Greenhouse Plant Production Journal 1(4) (2024) 36–48 gppj.araku.ac.ir

Greenhouse Plant Production Journal

The Impact of Arginine on the Fruit Quality Attributes of Strawberry (Fragaria × ananassa Duch.) cv. Queen Eliza

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Original Article

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Citation: Pakkish, Z. and Mohammadi, H. 2024. The Impact of Arginine on the Fruit Quality Attributes of Strawberry (Fragaria × ananassa Duch.) cv. Queen Eliza. Greenhouse Plant Production Journal, 1(4): 36–48.

https://10.61186/gppj.1.4.36

KEYWORDS

Amino Acids
Anthocyanin
Phenol content
Qualitative treaties
Spraying Time

ARTICLE HISTORY

Received: 07 December 2024
Revised: 24 December 2024
Accepted: 31 December 2024

ABSTRACT

Strawberries are one of the most important fruits in horticulture. In this research, the effects of arginine application timing and different concentrations on some qualitative and quantitative characteristics of the strawberry cultivar 'Queen Eliza' were investigated. Plants were treated with arginine at concentrations of 0, 250, and 500 µM at two stages (30 days after planting and at the first blooming) in a factorial experiment based on a randomized complete block design with four replications under greenhouse conditions. The results showed that the fruits of plants treated with arginine exhibited improved qualitative characteristics compared to the control treatment. Specifically, the application of different levels of arginine increased yield, primary and secondary fruit weight, and the number of achenes (quantitative characteristics). Additionally, it enhanced total soluble solids, reducing sugars, titratable acidity, anthocyanin, phenol, and vitamin C content (qualitative characteristics). The most effective treatment and optimal spraying time were 500 µM arginine and 30 days after planting, respectively, for improving both qualitative and quantitative characteristics. This study provides the first evidence that arginine enhances the quantitative characteristics of strawberry fruits, thereby improving overall fruit quality.

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Publisher: Arak University

1. Introduction

Strawberry (Fragaria × ananassa Duch.) is a delicious fruit with significant health benefits (Chung *et al*., 2002) and is cultivated worldwide, including in Iran. In Iran, strawberries are one of the most important crops, grown both for local consumption and export, particularly from December to April (Eshghi and Jamali, 2009). Strawberries are rich in natural antioxidants, such as carotenoids, vitamins, phenols, flavonoids, dietary glutathione, and endogenous metabolites. They exhibit high antioxidant capacity, effectively combating free radical species, including superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen (Wang and Jiao, 2000). The high antioxidant activity of strawberries contributes to reducing carcinogens in humans (Bickford *et al*., 2000; Chung *et al*., 2002; Kresty *et al*., 2001). Anthocyanins, which are present in high concentrations in strawberries, are believed to play a significant role in the fruit's total antioxidative activity (Wang *et al*., 1997). Both the total antioxidant activity and anthocyanin content can vary depending on the cultivar and cultivation techniques. These factors may influence the overall health-protective benefits of strawberries, making them a subject worthy of further investigation. The application of amino acids for foliar use is based on its requirement by plants in general and at critical stages of growth in particular. Plants absorb amino acids through stomatas and are proportional to environment temperature (Vasanth *et al*., 2006). Arginine is one of the essential amino acids (considered the main precursor of polyamines, agmatine and proline as well as the cell signaling molecules glutamine and nitric oxide (Chen *et al*., 2004; Liu *et al*., 2006; Wang *et al*., 2021), which produced by decarboxylation of arginine via arginine decarboxylase to form putrescine (Evans and Malmberg, 1989; Bocherueu, 1999). Polyamines and their precursor arginine have been implicated as vital modulators in a variety of growth, physiological and developmental processes in higher plants (Glastone and Kaur-sawhny, 1990). Polyamines are involved in the control of cell cycle, cell division, morphogenesis in phytochrome and plant hormone mediated process and the control of plant senescence, as well as in plant response to various stress factors (Almutairi *et al*., 2022; Walters, 2000). The application of arginine significantly promoted the growth and increased the fresh and dry weights, certain endogenous plant growth regulators, chlorophylls a and b and carotenoids in bean (Nassar *et al*., 2003; Nasibi *et al*., 2020); and wheat (El-Bassiouny *et al*., 2008). Moreover, researchers reported positive role of arginine in alleviating the inhibition occurs as the result of exposing plants to stress (Hassanein *et al*., 2008; Hussein *et al*., 2022; Khalil *et al*., 2009). In higher plants, it has also been proposed that both endogenous and exogenous arginine have roles in plant growth responses, (Li *et al*., 2024; Zeid, 2009; Nasibi *et al*., 2011; Song *et al*., 1999; Zheng *et al*., 2011). Positive effects of exogenous NO (Liu *et al*., 2011; Neill *et al*., 2003), PAs (He *et al*., 2002) and Prolin (Patton *et al*., 2007; Gothandam *et al*., 2010) on growth and development in stress conditions have been reported previously. However not any data are available on the effect of exogenous arginine as a precursor of these compounds in growth and development of plants such as strawberry.

