Effect of spraying potassium silicate, boron and vitamin C on levels of antioxidant enzymes and phytohormones in the black French grape *Vitis vinifera* L.

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Abstract

The research was conducted in one of the private orchards in Al-Musayyib District, Babylon province, Iraq for the seasons 2020 and 2021 to study the effect of spraying with potassium silicate, boron and vitamin C on levels of peroxidases and catalase enzymes and the phytohormones gibberellin (GA_3) and indole acetic acid (IAA) in the black French grapevine The experiment was conducted according to a randomized complete block design (RCBD) as a factorial experiment, and the means were compared using the least significant difference at the probability level of 0.05 to test the significant differences(L.S.D.). The results showed that spraying with potassium silicate at a concentration of 500 mg. l^{-1} with boron and ascorbic acid, both at a concentration of 50 mg.l^{-1}, resulted in a significant increase in the levels of peroxidase, catalylase, indole acetic acid and gibberellin in French black grape leaves, which gave (40.62 and 40.65) unit.100 g^{-1}, (0.093 and 0.091) **unit.g^{-1} protein**, (65.28 and 66.02 **µmol.g^{-1} fresh weight** and (32.14 and 32.74) **µmol g^{-1} fresh weight** for the first and second seasons, respectively, which was significantly superior to most of the interactions. These levels of the two enzymes and hormones studied are among the appropriate levels for the grape plant to exercise its physiological activity and carry out vital processes.

Keywords: **Grape, Potassium silicate, Boron, Vitamin C, GA_3, IAA, Catalase, Peroxidase.**

Introduction

Grapes belong to the genus Vitis, and it is one of the fourteen genera of the Vitaceae family (**11,** 8). Grapes are a rich source of nutritional and medical attributes that provide powerful health benefits. Each 100 gm of dry grapes contains 81% water, 67 calories, 0.6 g protein, 0.3 g fat, 18 g carbohydrates, 100 international units of vitamin A, 15 mg vitamin B_1, 20 mg vitamin B_2, 50 mg vitamin B_6, 7 mg vitamin C, 170 mg potassium, 3 mg sodium, 18 mg iron, 20 mg phosphorous and 12 mg calcium (37). Grapes are used as a stimulant for brain cells and heart muscles, tonic for the liver and kidneys, cardioprotective, and reduce the incidence of stomach, intestine, as well as urinary system diseases (21). The black French grape cultivar is one of the cultivars distributed in the central regions of Iraq, which is characterized by early clustering as well as the long production period that extends from July to November (19). One of the important indicators in the development of agriculture is the use of foliar nutrition, where research and experiments have proven the possibility of supplying plants with various nutrients by spraying them with solutions of these elements, which are absorbed by the leaves and other plant parts that appear above the surface of the soil, such as fruits (**34**).

The element silicon (Si) is one of the most significant and essential elements for plant growth. It has important roles in many physiological processes such as increasing the root absorption efficiency of nutrients,

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improving the photosynthesis rate, increasing the effectiveness of antioxidant enzymes, and reducing the toxicity of heavy metals (29). Potassium (K) functions as an activator of enzymes related to the photosynthesis process (30) and contributes to the assimilation of carbohydrates and proteins (23). Potassium also helps to transfer carbohydrates from the location of their synthesis to other parts of plants (27) and maintains the building of proteins, membrane permeability, and cellular pH homeostasis, as well as regulates the opening and closure of stomata apparatus (6). K also facilitates the transfer of nitrates from plant leaves to the roots and regulates the cellular osmotic pressure (7). Boron plays a major role in the exchange of proteins, carbohydrates, and nucleic acids, whereas, the lack of boron causes feeble growth, low production, reduced quality, low photosynthesis rate, and declined root growth thus lowering absorption of nutrients from the soil (26). Boron also has a crucial role in stimulating pollen germination and pollen tube growth, thus increasing the rate of fertilization and then increasing the number of berries in the cluster (22) at the level of global research, vitamins have been used, including vitamin C (Ascorbic acid) due to its role in metabolic activities including its regulation of reproductive growth, which is reflected in increasing the yield and improving its quality (17). It is one of the basic components in plants that helps to maintain normal growth and increases plant resistance to severe conditions. It also preserves chloroplast from oxidation since it functions as an antioxidant factor (24). Vitamin C also has a role in boosting vegetative growth with the same effect of growth regulators (5).

