

In vivo polyamine applications to globe artichoke: Effects on plant growth-development and some bioactive components

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Abstract

The globe artichoke, *Cynara cardunculus* var. *scolymus* (L.), is an important member of the *Asteraceae* family, being used as a food and due to it has certain health-improving qualities in the treatment of various disorders since ancient times. Low molecular weight organic compounds with a role in several crucial cellular and physiological processes of plants are known as polyamines. They participate in processes such as cell division, differentiation, and growth, the sprouting of seeds, root formation, and flower and fruit development. They have been identified as particularly efficient against biotic and abiotic stress factors. The present research aimed that the evaluation of effects of various polyamine (PA) types and concentrations on three different artichoke cultivars by biometric measurements and biochemical analyses. For this purpose, seedlings of three artichoke cultivars ('Sakız OP', 'Bayrampaşa OP', and 'Olympus F₁') were sprayed with various concentrations (0, 50, 100 and 250 mg L⁻¹) of three distinct types of significant polyamines, including putrescine, spermidine, and spermine. Several growth parameters were examined in the current research. The effects on the total phenol and antioxidant capacities, as well as changes in the amounts of cynarin and 1,5-*O*-dicafeoylquinic acid, were also evaluated. The results revealed that there were statistically differences based on cultivars and treatment concentrations. Among the three forms of PA spermine was potent at increasing total phenolic content, while putrescine was effective at increasing total antioxidant content. On the other hand, putrescine, spermine and, spermidine had no positive effects on cynarin and 1,5-*O*-dicafeoylquinic acid levels. Results clearly revealed that each biometric data and biochemical content could not be determined by a single polyamine type and concentration. It can be concluded that to improve the biometric values and biochemical content are depending on cultivar and dosage of various PAs.

Keywords: biochemical analyses; biometric measurements; putrescine; spermidine; spermine

Introduction

Globe artichoke [*Cynara cardunculus* var. *scolymus* (L.)] cultivation is currently quietly intense in the countries of the Mediterranean region as it is recognized one of the significant members of the *Asteraceae* family.

Polyamines are low molecular weight hydrocarbons that contain one or more amine groups and are essential for a number of vital cellular and physiological processes. They actively participate as regulatory

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molecules in seed germination, rooting, flower and fruit growth and development in addition to basic cellular functions, including protein and DNA synthesis, gene expression, cell division, differentiation, and growth. However, under biotic and abiotic stress circumstances, they have significant functions in plants (Amorim *et al.*, 2017; Mustafavi *et al.*, 2018; Wang *et al.*, 2019). Putrescine, spermidine, and spermine are the three most prevalent polyamines found in plants (Yousefi *et al.*, 2019).

According to research conducted so far, externally treated polyamines, particularly putrescine, positively influence a plant growth and development and may even boost the accumulation and/or production of already present bioactive components (Bais *et al.*, 1999). It is reported that external applications of polyamines may promote bioactive component production or accumulation in plants. Therefore, it is possible to acquire by-products from plants that have high added value as well as greater consumer attractiveness from the plants' viewpoint. This might also increase the value of plant components, such as leaves, that are not edible for using in nutraceuticals and agrochemicals, as well as in many other industrial sectors (Mostafaei *et al.*, 2018; Orlando *et al.*, 2019; Coman *et al.*, 2020; Collado-González *et al.*, 2021).

Several researchers examined the role of polyamines on plant growth and development. For example, in experiments conducted on pepper by Shawky (2003) and on gladiolus plants by Nahed *et al.* (2009), the beneficial effects of externally applied polyamines on plant vegetative development were clearly demonstrated. In a different research, orange trees were subjected to foliar treatment of putrescine and spermidine, and it was shown that this treatment increased nutrient intake via roots and encouraged plant development. In research conducted by Badawy *et al.* (2015), it was reported that foliar spraying 200 mg L⁻¹ of putrescine on *Antirrhinum majus* L. plants had a good impact on the plant root development. In another study by Danaee and Abdossi (2019), they assessed the impacts of polyamines such putrescine, spermidine, and spermine on basil plants, and reported that polyamines increased the fresh and dry mass of plant roots.

The current study is conducted to identify the type and concentration of polyamines on globe artichoke cultivars in order to see their effect on biometric measures, the number of leaves, stem length, stem diameter, root length, plant height, SPAD values (chlorophyll assay), and fresh-dry weights of the leaves, and some of the bioactive content such as cynarin, 1,5-*O*-dicaaffeoylquinic acid, total phenol and total antioxidant contents.

Materials and Methods

Plant materials and growing conditions

Trials were performed at the Akdeniz University, Faculty of Agriculture, and Department of Horticulture. Globe artichoke seeds were harvested from the plants grown in trial fields of stated department in 2022. Artichoke seeds were sown in plastic pots with a diameter of 35 cm and a length of 13 cm filled with combination of peat: perlite mortar (2:1 v/v). For each cultivar, three globe artichoke achenes were sown in each prepared mortar mixture, and when the first true leaves appeared, they were reduced to one plant in each pot in a greenhouse.