The simulative effect of arginine as polyamine precursor on growth and yield component may act as protective agent in plants adapted to extreme environment (Abd El-Monem, 2007; Heinemann and Hildebrandt, 2021). Paschalidis and Roubelakis-Angelakis (2005) reported that PAS, their precursor arginine and their biosynthetic enzymes are involved in stimulation of cell division, expansion and differentiation and vascular development in tobacco plant. Hassanein *et al*. (2008) found that arginine at 2.5 mM was the most effective in improving growth and yield of wheat plant exposed to high temperature stress. Moreover, researchers concluded that foliar application of arginine on wheat exhibited significant increments in the growth and all yield parameters in comparison to control treatment (Abd El-Monem, 2007; Li *et al*., 2023; Mostafa *et al*. 2009).

The aim of this study is to investigate the effects of exogenous arginine application on the growth, yield, and quality of strawberry (Fragaria × ananassa Duch.) cv. Queen Eliza. Given the high health value of strawberries, attributed to their rich antioxidant content, including anthocyanins, phenols, and vitamins, this research seeks to explore how arginine can enhance both qualitative and quantitative characteristics of strawberry fruits. Specifically, the study aims to determine the optimal concentration and timing of arginine application to improve yield, fruit weight, antioxidant capacity, and overall fruit quality. Also by addressing the lack of data on the role of arginine in strawberry cultivation, this research provides novel insights into its potential as a growth enhancer and protective agent, contributing to improved cultivation practices and increased consumer demand for this nutritionally valuable fruit.

2. Martial and Methods

This study was carried out at commercial greenhouse of Jiroft city, Iran. Induced and rooted daughter plants of strawberry cultivar Queen Eliza were planted in greenhouse. Greenhouse conditions (including temperature,22 °C, humidity 60-70%, hours of light (16h)_and darkness(8h). The strawberries were cultivated in rows on the surface of the greenhouse, and the soil used for their cultivation was loamy. No fertilizers were used during the growing season. The growth period of the strawberries was considered to be 3 months. The treatments were including: different arginine concentration (0, 250 and 500µM) and different times of spraying (30 days after planting and blooming stage). After applying the treatments, characteristics such as: yield, weight of primary and secondary fruits and number of their achenes (quantitative characteristics) total soluble solid, inducing sugar, titratable acidity, anthocyanin, phenol, dry weight, vitamin C (qualitative characteristics) was measured.

2.1. Measurements of Total soluble solids (TSS)

Total soluble solids (TSS), were taken on 5 fruit per plant at harvest using a hand-held refractometer (American Optical Co., Keene, N.H.). Titratable acidity and vitamin C measurement were determined by method of Basiouny and Woods (1992).

2.2. Assays of Reducing sugars content

Determination of reducing sugars extracted one gram sample of fruit tissue with 20 ml of 50% ethanol and then incubated in the oven at 60°C for 2h. One ml of the supernatant liquid was mixed with 0.5 ml of 0.1N hydrochloric acid and boiled for 15 min. This mixture was then mixed with 0.5 ml of 0.1N sodium hydroxide. One ml of the supernatant liquid was then taken for quantifying the reducing using Hodge-Hofreiter's method (Hodge and Hofreiter, 1962)

2.3. Assays of Anthocyanin Content

An aliquot of extract was combined with ethanolic HCI solution (0.25 M) to give a dilution 1:10. The solution was mixed thoroughly, and the absorbance at 520 nm (A520) was read after 5 min, using the ethanolic HCI solution as blank. Total anthocyanin content was determined as cyanin (cyanidin 3-O-glucoside) equivalents (CyE) per 100 g fresh tissue, using e = 26900 and MW = 449.2 (Kim *et al*., 2003).

