

Measuring of Antioxidant enzyme activity and some fruits quality characteristics in apple trees

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ABSTRACT

In this study, a factorial experiment was conducted using a Randomized Complete Block Design (RCBD) with three replicates to investigate the effects of silicon at four concentrations: 0, 2, 4, and 6 ml/L, designated as S0, S1, S2, and S3, respectively and a calcium-boron combination at three concentrations: 0, (0.5 g/L Ca-EDTA, + 10 mg/L B), and (1 g/L Ca-EDTA, + 20 mg/L B), designated as C0, C1, and C2, respectively. on the activity of antioxidant enzymes and some qualitative traits of fruits. The results indicated that the studied traits were significantly influenced by the factors. Silicon application notably increased enzyme activity, treatment S3 showed the highest activity levels for peroxidase (POD) and superoxide dismutase (SOD) reached (187.6 and 119.7) units g⁻¹ in fresh leaves, respectively. Additionally, fruits from the S3 treatment had the highest results in pectin content 1.14%, protein content 0.35%, sugar content 8.33 g per 100 g fresh weight, and a vitamin C content 18.26 mg per 100 g. In contrast, treatment S2 resulted in the highest carotene concentration in the fruits, 2.54 mg per 100 g.

The calcium-boron mixture also positively influenced the measured traits. Treatment C2 achieved the highest POD and SOD activities, with values of (169.6, 94.4) units g⁻¹ in fresh leaves, respectively. Furthermore, fruits from the C2 treatment had the best results in pectin content 1.1%, protein content 0.37%, carotene, total sugars, and vitamin C at 2.75 mg, 8.11 g, and 18.65 mg per 100 g, respectively.

The interaction between the silicon and calcium-boron treatments significantly affected the measured traits, with the combined treatment S3C2 showing the highest values for most traits evaluated.

Key words: ROS, environment, beneficial element

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Introduction

Environmental stress factors affect biological membranes, causing functional disruptions such as altered permeability and fluidity, as well as impaired enzyme activity within cellular membranes. These negative effects result from membrane degradation due to the increased presence of free radicals, which increase membrane permeability to various elements and substances [14]. Reactive

oxygen species (ROS) interact with other vital cellular components, such as photosynthetic pigments, proteins, and nucleic acids. Therefore, their concentration within cells must be continuously controlled and regulated. The regulation of ROS is achieved through antioxidant mechanisms, which include two main categories: non-enzymatic antioxidants (such as ascorbic acid [vitamin C],

glutathione, tocopherol [vitamin E], and carotenoids) and enzymatic antioxidants (such as peroxidase [POD], superoxide dismutase [SOD], and catalase). These antioxidants work synergistically to neutralize ROS, thereby protecting the cell from oxidative damage and maintaining cellular homeostasis [38]

Silicon is classified as a beneficial element for plants, influencing plant growth in two main ways. Firstly, it is involved in the construction of cell walls and accumulates beneath the cuticle layer in leaves, as well as in the intercellular spaces of branches, forming siliceous bodies called phytoliths. In this context, silicon acts as a structural support for the plant [8, 15,19]. Secondly, silicon has a physiological effect by enhancing the activity of antioxidant enzymes and promoting the synthesis of non-enzymatic antioxidants under environmental stress conditions [20,32]. According to [15] silicon is considered a quasi-essential element for plants because its deficiency leads to abnormalities in plant growth, development, and reproduction. Adequate silicon availability improves the plant's defensive mechanisms against environmental and biotic stressors, thereby positively impacting yield quantity and quality. Also [24] reported that treating apple trees with silicon increased leaf chlorophyll content and photosynthetic efficiency. Another study [3] found that spraying silicon on date palm trees increased leaf area, chlorophyll content, carbohydrates, and the levels of N, P, K, and Mg, as well as overall yield, fruit weight, soluble solids, and soluble sugars, while reducing total acidity and tannins.