Polyamine treatments

Three globe artichoke cultivars' namely 'Sakız OP', 'Bayrampaşa OP' and 'Olympus F₁' biometric measures and biochemical analyzes were subjected to factorial experimental design in a completely randomized manner with three replications to examine the impact of various kinds and concentrations of polyamines on these assays. Three types of polyamine (PA) namely putrescine, spermidine, and spermine (Sigma-Aldrich) were employed with four different concentrations (0 (control), 50 mg L⁻¹, 100 mg L⁻¹, and 250 mg L⁻¹). After the achenes of different artichoke cultivars were sown, PAs at the specified concentrations were sprayed when seedlings had 4-5 leaves and continued with 14 days intervals during two months. After each treatment

observations were recorded firstly 14 days after first foliar PA treatment while second observation was conducted 14 days after last foliar PA application.

Biometric measurements

The number of leaves, stem length, stem diameter, root length, plant height, SPAD values (chlorophyll assay), and fresh-dry weights of the leaves were some of the morphological parameters examined in the current research after foliar PA treatment. The number of leaves per plant was counted and a ruler was used to measure plant height, root length, and stem length, and a digital caliper was used to assess stem diameter while portable chlorophyll meter, the SPAD (SPAD-502, Minolta Corporation, Ltd., Osaka, Japan), was used to measure the quantity of chlorophyll in the leaves. Measurements were taken at the leaf center to determine SPAD values. For each application, twenty random readings were used, averaged, and converted into a single SPAD value. The fresh and dried weights of the leaves were determined using a digital scale (0.0001 g).

Biochemical analyses

Biochemical analyses of cynarin, and 1,5-*O*-dicaffeoylquinic acid, total phenol-antioxidant contents were conducted in randomly chosen leaf samples of artichoke cultivars.

By using the Singleton *et al.* (1999) method, the total phenol content of leaf samples was determined. Folin-Ciocalteu reagent (FCR) was utilized to serve the purpose. Briefly, the leaf sample extracts were diluted in ethanol at a 500 g mL concentration, and then 0.5 mL of FCR was added. The extracts were then homogenized by shaking 3 minutes, and then 0.5 mL of saturated Na₂CO₃ solution was added to the mixture. Later on, deionized water was added and diluted to 5 mL. Following these procedures, the resulting mixture was left in the dark for two hours and afterward absorbance values were measured using a spectrophotometer at 760 nm. By creating a gallic acid calibration graph, the results were expressed as gallic acid equivalent (mg GAE/g extract).

The total antioxidant content of leaf samples was determined as follows. Solutions of copper (II), neocuproin, and ammonium acetate buffer at 1 mL volume each were added to test tubes with leaf sample extracts in. Then antioxidant solution and distilled water were added to the same test tubes until the final volume reached to 4.1 mL. After they were shaken well, and left at room temperature for 30 minutes, absorbance values at 450 nm were calculated with the use of a spectrophotometer.

After weighing the stock solutions of cynarin and 1,5-*O*-dicaffeoylquinic acid with an accuracy of 0.01 mg, the standards were dissolved in pure methanol. The prepared stock solutions were stored at -18 °C until analyzing. Calibration curves required for quantitative measurement were prepared by making dilutions at various ratios from cynarin and 1,5-*O*-dicaffeoylquinic acid stock solutions and then injected into HPLC at concentrations of 5, 10, 25, 50, 100 and 250 mg kg⁻¹.

A liquid chromatography system (HP 1100 HPLC-DAD) was used to measure the concentrations of cynarin and 1,5-*O*-dicaffeoylquinic acid in the artichoke leaf extracts. At 330 nm, measurements were conducted by using DAD detector. The injection volume was 20 µL, the column oven temperature was 28 °C, and each injection lasted in 70 minutes, including the time required to prepare for the next injection. Mobile phases A and B for the HPLC analysis were acetonitrile and water containing 5% acetic acid, respectively.

Statistical analyses

The current experiment was performed as a completely randomized factorial design with three replications. The JMP version 5.0.1 statistical package program (SAS Institute Inc., Cary, NC, USA) was used to analyse the data obtained. The data were subjected to one-way analysis of variance (ANOVA) followed by Least Significant Difference (LSD) test at the 5% significance level.

Results and Discussion

In the present study, according to observations made after the first foliar PA treatment and 14 days after the last foliar PA treatment, statistically significant differences were recorded in terms of the evaluated parameters.

Biometric measurements

There were statistically significant variations among cultivars and PA treatment in terms of number of leaves after to the first foliar PA treatment. The highest number of leaves was recorded in ‘Olympus F₁’ and ‘Sakız OP’ cultivars that reacted best in terms of number of leaves while 50 mg L⁻¹ putrescine and 50 mg L⁻¹ spermine treatments resulted with the highest number of leaf formation (Figure 1a). Considering the measurements observed after last foliar PA treatment, ‘Sakız OP’ was found the best responsive among the cultivars, while 50 mg L⁻¹ spermine and 250 mg L⁻¹ putrescine was the best regarding the PA types and concentrations (Figure 1b). According to Yousefi *et al.* (2021), treatment with putrescine or spermidine at various doses led to the biggest rise in the number of leaves, whereas spermine produced the fewest leaves in their study conducted on rose. Regarding that, Mahgoub *et al.* (2011) reported that foliar application of different types of PAs increased the number of leaves of *Dahlia pinnata*. Other research, like those on *Populus euramericana* by Habba *et al.* (2016) and *Antirrhinum majus* by Badawy *et al.* (2015), revealed positive polyamine impacts on number of leaves, as in the current study.