$$\%W/W = \frac{A}{\varepsilon l} \times MW \times DF \times \frac{V}{WT} \times 100$$

A_{520}: (Absorbance)

ε (epsilon): (Molar Extinction Coefficient) = 26900

%W/W: (Weight/Weight Percentage)

MW: (Molecular Weight) cyanidin 3-O-glucoside = 449.2 (g/mol)

DF: (Dilution Factor) = 10

2.4. Assays of Phenol Compound

Measurements were carried out according to a previously published protocol (Arnous, *et al*., 2002), employing the Folin-Ciocalteu methodology. Gallic acid was used as the reference standard, and results were expressed as mg gallic acid equivalents (GAE) per 100 g of fresh tissue. An aliquot of extract was combined with ethanolic HCI solution (0.25 M) to give a dilution 1:10. The solution was mixed thoroughly, and the absorbance at 520 nm (A520) was read after 5 min, using the ethanolic HCI solution as blank.

2.5. Statistical analysis

The experimental design was a factorial experiment based on completely randomized design with single plant and four replications. Data were analyzed by analysis of variance (ANOVA) and the means were compared (p≤0.05) by Dancan's multiple rang test (DMRT). All analyses were performed by using SAS (ver 9.1).

3. Results and Discussion

3.1. Yield

Results showed that fruits of treated plants by arginine, compared to control treatment (Spraying with distilled water) improved qualitative characteristics. So, application arginine in levels concentration application were increased yield (Fig 1), weight of primary and secondary fruits (Fig 2A and B) and number of their achenes (Fig 3A and B) (quantitative characteristics), and effective treatment and best spraying time was arginine 500 µM and 30 days after planting, respectively for qualitative characteristics improvement.

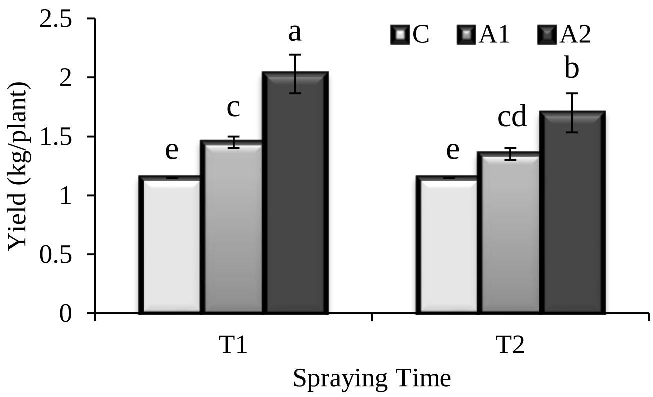

Fig. 1 Effect of arginine on yield of strawberry Queen Eliza cultivar. (C: control, A1: Arginine 250µM, and A2: Arginine 500µM and T1: 30 days after planting and T2: first blooming. Means followed by same letter are not significantly different at P<0.05 according to Duncan's multiple range tests.

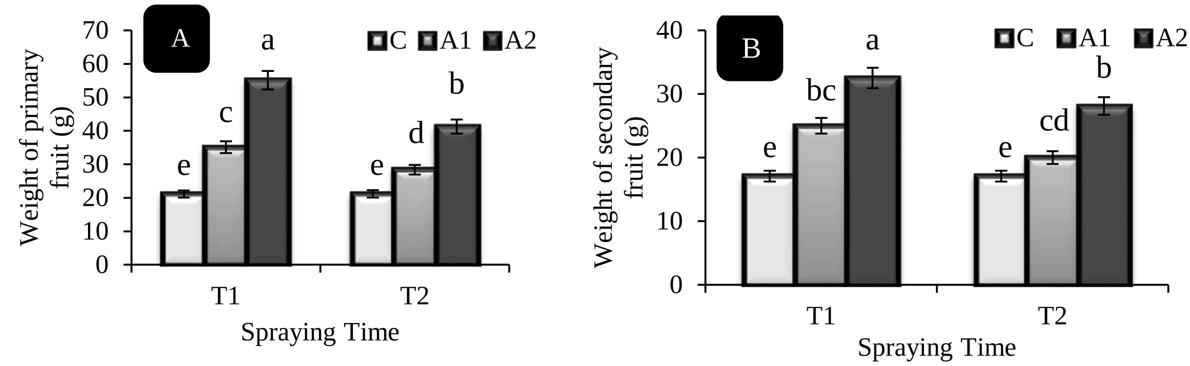

Fig. 2A and B Effect of arginine on weight of primary (A) and secondary (B) fruit of strawberry Queen Eliza cultivar. (C: control, A1: Arginine 250µM, and A2: Arginine 500µM and T1: 30 days after planting and T2: first blooming. Means followed by same letter are not significantly different at P<0.05 according to Duncan's multiple range tests.