Plants have a defense mechanism to limit the toxicity of oxidative stress, and this mechanism includes two types of systems, the first is non-enzymatic systems such as ascorbate and carotenoids, and the second is enzymatic systems such as peroxidase (POD) and catalase (CAT) (31). The study aimed to investigate the influence of foliar application of potassium silicate, boron, and vitamin C on the magnitudes of peroxidase and catalase enzymes, gibberellin, and indole acetic acid in the black french grape.

Materials and methods

The experiment was carried out for two consecutive seasons in February 2019-2020 and 2020-2021 in a private orchard belonging to Al-Musayyib / Babil district, planted with the variety french black, 12 years old, with stripes and dimensions (2 * 3) m, which was propagated by cuttings and raised on homemade wire cages.

54 vines that were as homogeneous as possible were chosen to conduct the experiment. The winter pruning process was carried out in mid-January for both experimental seasons, by leaving an equal number of canes for all the vines. Seven fruiting canes were left, each stem containing 12 eyes, so the total number of eyes was 84 eyes.

The experiment was implemented as a global experiment following a randomized complete block design (RCBD) with three factors(3).

The research was conducted in one of the private orchards in the Abu Al-Jassem area in the Musayyib district / Babylon province during the growing seasons of 2020 and 2021 to study the effect of spraying with potassium silicate, boron, and vitamin C on levels of peroxidase and catalase enzymes and plant hormones in grapevine of the black French cultivar and bred on wireframes. An analysis of the orchard soil was tested for the physical and chemical properties of the soil (Table 1). The analysis was conducted in the laboratories of the College of Science - University of Baghdad, Iraq.

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Table 1. Chemical and physical analyses of orchard soil properties

Traits	Units	Values
pH reaction degree	-----	8.0
Electrical Conductivity (EC)	$dS.m^{-1}$	3.7
Organic matter	$g.kg^{-1}$	6.30
Total nitrogen	$g.kg^{-1}$	1.27
Available phosphorous	$mg.kg^{-1}$	6.10
Available potassium	$mg.kg^{-1}$	3.60
Boron	$mg.kg^{-1}$	1.15
Clay	$g.kg^{-1}$	415
Sand	$g.kg^{-1}$	186
Silt	$g.kg^{-1}$	399
Soil Texture	Silty clay	

This experiment included the following treatments and their interactions:

1- Spraying with potassium silicate K_2SiO_3 (35% potassium silicate, which contains 12% potassium oxide K_2O,) in three concentrations (0, 250 and 500 $mg.l^{-1}$) and symbolized by the symbol (A) .

2- Spraying with boron in the form of boric acid H_3BO_3 (B 17%) before flowering opens with three concentrations (0, 50 and 100 $mg.l^{-1}$) and symbolized by the symbol (B).

3- Spraying with vitamin C in the form of ascorbic acid in two concentration of (0 and 50 $mg.l^{-1}$) and symbolized by the symbol (C).

Spraying dates

Spraying was carried out in the early morning on the foliage of the vines using a hand sprinkler until the vine was completely wet, with the addition of a 0.1% diffuser to reduce the surface tension of the water molecules (4).

The first spraying: before the flowers open in their maturity stage and on the 5th of April for the first season March 27th for the second season.

The second spraying: The vines were sprayed immediately after the setting, that is, after the completion of petal fall, on May 7th for the first season and April 29th for the second season. (16).

Third spray: The vines were sprayed three weeks after the second spray.