Calcium has various structural and physiological roles in plants. It is involved in the composition of plant cell walls and acts to bind components such as pectin and polysaccharides. This means that an adequate

supply of calcium provides plants with structural strength, enhances overall growth speed, and improves crop quality specifically [13.]

Additionally, calcium plays a crucial role in the stability and permeability of plasma membranes and is notable for its role in signal transduction within the plant, especially under environmental stress conditions like heat and drought [12.]

[6]have confirmed that spraying apple trees of the Golden Delicious and Granny Smith varieties with chelated calcium at concentrations of 2 and 4 mg/L significantly increases the weight and firmness of the fruits, total soluble solids, total acidity, and calcium content of the fruits, thereby increasing the overall yield.

Furthermore, [30] has found that spraying fig trees with calcium in the form of CaO 14% + amino acids at a concentration of 5 ml/L significantly increases the fresh weight of vegetative growth, moisture content, leaf area, and the content of elements (N, K, Ca, Mg, B) in the leaves. Regarding fruit characteristics, it increases the fresh and dry weight, moisture content, fruit size, firmness, total soluble solids, total acidity, ascorbic acid, and anthocyanin pigments.

Boron is one of the essential micronutrients for the growth and production of plants in general. It plays a variety of physiological and structural roles, such as sugar transport, cell wall formation, lignification of cell walls, RNA synthesis, respiration, and the production of the hormone auxin and phenols. Boron is also crucial for the pollination process and for transporting calcium within the plant from the roots to the upper parts [36, 27, 28.]

A study by [22] on Anna apple trees indicated that spraying the trees with borax (17.5% B) at a concentration of 300 mg/L led to increased

branch length, leaf area, and the boron and chlorophyll content in the leaves. It also reduced the number of unopened buds and increased the percentage of fruit set, fruit weight, firmness, total yield, and the percentage of reducing, non-reducing, and total sugars, as well as anthocyanin content, while reducing fruit acidity.

Material and methods

The experiment was conducted in an apple orchard with the Anna variety, grafted onto quince rootstock, aged 4 years. The orchard is located at the College of Agricultural Engineering Sciences, University of Baghdad, Jadriya, Research Station A. The orchard consists of three rows, each row containing 15 trees, with a spacing of 4 meters between rows and 2.5 meters between trees.

A factorial experiment with two factors was carried out using a randomized complete block design (RCBD) with three replicates. Each experimental unit consisted of a single tree, making the total number of trees 36.

The first factor involved foliar spraying with potassium silicate (K_2SiO_3) as a source of silicon (26.5% Si_2O and 12.65% K_2O) at four concentrations: 0, 2, 4, and 6 ml/L [25], designated as S0, S1, S2, and S3, respectively.

The second factor involved spraying a combination of calcium as Ca-EDTA (9.7% Ca) and boron as boric acid (H_3BO_3 , 17% B) at three concentrations: 0, (0.5 g/L Ca-EDTA, half the company's recommendation + 10 mg/L B), and (1 g/L Ca-EDTA, the company's recommendation + 20 mg/L B) [5], designated as C0, C1, and C2, respectively.

Thus, there were 12 treatment combinations in total. The treatments were applied on the first of April, the first of May, and the first of June in 2018.

Studied Traits

Estimation of Superoxide Dismutase (SOD) Activity in Leaves (Unit g^{-1})

Mature leaves were collected from the experimental units in mid-June and transported in a cooled state to the laboratory, where the sample was prepared according to the method described in [16].

Estimation of Peroxidase (POD) Activity in Leaves (Unit g^{-1})

The sample was prepared, and the analysis was conducted following the method outlined in [4].

Estimation of Pectin Percentage in Fruits(%)

Five fruits were taken and sliced into pieces, from which 300 grams were randomly selected. The pectin was precipitated and estimated as per [33].

Estimation of Nitrogen Content in Fruits

The nitrogen content in the fruits was determined using the Micro-Kjeldahl method as described in [1].