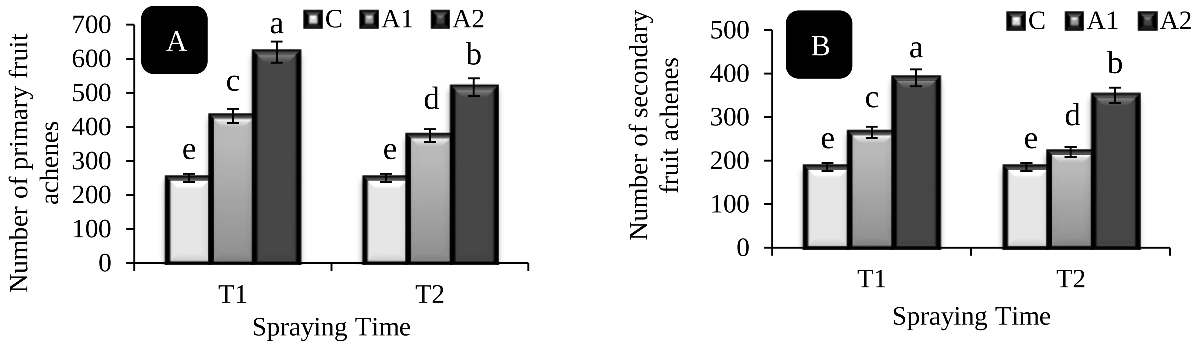

Fig. 3A and B Effect of arginine on fruit achens number in of primary (A) and secondary (B) fruit of strawberry Queen Eliza cultivar. (C: control, A1: Arginine 250µM, and A2: Arginine 500µM and T1: 30 days after planting and T2: first blooming. Means followed by same letter are not significantly different at P<0.05 according to Duncan's multiple range test.

3.2. Total soluble solid and inducing sugar

Strawberries treated with arginine had higher total soluble solids and inducing sugar compared to the control treatment and effective treatment and best spraying time was arginine 500 µM and 30 days after planting (Fig 4A and B).

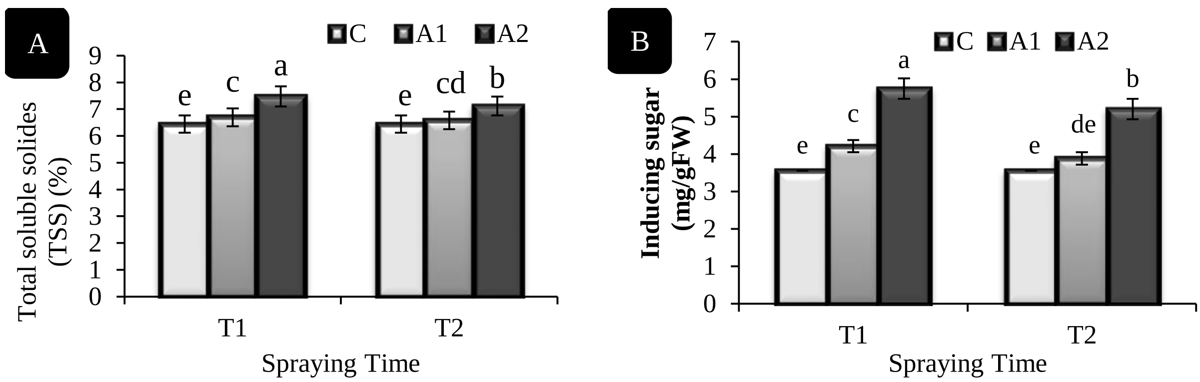

Fig. 4 A and B Effect of arginine on fruit total soluble solids (TSS) (A) and inducing sugar (B) of strawberry Queen Eliza cultivar. (C: control, A1: Arginine 250µM, and A2: Arginine 500µM and T1: 30 days after planting and T2: first blooming. Means followed by same letter are not significantly different at P<0.05 according to Duncan's multiple range tests.