Measurements

Enzymatic activity in peroxidase and catalase in grape leaves::

1-The activity of the peroxidase enzyme(unit.(100 g protein) $^{-1}$) was estimated according to the method of (28).

2-The activity of the enzyme catalase (unit.g^{-1} protein was estimated according to (1).

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Concentration of Indole-3-acetic acid (IAA) and gibberellins(GA_3) in leaves (µmol.g $^{-1}$ fresh weight):

The quantitative estimation of the two plant growth hormones (Auxin IAA and gibberellins GA_3) was carried out in mid-July by taking samples from grape leaves weighing 1 g fresh weight. Then each leaf sample was prepared by adding 12 ml of methane and 5 ml of chloroform, then ammonium hydroxide(2N) was added in avolume of 3 ml Afterward, the volume was completed to 25 ml of distilled water, and the acidity of the solution was adjusted to pH 2.5 using drops of hydrochloric acid (1 N) or sodium hydroxide(1N) with sodium nitrate and sodium nitrate on the scale. As for gibberellin and auxin, a standard curve was tested for the two hormones separately as reported by (13).

Results

Efficiency of enzymes:

1- Peroxidase activity (POD)

The results of Table (2) indicate that there is a significant effect of potassium silicate on the activity of the peroxidase enzyme. The highest effectiveness when treated without potassium silicate reached (38.78 and 38.87) unit. (100 g protein) $^{-1}$ for the first and second seasons, respectively. The lowest values of the peroxidase activity were at a concentration of 250 mg.l^{-1} of potassium silicate giving (36.03 and 36.17) unit.100 g $^{-1}$for the first and second seasons, consecutively.

It is noted from the same table that spraying with boric acid had a significant effect on the activity of the peroxidase enzyme, and the highest effectiveness was at a concentration of 50 mg. l^{-1} of boron reached 38.65 and 38.68 unit. (100 g protein) $^{-1}$for the first and second seasons, respectively, and the lowest values were found at a concentration of 100 mg. l^{-1} of boron reached 36.57 and 36.70 unit. (100 g protein) $^{-1}$for the first and second seasons, respectively.

The results of the same table show that vitamin C does not have a significant effect on this characteristic for both seasons of the experiment.

The binary interaction between potassium silicate and spraying with boric acid significantly affected the activity of the peroxidase enzyme, giving the highest effectiveness at a concentration of 500 mg. l^{-1} of cross-linked potassium silicate with a concentration of 50 mg. l^{-1} of boron reached 39.82 and 39.85 unit. (100 g protein) $^{-1}$ for the first and second seasons, respectively, and the lowest values for this characteristic were at a concentration of 250 mg. l^{-1} of potassium silicate + 100 mg. l^{-1} concentration of boron reached 33.18 and 33.53 unit. (100 g protein) $^{-1}$for the first and second seasons, respectively.

The binary interaction between potassium silicate and vitamin C also had a significant effect on the activity of the peroxidase enzyme, with the highest activity recorded at a concentration of 500 mg.l^{-1} of intercalated potassium silicate with a concentration of 50 mg. l^{-1} Vitamin C reached 39.44 and 39.46 unit.100 g $^{-1}$ for the first and second seasons, respectively, compared to the least effective peroxidase (35.12 and 35.37) unit. (100 g protein) $^{-1}$ recorded when spraying treatment with potassium silicate overlapped at a concentration of 250 mg.l^{-1} and vitamin C at a concentration of 50 mg.l^{-1} for the first and second seasons respectively.

The results of the interaction between spraying with boric acid and vitamin C indicate that there is no significant effect on the effectiveness of the peroxidase enzyme for both seasons of the experiment.