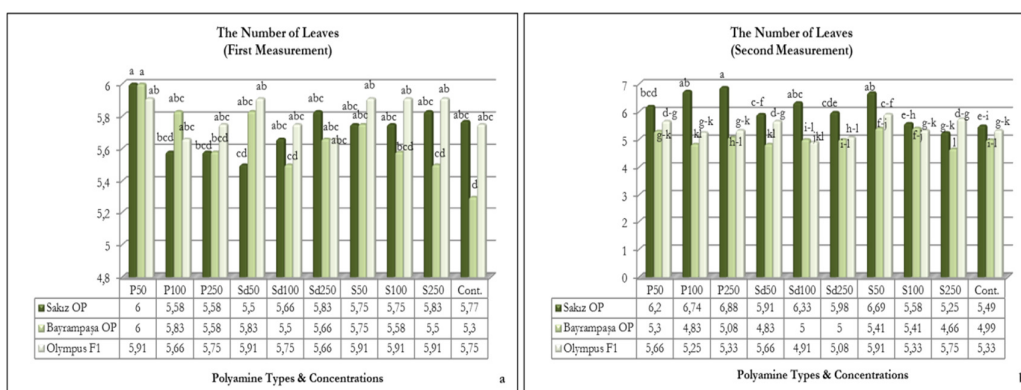


Figure 1. Effect of polyamine on; the number of leaves: (a) First measurement; (b) Second measurement (1): Differences between the means are shown with separate letters (2): LSD test, N.S.: Non-Significant ***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$; (a) $LSD_c^* = 0,113$; $LSD_t = N.S.$; $LSD_{c \times t} = N.S.$; (b) $LSD_c^* = 0,175$; $LSD_t^* = 0,321$; $LSD_{c \times t}^* = 0,556$ (c: cultivar; t: treatment; $c \times t$: cultivar \times treatment) (3): Abbreviations: P50= 50 mg L⁻¹ putrescine; P100= 100 mg L⁻¹ putrescine; P250= 250 mg L⁻¹ putrescine; Sd50= 50 mg L⁻¹ spermidine; Sd100= 100 mg L⁻¹ spermidine; Sd250= 250 mg L⁻¹ spermidine; S50= 50 mg L⁻¹ spermine; S100= 100 mg L⁻¹ spermine; S250= 250 mg L⁻¹ spermine; Cont.= Control

In terms of stem length, according to the observations made after the first foliar PA application, statistically significant differences were determined among cultivars and PA types and concentrations. Accordingly, while ‘Sakız OP’ and ‘Olympus F₁’ stood out among the cultivars, 250 mg L⁻¹ spermidine and 50 mg L⁻¹ spermine applications resulted with the highest stem length (Figure 2a). According to the second measurement in terms of stem length, among cultivars ‘Olympus F₁’ and 100 mg L⁻¹ putrescine, 250 mg L⁻¹ spermidine, 100 mg L⁻¹ spermine and 250 mg L⁻¹ spermine among PA treatments were found statistically significant. In the relation among cultivars and PA types and concentrations, ‘Olympus F₁’ with 250 mg L⁻¹ spermidine treatment came to the fore (Figure 2b). As in the present study, the studies conducted by several researchers on different plants have indicated that PAs positive impact on plant growth and development such

as the number of leaves, stem lengths, and plant heights (Mahgoub *et al.*, 2011; Dastyaran and Hosseini Farahi, 2014; Badawy *et al.*, 2015).

When the observation data of the stem diameter after the first foliar PA application were evaluated, differences were determined among cultivars and PA types and concentrations. Accordingly, while ‘Bayrampaşa OP’ among cultivars and 50 mg L⁻¹ spermidine among PA treatments stood out, it was determined that ‘Bayrampaşa OP’ cultivar responded well to 250 mg L⁻¹ putrescine compared to the others regarding the relationship among cultivars and PA types and concentrations (Figure 2c). According to the measurements made after the second foliar PA application, it was determined that ‘Sakız OP’ and ‘Bayrampaşa OP’ cultivars better responded than Olympus F₁ cultivar in terms of stem diameter, and 250 mg L⁻¹ spermine application came to the fore among the PA applications (Figure 2d). Polyamines are regarded as a novel class of in-plant growth-causing bioregulators for plants. It was reported that endogenous polyamine levels directly affect the pace of plant development, and when their manufacture fails, plant growth is either slowed down or completely stopped. Thus, it is thought that the increased activity of cell division may be responsible for stem growth (Ashari *et al.*, 2008) and it is thought that the current study is in compliance with it.