3.3. Organic acids

The organic acids in the treated strawberries increased, but no such improvement in organic acids was observed in the control strawberries and effective treatment and best spraying time was arginine 500 µM and 30 days after planting (Fig 5).

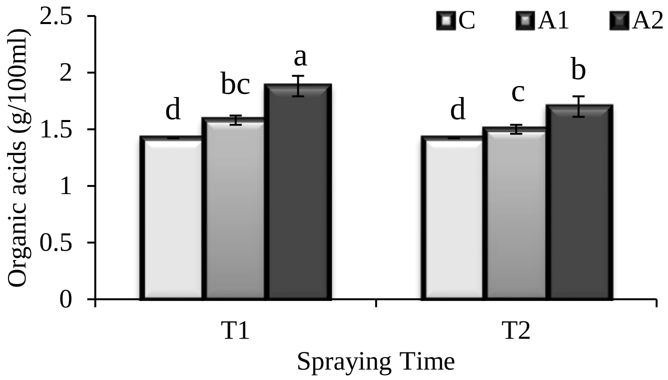

Fig. 5 Effect of arginine on fruit organic acids of strawberry Queen Eliza cultivar. (C: control, A1: Arginine 250µM, and A2: Arginine 500µM T1: 30 days after planting and T2: first blooming. Means followed by same letter are not significantly different at P<0.05 according to Duncan's multiple range tests.

3.4. Anthocyanin and phenol content

The levels of anthocyanin and phenol in the strawberries under this study changed such that, according to the obtained results, strawberry plants treated with the amino acid arginine had significantly higher anthocyanin pigment compared to the control treatment, showing a significant difference between them. Regarding phenolic compounds, the control treatment had the lowest amount, while the treatment with the amino acid arginine showed the highest phenol content and effective treatment and best spraying time was arginine 500 µM and 30 days after planting, respectively (Fig 6 and 7).

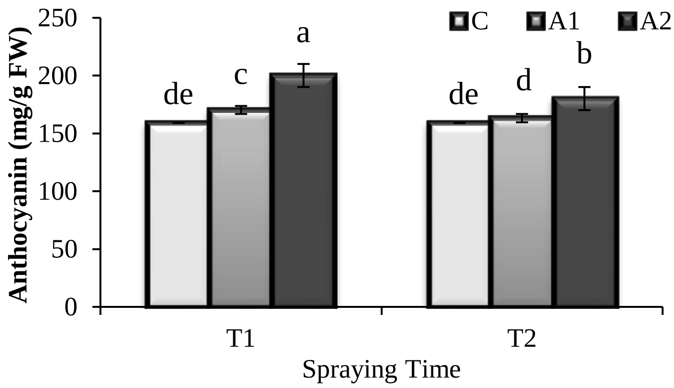

Fig. 6 Effect of arginine on fruit anthocyanin of strawberry Queen Eliza cultivar. (C: control, A1: Arginine 250µM, and A2: Arginine 500µM and T1: 30 days after planting and T2: first blooming. Means followed by same letter are not significantly different at P<0.05 according to Duncan's multiple range tests.

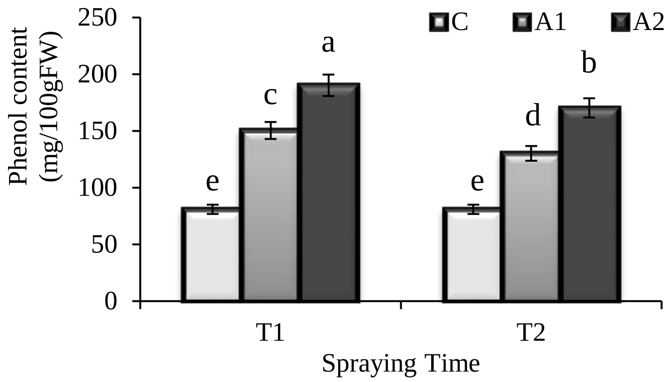

Fig. 7 Effect of arginine on fruit phenol content of strawberry Queen Eliza cultivar. (C: control, A1: Arginine 250µM, and A2: Arginine 500µM and T1: 30 days after planting and T2: first blooming. Means followed by same letter are not significantly different at P<0.05 according to Duncan's multiple range tests.