While the triple intervention had a significant effect on the effectiveness of the

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peroxidase enzyme for both seasons of the experiment, the highest rate of peroxidase activity was recorded at the 500 $mg.l^{-1}$ intervention of potassium silicate with a concentration of 50 $mg.l^{-1}$ boric acid, concentration of 50 $mg.l^{-1}$ of vitamin C for both seasons was 40.62 and 40.65 unit. (100 g protein) $^{-1}$, so each treatment within its year outperformed the least effective peroxidase enzyme for both the first and second seasons, 31.32 and 32.00 unit. (100 g protein) $^{-1}$, respectively, when the triple interaction of the experimental factors was 250 $mg.l^{-1}$ of potassium silicate with a concentration of 100 $mg.l^{-1}$ boric acid, concentration of 50 $mg.l^{-1}$ Vitamin C.

Table (2) Effect of spraying with potassium silicate, boron, and vitamin C and their interactions on the activity of peroxidase enzyme (unit. (100 g protein) $^{-1}$) for black French grape variety for both seasons 2020 and 2021

Season 2019 – 2020				
A	**B**	C		**A x B**
		C_1	**C_2**	
A_1	**B_1**	39.81	39.42	**39.62**
	B_2	38.23	38.50	**38.37**
	B_3	38.22	38.50	**38.36**
A_2	**B_1**	37.30	37.02	**37.16**
	B_2	38.50	37.03	**37.77**
	B_3	35.03	31.32	**33.18**
A_3	**B_1**	35.99	39.03	**37.51**
	B_2	39.02	40.62	**39.82**
	B_3	37.69	38.66	**38.18**
LSD $_{0.05}$		**3.182**		**2.234**
		C_1	**C_2**	**A**
A x C	**A_1**	38.75	38.81	**38.78**
	A_2	36.94	35.12	**36.03**
	A_3	37.57	39.44	**38.50**
LSD $_{0.05}$		**2.397**		**1.71**
		C_1	**C_2**	**B**
B x C	**B_1**	37.70	38.49	**38.10**
	B_2	38.58	38.72	**38.65**
	B_3	36.98	36.16	**36.57**
LSD $_{0.05}$		**N.S.**		**1.713**
		C_1	**C_2**	**C**
N.S.		37.75	37.79	

season 2020 -2021				
A	**B**	**C**		**A x B**
		C_1	**C_2**	
A_1	**B_1**	40.23	39.45	**39.84**
	B_2	38.25	38.52	**38.39**
	B_3	38.25	38.52	**38.39**
A_2	**B_1**	37.32	37.05	**37.19**
	B_2	38.52	37.06	**37.79**
	B_3	35.06	32.00	**33.53**
A_3	**B_1**	36.99	39.05	**38.02**
	B_2	39.05	40.65	**39.85**
	B_3	37.72	38.67	**38.20**
LSD $_{0.05}$		**3.265**		**2.441**
		C_1	**C_2**	**A**
A x C	**A_1**	38.91	38.83	**38.87**
	A_2	36.97	35.37	**36.17**
	A_3	37.92	39.46	**38.69**
LSD 0.05		**2.473**		**1.834**
		C_1	**C_2**	**B**
B x C	**B_1**	38.18	38.52	**38.35**
	B_2	38.61	38.74	**38.68**
	B_3	37.01	36.40	**36.70**
LSD $_{0.05}$		**N.S.**		**1.934**
		C_1	**C_2**	**C**
N.S.		37.93	37.89	

Non-significance denoted by N.S.

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2- Activity of the enzyme catalase (CAT)

The results of table (3) indicate that there is a significant effect of potassium silicate on the effectiveness of the catalase enzyme, as the highest effectiveness of the catalase enzyme was found when treated without potassium silicate, amounting to 0.069 and 0.072 unit.g^{-1} protein for the first and second seasons, respectively, and the lowest effectiveness at a concentration of 250 mg. l^{-1} of potassium silicate amounted to 0.058 and 0.061 unit.g^{-1} protein for the first and second seasons, respectively.