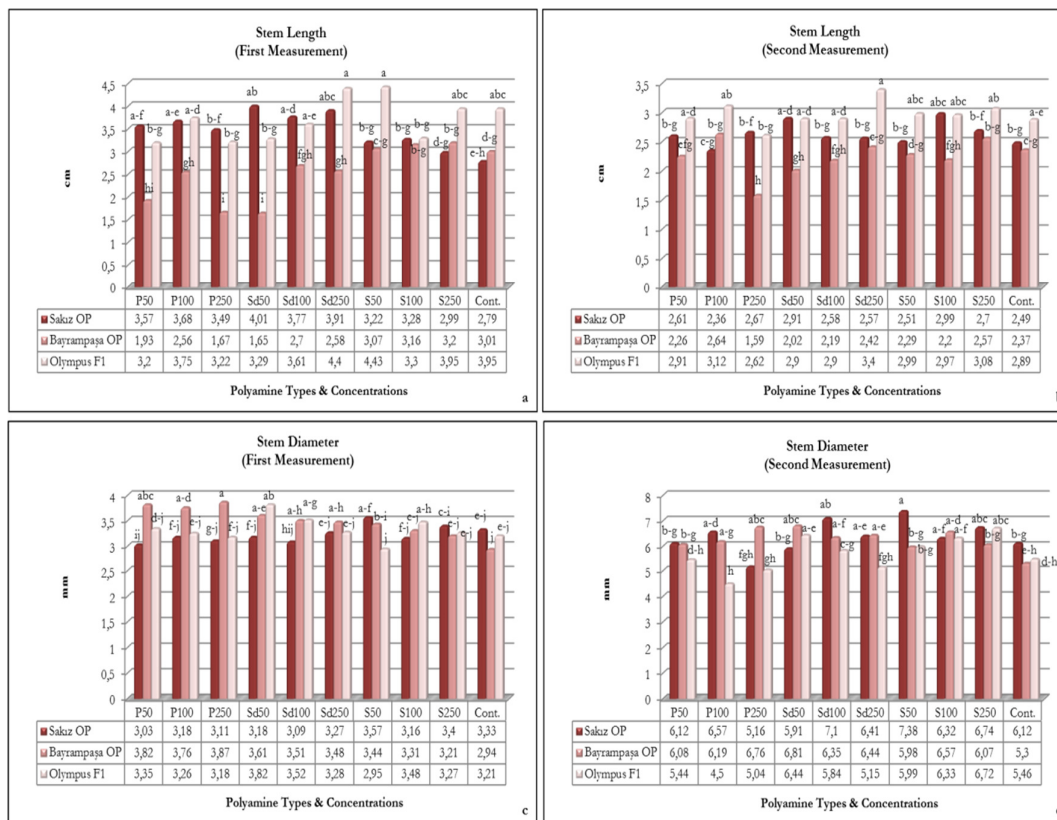


Figure 2. Effect of polyamine on; stem length and diameter: Stem length (a) First measurement; (b) Second measurement; Stem diameter (c) First measurement; (d) Second measurement

(1): Differences between the means are shown with separate letters

(2): LSD test, N.S.: Non-Significant ***:p<0.001; **:p<0.01; *:p<0.05; (a) LSDc* = 0,282; LSDt* = 0,515; LSDc×t* = 0,892; (b) LSDc* = 0,199; LSDt = N.S.; LSDc×t = N.S.; (c) LSDc* = 0,131; LSDt = N.S.; LSDc×t* = 0,416; (d) LSDc* = 0,385; LSDt* = 0,703; LSDc×t* = 1,218 (c: cultivar; t: treatment; c×t: cultivar×treatment)

(3): Abbreviations: P50= 50 mg L⁻¹ putrescine; P100= 100 mg L⁻¹ putrescine; P250= 250 mg L⁻¹ putrescine; Sd50= 50 mg L⁻¹ spermidine; Sd100= 100 mg L⁻¹ spermidine; Sd250= 250 mg L⁻¹ spermidine; S50= 50 mg L⁻¹ spermine; S100= 100 mg L⁻¹ spermine; S250= 250 mg L⁻¹ spermine; Cont.= Control

Based on the observations made after the first foliar PA treatments in terms of root length, statistical differences were found among the cultivars, while ‘Sakız OP’ and ‘Bayrampaşa OP’ responded well than ‘Olympus F1’ cultivar. When the PA types and concentrations were evaluated, 100 mg L⁻¹ putrescine, 250 mg L⁻¹ spermidine, and 100 mg L⁻¹ spermine had positive effects on root length. Considering the relationship among cultivars and the foliar PA treatments, the ones that came to the fore were ‘Sakız OP’ with 250 mg L⁻¹ spermidine and ‘Sakız OP’ with control applications (Figure 3a). On the other hand, the observation after the last foliar PA application were evaluated, in terms of root length ‘Sakız OP’ among the cultivars and 100 mg L⁻¹ putrescine among the PA treatments came to the fore (Figure 3b). In previous researches, polyamines’ beneficial influence on root development was also reported. Yousefi *et al.* (2019) found that the different types and doses of polyamines effected root length as the highest root length was obtained from spermidine and putrescine. When *Antirrhinum majus* L. and *Populus euramericana* plants were treated with putrescine, it was discovered that all doses of putrescine increase root length in comparison to the control (Badawy *et al.*, 2015; Habba *et al.*, 2016). Polyamines are the precursors to numerous alkaloids. Some of these alkaloids play a part in the growth of the root. It was reported that polyamines are found in roots and contribute to cell division by interacting favourably with auxins. It is therefore possible to claim that polyamines regulate root formation and elongation in plants, as well as enhancing fresh and dry root weights, in light of all of the functions it performs (Couée *et al.*, 2004; Yousefi *et al.*, 2019).