3.5. Vitamin C

The vitamin C content in strawberries treated with arginine showed a significant increase compared to the control and effective treatment and best spraying time was arginine 500 µM and 30 days after planting (fig 8).

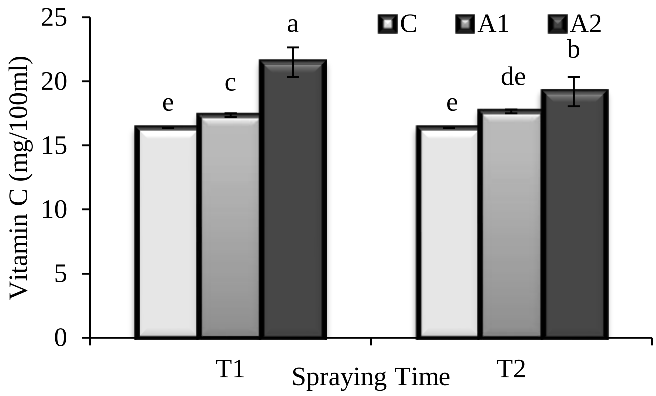

Fig. 8 Effect of arginine on fruit vitamin C of strawberry Queen Eliza cultivar. (C: control, A1: Arginine 250µM, and A2: Arginine 500µM and T1: 30 days after planting and T2: first blooming. Means followed by same letter are not significantly different at P<0.05 according to Duncan's multiple range tests.

4. Discussion

So, the simulative effect of arginine as polyamine precursor on growth and yield component may act as protective agent in plants adapted to extreme environment (Kuhen *et al.*, 1990; Abd El-Monem, 2007). Paschalidis and Roubelakis-Angelakis (2005) reported that PAs (Poly Amines), their precursor arginine and their biosynthetic enzymes are involved in stimulation of cell division, expansion and differentiation and vascular development in tobacco plant. Hassanein *et al.* (2008) found that arginine was the most effective in improving growth and yield of wheat plant exposed to high temperature stress. Moreover, researchers concluded that foliar application of arginine on wheat exhibited significant increments in the growth and all yield parameters in comparison to control treatment (Abd El-Monem,2007; Mostafa *et al.* 2009).

Foliar applied arginine due to syntheses polyamines and polyamines act as growth regulator is able to modulate the plant metabolism and the production of metabolites involved in stress tolerance (Anthony *et al.*, 2007; Tassoni *et al.*, 2002: Francisco *et al.*, 2006).

Aarginine is one of the most functionally diverse amino acids in living cells. In addition to serving as a constituent of proteins, arginine is a precursor for biosynthesis of polyamines, and proline, that there molecules act as development and growth regulator (Chen *et al*., 2004; Liu *et al*., 2006). The role of amino acids in growth and differentiation is known to a considerable extent (Yamada *et al.*, 1986).

Mechanism of arginine action to improve yield and development in plants include: arginine is precursor of growth factors such as polyamines, and proline (Chen *et al*., 2004; Liu *et al*., 2006; Liu *et al*., 2023). Polyamines have been suggested to be associated with cell division. Therefore, they can be utilized to regulate fruit development. Exogenous application of PAs has also been reported to promote reproductive development under normal growth conditions and offer protection to reproductive structures against abiotic stress (Alburquerque *et al*., 2006; Bibi *et al*., 2010; Malik and Singh, 2006; Nayyar, 2005; Ndayiragije and Lutts, 2007; Sun *et al*., 2023). The general positive effects observed at this study on yield and fruit characteristics of strawberry fruits as a result of applying arginine could be attributed to enhancement effects of polyamines synthesis on floral developments which resulted on both fruit set and fruit yield. In addition, accumulating evidences has showed that endogenous polyamine is an important for pollen germination and pollen tube growth (Wolukau *et al*., 2004). Furthermore, putrescine enhanced pollen tube ovule penetration and delayed ovule senescence without affecting flower ethylene production (Cheng *et al*., 2023; Crisosto *et al*., 1988; Crisosto *et al*., 1992). Also, the effect of polyamines has been ascribed to increase viability of ovule and prolonged pollination period (Kitashiba *et al*., 2005). Bagni *et al.*(1981) informed that, biosynthesis of polyamines from arginine took place before pollen tube emergence. On the other hand, the influence of polyamines in increasing fruit set has been observed in apples, and pears (Crisosto *et al*., 1988; Crisosto *et al*., 1992, Costa and Bagni, 1983, Li and Hoppe, 2023; Costa *et al*., 1986, Franco-Mora *et al*., 2005). The same results were reported by Biasi and Bagni (1991) on litchi and costa and Bagni (1983) on olives. As fruit characteristics, our abovementioned results cleared that application of arginine had a positive effect in fruit quntitative and chemical characteristics this effects were agreement with results obtained by Bregoli *et al.*(2002) in peach and Torrigiani *et al.*(2004) in nectarine. In the initial stage of fruit development, active cell division occurs, which possibly needs sufficient polyamines (Bouchereau *et al*.,1999 Hildebrandt *et al*., 2015). At the later stage of fruit development, cell division gives way to cell enlargement, in which polyamine synthesis is reduced. On the other hand, decrease in polyamines at the late stage of fruit development has been regarded as a signal for fruit ripening. Moreover, since polyamines (especially Spd (Spermide) and Spm (Spermidine)) share the same precursor (arginine), have demonstrated their function for improving apricot fruit (Paksasorn *et al*.,1995), or increasing physical and chemical characteristics in peaches (Bregoli *et al.* 2002], nectarine (Torrigiani *et al.* 2004) and pear(Franco-Mora *et al*., 2005). Bagni *et al.*(1981) reported that apple fruits treated with Spm and Put reached higher sugar content. While, Plum fruits treated with put showed lower soluble solutions and tetratable acids(Serrano *et al*., 2003).