Estimation of Carotene Content in Fruits (mg $100g^{-1}$)

Samples were taken from three randomly selected fruits per branch, and the carotenoid content was estimated according to the method mentioned in [33].

Estimation of Total Sugars in Fruits (g $100g$ Fresh Weight $^{-1}$)

The sugars in the fruits were estimated using the method described in [21], using a spectrophotometer at a wavelength of 490 nm.

Estimation of Vitamin C in Fruits (mg $100g^{-1}$)

A specific weight of the fruits was taken, and the concentration of Vitamin C was determined according to the method in [33].

Result and discussion

Superoxide dismutase activity in leaves unit g^{-1}

The activity of the enzyme superoxide dismutase in leaves (units g^{-1}) was

significantly affected by silicon. According to Table 1, treatment S3 resulted in the highest activity at 119.71 units g⁻¹, while treatment S0 showed the lowest activity at 59.89 units g⁻¹. The combination of calcium and boron also significantly influenced enzyme activity. Treatment C2 had the highest activity at 94.40 units g⁻¹, compared to treatment C0, which

had the lowest activity at 42.17 units g⁻¹. Additionally, the interaction between silicon and calcium-boron had a significant effect on increasing enzyme activity, with the S3C2 combination showing the highest activity at 130.80 units g⁻¹, compared to the control treatment S0C0, which had the lowest activity at 42.17 units g⁻¹.

Table1. effects of silicon and calcium boron combination on Superoxide dismutase activity in leaves (unit g⁻¹)

S	C C0	C1	C2	Average
0S	42.17	65.67	71.83	59.89
1S	75.10	75.08	78.06	76.08
2S	84.18	93.79	96.91	91.63
3S	102.1	126.1	130.8	119.7
Average	75.90	90.17	94.40	
LSD	S = 4.41	C = 3.82	S*C= 7.64	

peroxidase enzyme activity in leaves (unit g⁻¹)
The results in Table 2 show that silicon significantly increased the activity of the enzyme peroxidase in leaves. Treatment S3 had the highest activity at 187.6 units g⁻¹, while treatment S0 had the lowest at 66.2 units g⁻¹. Additionally, the combination of calcium and boron significantly enhanced enzyme activity, with treatment C2 showing the highest activity at 169.6 units g⁻¹, compared

to treatment C0 which had the lowest activity at 61.4 units g⁻¹. The interaction between silicon and the calcium-boron combination significantly increased peroxidase activity, with the S3C2 combination having the highest activity at 228.4 units g⁻¹, compared to the control treatment S0C0, which had the lowest activity at 61.4 units g⁻¹.

Table2. effects of silicon and calcium boron combination on peroxidase enzyme activity in leaves (unit g⁻¹)

S	C C0	C1	C2	Average
S0	61.4	64.7	72.4	66.2
S1	121.3	153.9	178.6	151.3
S2	175.3	178.1	199.0	184.1
S3	141.8	192.5	228.4	187.6
Average	125.0	147.3	169.6	
LSD	S = 8.42	C = 7.29	S*C= 14.58	

pectin percentage in fruits(%)

The results in Table 3 show that silicon significantly increased the percentage of pectin in fruits. Treatment S3 had the highest percentage at 1.14%, while treatment S0 had the lowest at 0.73%. Additionally, the combination of calcium and boron significantly increased the pectin percentage, with treatment C2 showing the highest percentage at 1.10% compared to treatment

C0, which had the lowest percentage at 0.77%. The interaction between silicon and the calcium-boron combination also significantly increased the pectin percentage in fruits, with the S3C2 combination having the highest percentage at 1.41%, compared to the control treatment S0C0, which had the lowest percentage at 0.34%.