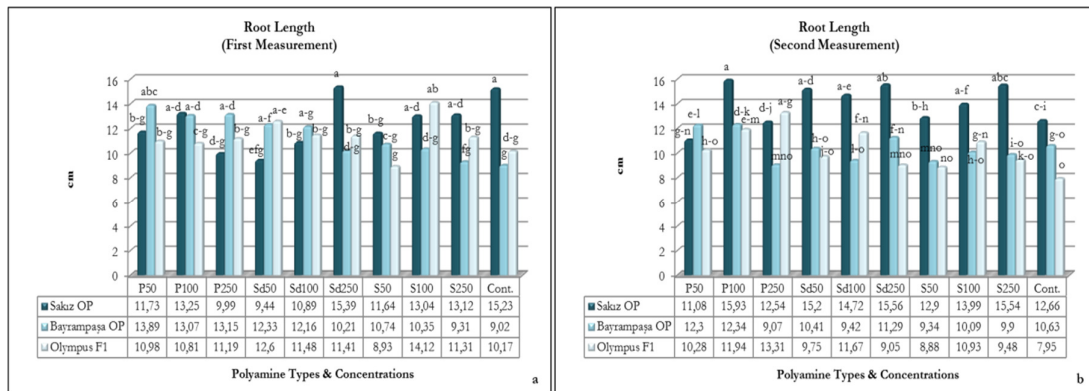


Figure 3. Effect of polyamine on; root lengths; (a) First measurement; (b) Second measurement

(1): Differences between the means are shown with separate letters

(2): LSD test, N.S.: Non-Significant ***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$; (a) $LSD_c = N.S.$; $LSD_t = N.S.$; $LSD_{c \times t} = 3,265$; (b) $LSD_c = 0,911$; $LSD_t = 1,665$; $LSD_{c \times t} = 2,883$ (c: cultivar; t: treatment; $c \times t$: cultivar \times treatment)

(3): Abbreviations: P50= 50 mg L⁻¹ putrescine; P100= 100 mg L⁻¹ putrescine; P250= 250 mg L⁻¹ putrescine; Sd50= 50 mg L⁻¹ spermidine; Sd100= 100 mg L⁻¹ spermidine; Sd250= 250 mg L⁻¹ spermidine; S50= 50 mg L⁻¹ spermine; S100= 100 mg L⁻¹ spermine; S250= 250 mg L⁻¹ spermine; Cont.= Control

When the data of the first foliar PA treatment was evaluated on plant height, it was observed that there were statistically significant differences among cultivars, PA type and concentrations (Figure 4a). After the last foliar PA application on plant height, differences were determined among the cultivars and the ‘Sakız OP’ cultivar responded well than others. There were also statistically significant differences among the treatments, with 250 mg L⁻¹ spermidine and 100 mg L⁻¹ spermine coming to prominence (Figure 4b). Yousefi *et al.* (2021) reported that putrescine effect on plant height was more than spermine and spermidine in their study conducted on rose. For physiological functions of plants, such as organogenesis, embryogenesis, flowering, leaf aging, development of pollen tube and fruit, and reaction to different biotic and abiotic stresses, polyamines are essential (Kusano and Suzuki, 2015). Growing tissues have very high levels of polyamines and the biosynthetic enzymes that produce them. Triggering biosynthesis pathways of polyamines leads to a rise in their concentration when growth and differentiation are initiated in the cell. All of these data make it clear how

polyamines play a role in cell development and differentiation (Yousefi *et al.*, 2021). Polyamines alter growth and cell differentiation as evidenced by the increased plant height, the number of leaves, stem length and diameter, and root length in the current study.