Arginine induces synthesis of flower and fruit related hormones (stimulates phytohormones synthesis) (Tarenghi and Martin-Tanguy 1995: Zeid, 2009), promotes uptake of macro and micro nutrients, chelating effect and rooting (Jones *et al*., 2002; Jones *et al*., 2005;Orlikowska 1992; Sarropoulou *et al*., 2014), promotes enzymatic activity by acting as natural stimulates photosynthesis (argenine get readily absorbed through leaves and aid in protein synthesis. It also leads to better synthesis of chlorophyll and helps to improve yield (Yagi and Abdulkareem 2006), action on the stomas (El-Bassiouny and Bekheta, 2001; He *et al*., 2002), stimulates proteins and enzymes synthesis(Bibi *et al*., 2010)., induces flowering and fruit set related hormones (Vasanth *et al*., 2006), increase stress resistance (Liu *et al*., 2006; Liu *et al*., 2011; Nasibi *et al*., 2011), improve pollination and fruit formation (Anthony *et al*., 2007; Mostafa *et al*., 2009). So, antioxidative properties of arginine -treated strawberry plants improved by arginine. In this respect, both concentration of arginine used in the present investigation increased this property in terms of stimulating the accumulation of some antioxidants as ascorbic acid, phenol compounds, titratable acidity, and anthocyanin (Fig 4-9). Perhaps, arginine by motioned reasons increases yield and improving qualitative characteristics of crops such as strawberry. This result is in agreement with the results obtained by (El-Bassiouny *et al*., 2008, Hassanein *et al*., 2008, Khalil *et al*., 2009, Liu *et al*., 2006, Mostafa *et al*., 2009, Nasibi *et al*., 2011, Sarropoulou *et al*., 2014, Vasanth *et al*., 2006, Yagi and A-abdulkareem, 2006,Zeid, 2009).Therefore, the goal of the present work is achieved through improving the yield and the quality of strawberry by foliar application of arginine, and consequently maximizing the endogenous antioxidant content in strawberry fruit necessary for human health.

Conclusion

The findings of this study demonstrate that foliar application of arginine significantly enhances both the quantitative and qualitative characteristics of strawberry plants (cv. Queen Eliza) grown under greenhouse conditions. Treatment with arginine at concentrations of 250 and 500 µM, applied at two critical growth stages—30 days after planting and first blooming resulted in notable improvements in yield, fruit weight, and overall fruit quality. The most effective treatment was observed with 500 µM arginine applied 30 days after planting, which optimized key parameters such as total soluble solids, anthocyanin content, phenol levels, vitamin C, and antioxidant activity. These results highlight the potential of arginine as a powerful tool for improving strawberry production and fruit quality, offering valuable insights for growers aiming to meet increasing consumer demand for high-quality, nutritionally rich strawberries. This study provides the first evidence of arginine's role in enhancing strawberry cultivation, paving the way for its broader application in horticultural practices.

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