Table3. effects of silicon and calcium boron combination on pectin percentage in fruits(%)

S	C			
	C0	C1	C2	Average
S0	0.34	0.78	1.06	0.73
S1	0.65	0.91	1.14	0.90
S2	0.70	1.05	1.35	1.03
S3	1.41	1.17	0.85	1.14
Average	0.77	0.98	1.10	
LSD	S = 0.10	C = 0.05	S*C= 0.06	

Protein percentage in fruits (%)

The results of Table 4 show that silicon significantly increased the percentage of proteins in the fruits. Treatment S3 gave the highest rate of 0.35%, while treatment S0 gave the lowest rate of 0.30%. Additionally, the combination of calcium and boron significantly increased this trait, with treatment C2 giving the highest rate of 0.37% compared to treatment C0, which gave the lowest rate of 0.27%.

The interaction between the two factors, silicon and the combination of calcium and boron, also significantly increased the percentage of proteins in the fruits. The interaction treatment S3C2 gave the highest rate of 0.39% compared to the control treatment S0C0, which gave the lowest rate of 0.22%.

Table 4. effects of silicon and calcium boron combination on protein percentage in fruits(%)

S	C			
	C0	C1	C2	Average
S0	0.22	0.32	0.35	0.30
S1	0.28	0.34	0.37	0.33
S2	0.29	0.36	0.38	0.34
S3	0.31	0.36	0.39	0.35
Average	0.27	0.34	0.37	
LSD	S = 0.02	C = 0.02	S*C= 0.04	

carotene percentage in fruits (%)

The results of Table 5 show that silicon significantly increased the carotene content in the fruits. Treatment S2 gave the highest rate of 2.54 mg 100 g⁻¹, while treatment S0 gave the lowest rate of 2.29 mg 100 g⁻¹. Additionally, the combination of calcium and boron significantly increased this trait, with treatment C2 giving the highest rate of 2.75 mg 100 g⁻¹ compared to treatment C0 which

gave the lowest rate of 1.84 mg g⁻¹. The interaction between the two factors, silicon and the combination of calcium and boron, also significantly increased the carotene content in the fruits. The interaction treatment S2C2 gave the highest rate of 2.94 mg 100 g⁻¹ compared to the control treatment S0C0, which gave the lowest rate of 1.84 mg 100 g⁻¹

Table 5. effects of silicon and calcium boron combination on carotene percentage in fruits(%)

S	C			Average
	C0	C1	C2	
S0	1.84	2.39	2.63	2.29
S1	1.93	2.43	2.65	2.34
S2	1.98	2.71	2.94	2.54
S3	2.04	2.63	2.77	2.48
Average	1.95	2.54	2.75	
LSD	S = 0.10	C = 0.09	S*C= 0.18	

Total sugar in fruits (g 100 g⁻¹ fresh wight(

The results of Table 6 show that silicon significantly increased the total sugar content in the fruits. Treatment S3 gave the highest rate of 8.33 g 100 g⁻¹, while treatment S0 gave the lowest rate of 7.38 g 100 g⁻¹. Additionally, the combination of calcium and boron significantly increased this trait, with treatment C2 giving the highest rate of 8.11 g 100 g⁻¹ compared to treatment C0, which gave the lowest rate of 7.53 g 100 g⁻¹.

The interaction between the two factors, silicon and the combination of calcium and boron, also significantly increased the total sugar content in the fruits. The interaction treatment S3C2 gave the highest rate of 8.88 g 100 g⁻¹ compared to the control treatment S0C0, which gave the lowest rate of 7.12 g 100 g⁻¹

Table 6. effects of silicon and calcium boron combination on Total sugar in fruits (g 100 g-1 fresh wight(

S	C			
	C0	C1	C2	Average
S0	7.12	7.42	7.62	7.38
S1	7.35	7.53	7.86	7.58
S2	7.72	7.39	8.10	7.74
S3	7.93	8.20	8.88	8.33
Average	7.53	7.63	8.11	
LSD	S = 0.23	C = 0.20	S*C= 0.40	

Vitamin C concentration in fruits (g 100 g-1 fresh wight(

The results presented in Table 7 indicate that the application of silicon significantly increased the measured attribute. Treatment S3 resulted in the highest value of 18.28 mg per 100 g, while treatment S0 had the lowest value of 14.26 mg per 100 g. Similarly, the combination of calcium and boron significantly enhanced this attribute, with treatment C2 achieving the highest value of 18.65 mg per 100 g compared to treatment C0,

which had the lowest value of 14.01 mg per 100 g.