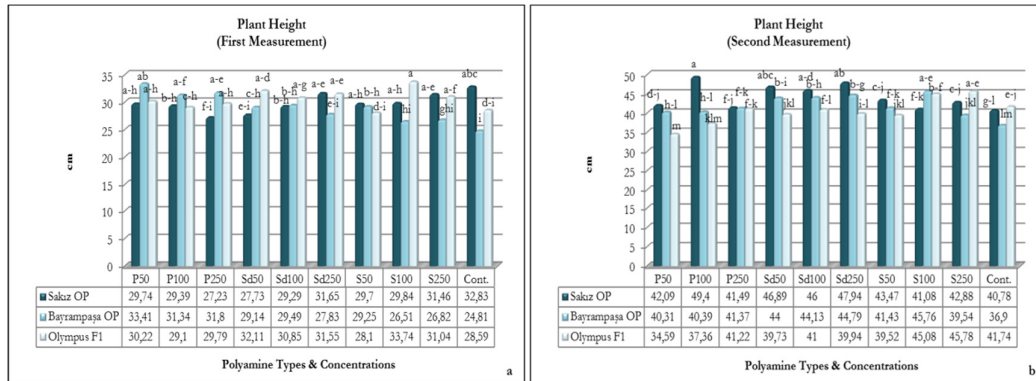


Figure 4. Effect of polyamine on; plant height; (a) First measurement; (b) Second measurement
 (1): Differences between the means are shown with separate letters
 (2): LSD test, N.S.: Non-Significant ***:p<0.001; **:p<0.01; *:p<0.05; (a) LSDc= N.S.; LSDt= N.S.; LSDc×t= 4,210; (b) LSDc= 1,306; LSDt= 2,384; LSDc×t= 4,130 (c: cultivar; t: treatment; c×t: cultivar×treatment)
 (3): Abbreviations: P50= 50 mg L⁻¹ putrescine; P100= 100 mg L⁻¹ putrescine; P250= 250 mg L⁻¹ putrescine; Sd50= 50 mg L⁻¹ spermidine; Sd100= 100 mg L⁻¹ spermidine; Sd250= 250 mg L⁻¹ spermidine; S50= 50 mg L⁻¹ spermine; S100= 100 mg L⁻¹ spermine; S250= 250 mg L⁻¹ spermine; Cont.= Control

Regarding SPAD values, there was statistically difference among cultivars, but there was no statistically difference among cultivars, PA types and concentrations (Figure 5a). With respect to SPAD data, after the last foliar PA application, no statistically significant difference was determined among the cultivars, while 250 mg L⁻¹ spermidine was effective among all PA treatments (Figure 5b). It was stated that increases in the number of leaves, fresh and dry leaf weights lead to an increase in the amount of photosynthetic pigment, thus increasing the biosynthesis of chlorophyll molecules and photosynthetic capacity and protecting the cell (Savvas and Ntatsi, 2015). Considering the findings of the current study, it is possible to say that the effect of polyamines on leaf development and growth and photosynthetic pigments is positive.

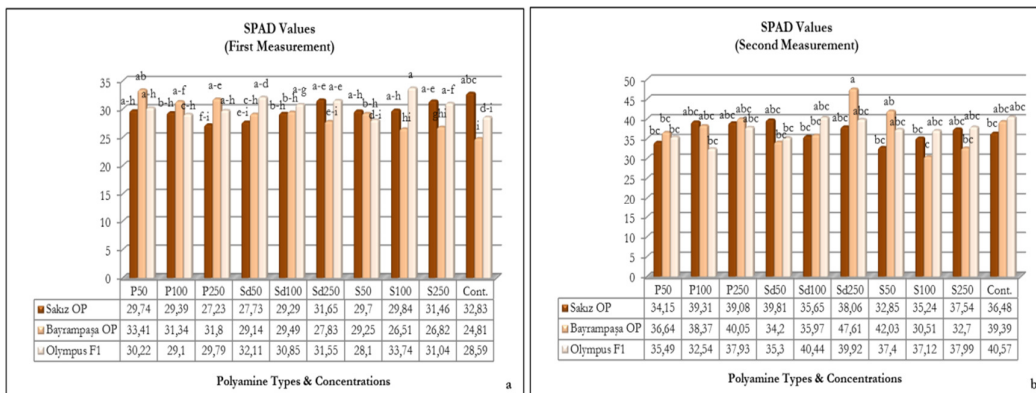


Figure 5. Effect of polyamine on; SPAD values; (a) First measurement; (b) Second measurement
 (1): Differences with separate letters
 (2): LSD test, N.S.: Non-Significant ***:p<0.001; **:p<0.01; *:p<0.05; (a) LSDc= 1,648; LSDt= N.S.; LSDc×t= N.S.; (b) LSDc= N.S.; LSDt= N.S.; LSDc×t= N.S. (c: cultivar; t: treatment; c×t: cultivar×treatment)
 (3): Abbreviations: P50= 50 mg L⁻¹ putrescine; P100= 100 mg L⁻¹ putrescine; P250= 250 mg L⁻¹ putrescine; Sd50= 50 mg L⁻¹ spermidine; Sd100= 100 mg L⁻¹ spermidine; Sd250= 250 mg L⁻¹ spermidine; S50= 50 mg L⁻¹ spermine; S100= 100 mg L⁻¹ spermine; S250= 250 mg L⁻¹ spermine; Cont.= Control

The fresh and dry weights of the leaves (fresh leaf weight 'FLW', dry leaf weight 'DLW') were also measured and recorded. The alteration in the weights with regard to cultivars and PA types and concentrations is presented in the chart below (Figure 6). In previous studies, findings supporting the positive effects of polyamines on leaf growth were reported in different plants, as in the current study (Mahgoub *et al.*, 2011; Badawy *et al.*, 2015; Habba *et al.*, 2016). Plant growth regulators notably auxin, cytokinin, and gibberellic acid had an impact on the development of leaves in plants (Kusano and Suzuki, 2015). By inducing cell division, polyamines are hypothesized to indirectly alter plant growth regulators, increasing leaf size, thickness, and fresh and dry weight (Yousefi *et al.*, 2021).

