pomology. Timber press

Wilde S, Corey R, Lyer I, Voigt G (1985) Soil and plant analysis for tree culture, 3rd edn. Oxford & IBH publishing Co, New Delhi, pp 1–218