Moreover, the interaction between silicon and the combination of calcium and boron significantly increased the vitamin C content in the fruits. The interaction treatment S2C2 produced the highest vitamin C content, measuring 20.98 mg per 100 g, in contrast to the control treatment S0C0, which had the lowest value of 12.27 mg per 100 g.

Table 7. effects of silicon and calcium boron combination on Vitamin C concentration in fruits (g 100 g-1 fresh wight(

S	C			
	C0	C1	C2	Average
S0	12.27	14.91	15.60	14.26
S1	13.80	15.52	17.80	15.71
S2	14.34	18.53	20.98	17.95
S3	15.62	18.95	20.22	18.26
Average	14.01	16.98	18.65	
LSD	S = 1.31	C = 1.13	S*C= 2.27	

The results of Tables 1 and 2 indicate that the study elements had a direct effect on the effectiveness of antioxidant enzymes. This could be due to the role of silicon in increasing the absorption of nutrients (18). Silicon enhances the plant's uptake of calcium and potassium, especially under stress conditions, thereby affecting water movement within cells

and the function and stability of cell membranes. Moreover, silicon increases the concentration of chlorophyll and the microstructure of plastids, preventing the breakdown of grana under environmental stress (42,23.(

The results also confirm that the combination of calcium and boron significantly affected the

activity of SOD and POD enzymes. This effect may be attributed to the role of calcium in cell wall construction, thereby enhancing the plant's ability to withstand external conditions [13]. Calcium also plays a crucial role in the stability of cell membranes and signal transmission within the plant [12]. It regulates ion pump activity by stimulating the calmodulin enzyme, which activates the pumps and thus controls the absorption and movement of ions within cells [17]. Additionally, calcium is involved in the opening and closing of stomata [7].

Regarding boron, its effect on enzyme activity may be due to its influence on the synthesis of RNA and the production of auxin hormones and phenolics [28,27,36]. Boron also affects the water balance of plant cells, increases the content of vitamins B and C, and regulates the function of plant membranes and hormones [26,10,9].

As for the impact of silicon on the qualitative traits of the yield (Tables 3, 4, 5, 6, and 7), it can be attributed to its role in improving the efficiency of photosynthesis, which reflects on the quantity of manufactured nutrients [42]. Silicon also enhances the absorption of beneficial nutrients and restricts the uptake of toxic heavy metals [18,35]. Additionally, it increases the plant's resistance to biotic and abiotic stress by improving the activity of antioxidant enzymes and boosting the production of antioxidants [38,39], while reducing the activity of poly galacturonans, an enzyme responsible for pectin degradation [43]. The effect might also stem from silicon's structural roles in plants, as it is part of the cell wall composition [15]. These findings are consistent with those of [3,29,25,44].

The role of calcium and boron in the qualitative traits of fruits is related to their contribution to cell wall construction [36,13],

their inclusion in cell membrane composition and permeability regulation [40,12]. These elements play a role in cell division and tissue formation and are involved in the synthesis and regulation of the auxin hormone [7,11]. They also regulate the plant's water content [26,41], nitrogen absorption, nitrate reductase enzyme activity, and protein synthesis [10,11,31]. Furthermore, they contribute to pollen tube growth and pollen viability [7,37]. Boron, in particular, is important for the synthesis and transport of carbohydrates and sugars within the plant to growth sites, RNA synthesis, reducing pectinase enzyme activity, and increasing the concentration of vitamins B and C [28,27,36,26,7]. These results are consistent with the findings of (5,2).

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