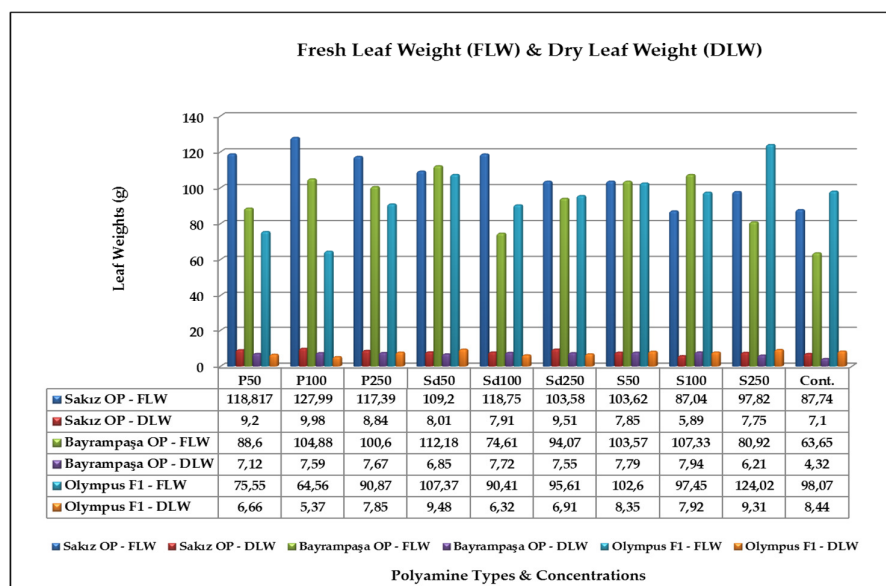


Figure 6. Effect of polyamine on; fresh and dry leaf weights

Biochemical analyses

Regarding the data on cynarin and 1,5-*O*-dicafeoylquinic acid values, differences were determined among the cultivars. Accordingly, 'Olympus F1' cultivar responded well relating to PA types and concentrations than other cultivars. However, among the PA treatments, the control group ended with more significant results in terms of these two bioactive components compared to all other PA types and concentrations. So, there was obtained any positive response to different types and concentrations of PA (Figures 7a, 7b). The antioxidant capacity and compounds with antioxidant capabilities rise in response to stress in order to save the plant. In non-stress circumstances, polyamines do not boost antioxidant compounds but rather help the plant to maintain development. It was concluded that foliar treatments of different types of polyamines (putrescine, spermidine, and spermine) within particular concentrations could not lead to a significant increase in cynarin and 1,5-*O*-dicafeoylquinic acid contents compared to the control plants, considering that the current study was conducted under a non-stress condition.

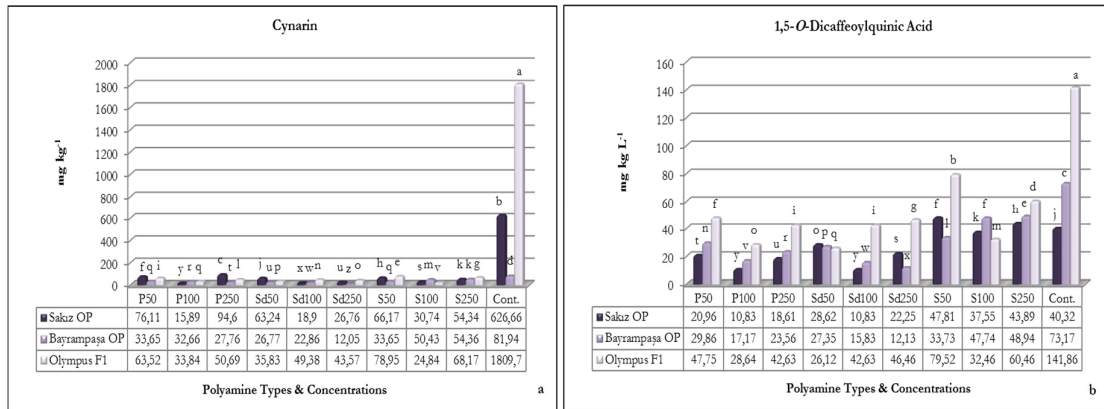


Figure 7. Effect of polyamine on; (a) Cynarin; (b) 1,5-O-dicaffeoylquinic acid values

(1): Differences between the means are shown with separate letters

(2): LSD test, N.S.: Non-Significant ***:p<0.001; **:p<0.01; *:p<0.05; (a) LSDc*= 0,072; LSDt*= 0,131; LSDc×t*= 0,228; (b) LSDc*= 0,051; LSDt*= 0,094; LSDc×t*= 0,163 (c: cultivar; t: treatment; c×t: cultivar×treatment)

(3): Abbreviations: P50= 50 mg L⁻¹ putrescine; P100= 100 mg L⁻¹ putrescine; P250= 250 mg L⁻¹ putrescine; Sd50= 50 mg L⁻¹ spermidine; Sd100= 100 mg L⁻¹ spermidine; Sd250= 250 mg L⁻¹ spermidine; S50= 50 mg L⁻¹ spermine; S100= 100 mg L⁻¹ spermine; S250= 250 mg L⁻¹ spermine; Cont.= Control

Regarding the total phenolic content, there was determined that the statistically significant differences among cultivars, PA types and concentrations. Accordingly, ‘Olympus F₁’ among cultivars, spermine concentration at 250 mg L⁻¹ among PA treatments, ended with important results (Figure 8a).