Table (3) Effect of spraying with potassium silicate, boron, and vitamin C and their interactions on the activity of the catalase (CAT) enzyme unit.g^{-1} protein for the black French grape variety for both seasons 2020 and 2021

First Season 2019 - 2020				
A	B	C: C_1	C: C_2	A x B
A_1	B_1	0.070	0.082	**0.076**
	B_2	0.069	0.071	**0.070**
	B_3	0.050	0.073	**0.062**
A_2	B_1	0.070	0.061	**0.066**
	B_2	0.062	0.059	**0.061**
	B_3	0.060	0.038	**0.049**
A_3	B_1	0.059	0.060	**0.060**
	B_2	0.042	0.093	**0.068**
	B_3	0.042	0.070	**0.056**
LSD 0.05		**0.0033**		**0.0023**
		C_1	C_2	A
A x C	A_1	0.063	0.075	**0.069**
	A_2	0.064	0.053	**0.058**
	A_3	0.048	0.074	**0.061**
LSD 0.05		**0.0022**		**0.0014**
		C_1	C_2	B
B x C	B_1	0.066	0.068	**0.067**
	B_2	0.058	0.074	**0.066**
	B_3	0.051	0.060	**0.056**
LSD 0.05		**0.0019**		**0.0015**
		C_1	C_2	C
0.0011		0.058	0.067	

Second Season 2021 - 2020				
A	B	C: C_1	C: C_2	A x B
A_1	B_1	0.072	0.086	**0.079**
	B_2	0.071	0.073	**0.072**
	B_3	0.051	0.078	**0.065**
A_2	B_1	0.073	0.064	**0.069**
	B_2	0.064	0.061	**0.063**
	B_3	0.062	0.041	**0.052**
A_3	B_1	0.056	0.061	**0.059**
	B_2	0.045	0.091	**0.068**
	B_3	0.044	0.076	**0.060**
LSD 0.05		**0.0026**		**0.0012**
		C_1	C_2	A
A x C	A_1	0.065	0.079	**0.072**
	A_2	0.066	0.055	**0.061**
	A_3	0.048	0.076	**0.062**
LSD 0.05		**0.0008**		**0.0006**
		C_1	C_2	B
B x C	B_1	0.067	0.070	**0.069**
	B_2	0.060	0.075	**0.067**
	B_3	0.052	0.065	**0.059**
LSD 0.05		**0.0008**		**0.006**
		C_1	C_2	C
0.0005		0.060	0.070	

It is noted from the same table that spraying with boric acid had a significant effect on reducing the effectiveness of the catalase enzyme. The highest rate of catalase activity was recorded in the comparison treatment, amounting to 0.067 and 0. unit.g^{-1} protein for the first and second seasons, respectively, which exceeded the effectiveness of 0.056 and 0.057 unit.g^{-1} protein for the first and second seasons, respectively, at the highest concentration of boric acid 100 mg. l^{-1}. The presented results show that adding vitamin C had a significant effect on increasing the effectiveness of the catalase enzyme at a concentration of 50 mg. l^{-1} of vitamin C, with an effectiveness rate of 0.068 and 0.070 unit.g^{-1} protein for the first and second seasons, respectively, which was significantly superior to the comparison treatment, both according to its season, in

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which the effectiveness rate was 0.058 and 0.060 unit.g-1 protein for the two seasons, respectively.

As for the dual interaction between the experimental procedures, potassium silicate and spraying with boric acid, it had a significant effect on reducing the effectiveness of the catalase enzyme. The highest rate was recorded in the comparison treatment for the two workers, 0.076 and 0.079 unit.g^{-1} protein for the first and second seasons, respectively. Each treatment outperformed the least effective enzyme in its season, 0.049. And 0.051 unit.g^{-1} protein for the first and second seasons, respectively, when treated with 250 mg. l^{-1} overlap of potassium silicate, concentration of 100 mg. $l^{-1}$of boron.