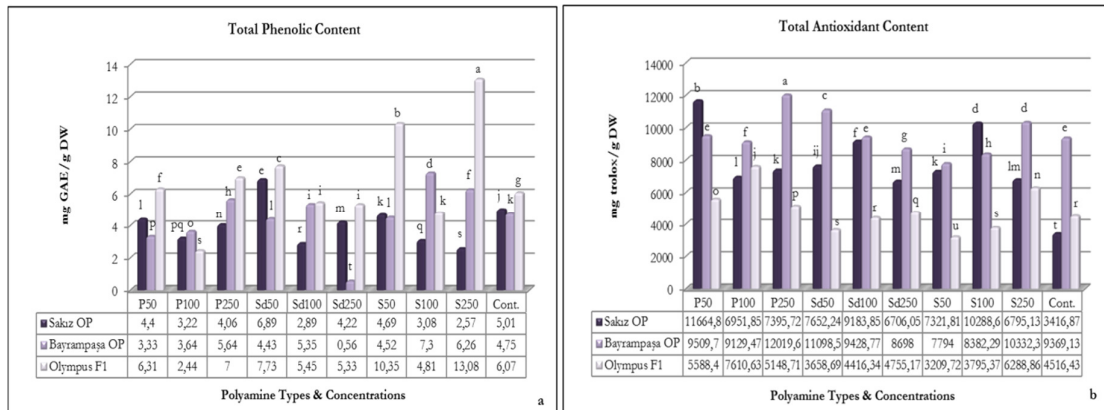


Figure 8. Effect of polyamine on; (a) Total phenolic content; (b) Total antioxidant content

(1): Differences between the means are shown with separate letters

(2): LSD test, N.S.: Non-Significant ***:p<0.001; **:p<0.01; *:p<0.05; (a) LSDc*= 0,046; LSDt*= 0,084; LSDc×t*= 0,146; (b) LSDc*= 57,904; LSDt*= 105,719; LSDc×t*= 183,111 (c: cultivar; t: treatment; c×t: cultivar×treatment)

(3): Abbreviations: P50= 50 mg L⁻¹ putrescine; P100= 100 mg L⁻¹ putrescine; P250= 250 mg L⁻¹ putrescine; Sd50= 50 mg L⁻¹ spermidine; Sd100= 100 mg L⁻¹ spermidine; Sd250= 250 mg L⁻¹ spermidine; S50= 50 mg L⁻¹ spermine; S100= 100 mg L⁻¹ spermine; S250= 250 mg L⁻¹ spermine; Cont.= Control

By deactivating reactive oxygen species, antioxidants assist in preventing oxidative damage under oxidative stress circumstances. Phenols are a class of secondary metabolites having antioxidant capabilities that were found in all plants (Yousefi *et al.*, 2021). Phenols are compounds having antioxidant characteristics. It was reported that when the phenol contents were increased, the antioxidant content rise. Therefore, it was assumed that there was a linear connection between the phenolic compounds and antioxidant content (Razzaq *et al.*,

2014). It was also seen in the current study that high PA concentration treated to plants was found to be more effective in increasing the phenolic content.

Regarding total antioxidant content foliar treatments of both PA types and effective dosage showed variation based on cultivar. In that sense, 'Bayrampaşa OP' among cultivars and 50 mg L⁻¹ putrescine among foliar PA treatments came forward (Figure 8b). In the current study contrary to phenolic content, it was found that the lowest PA concentration was effective in antioxidant content. According to research conducted by Yousefi *et al.* (2019), the findings of the analysis of variance, the polyamine treatments had no effect on antioxidant enzymes. Under stress conditions, the main defence mechanism of plants is to prevent the production of free radicals (Baniasadi *et al.*, 2018). Plant antioxidants are thought to postpone the aging process and protect against a number of diseases. By lowering oxidative activity, polyamines interact with plant membranes in this way and help shield plants from the harmful effects of stress (Minocha *et al.*, 2014). Polyamines are similar to antioxidants as they also shield membrane lipids from oxidative degradation. An increase in total phenolic chemicals is responsible for higher antioxidant activity (Aronova *et al.*, 2005). It was reported that important polyamines putrescine, spermidine, and spermine involved in controlling plants' antioxidant activity (Ahmad and Tahir, 2018).

Conclusions

In conclusion, it can be inferred that the use of polyamines can significantly affect plant growth and development traits and biochemical contents in a number of ways. Among three types of PA, spermine was effective on total phenolic content, while putrescine had a potent effect on total antioxidants. Similarly, putrescine and spermine had positive effects on cynarin and 1,5-*O*-dicaffeoyquinic acid contents, respectively, following control treatments. In other words, putrescine, spermine and, spermidine had no positive effects on cynarin and 1,5-*O*-dicaffeoyquinic acid levels. For further studies, different concentrations of putrescine, spermidine and spermine should be tested to check their effect on increasing total phenolic contents and total antioxidants. It was not possible to determine one single polyamine type and concentration for each biometric measurement and biochemical content. The results of the research studies clearly revealed that both the type and the dose of PA should be determined according to the cultivars to be used in future studies.

Authors' Contributions

The author read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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