The binary interaction between potassium silicate and vitamin C had a significant effect on the effectiveness of the catalase enzyme, as its highest effectiveness was recorded when the comparison treatment of potassium silicate interacted with a concentration of 50 mg. l^{-1} of vitamin C reached 0.075 unit.g^{-1} for the first season and 0.079 unit.g^{-1} for the second season, both of which exceeded the lowest effective rate of interaction when treated with 250 mg. $l^{-1}$for potassium silicate, the concentration is 50 mg. l^{-1}vitamin C for the first and second seasons (0.053 and 0.055) unit.g^{-1} protein for the first and second seasons, respectively.

The results of the interaction between spraying with boric acid and vitamin C indicate that there is a significant effect of the interaction on the effectiveness of the catalase enzyme, and the highest reading was when the interaction concentration was treated with 50 mg. l^{-1} of both boron and vitamin C amounted to 0.074 and 0.075 unit.g^{-1} for the first and second seasons, respectively, and both were superior to the least effective enzyme, 0.051 and 0.052 unit.$g^{-1}$for the first and second seasons, respectively, at an overlapping concentration of 100 mg. l^{-1} of boron is a comparative treatment for vitamin C.

The triple interaction of factors had a significant effect on the effectiveness of the catalase enzyme, with the highest effectiveness recorded at 0.093 and 0.091 units.gm-1 for the first and second seasons, respectively, at an interaction of 500 mg. l^{-1} of potassium silicate + 50 mg. l^{-1} of boron + 50 mg. $l^{-}$of vitamin C, both of which were significantly superior to the lowest activity of the catalase enzyme, recording 0.038 and 0.041 unit.g^{-1} protein for the first and second seasons, respectively, when potassium silicate was mixed with 250 mg. l^{-1} with 100 mg. l^{-1} boron and 50 mg. l^{-1} Vitamin C.

Concentration of plant hormones in vine leaves:

3- IAA concentration (µmol.g^{-1} fresh weight)

The results in Table (5) indicate that there is a significant effect of potassium silicate on the concentration of the IAA hormone. The highest concentration of indole acetic acid reached 60.48 and 59.87 µmol.g^{-1} fresh weight at a concentration of 500 mg. l^{-1} of potassium silicate for the first and second seasons, respectively, which were superior to Significantly compared to the comparison treatment for the two seasons (37.32 and 37.46) µmol.g^{-1} fresh weight, first and second, respectively.

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Table (4) Effect of spraying with potassium silicate, boron, and vitamin C and their interactions on the concentration of Indole-3-acetic acid(IAA) hormone (µmol.g^{-1} fresh weight) for black French grape variety for both seasons 2020 and 2021

Season 2019 – 2020

A	B	C: C_1	C: C_2	A x B
A_1	B_1	20.51	33.03	**26.77**
	B_2	44.88	43.45	**44.17**
	B_3	39.38	42.65	**41.02**
A_2	B_1	52.63	54.69	**53.66**
	B_2	50.31	55.52	**52.92**
	B_3	64.49	56.42	**60.46**
A_3	B_1	53.93	59.87	**56.90**
	B_2	59.77	65.28	**62.53**
	B_3	62.58	61.45	**62.02**
LSD $_{0.05}$		**2.748**		**2.186**
		C_1	C_2	A
A x C	A_1	34.92	39.71	**37.32**
	A_2	55.81	55.54	**55.68**
	A_3	58.76	62.20	**60.48**
LSD $_{0.05}$		**2.121**		**1.921**
		C_1	C_2	B
B x C	B_1	42.36	49.20	**45.78**
	B_2	51.65	54.75	**53.20**
	B_3	55.48	53.51	**54.50**
LSD $_{0.05}$		**2.794**		**1.942**
		C_1	C_2	C
1. 786		49.83	52.48	

Season 2020-2021

A	B	C: C_1	C: C_2	A x B
A_1	B_1	17.75	33.76	**25.76**
	B_2	45.68	44.21	**44.95**
	B_3	40.18	43.15	**41.67**
A_2	B_1	53.18	55.19	**54.19**
	B_2	50.54	55.86	**53.20**
	B_3	62.18	57.27	**59.73**
A_3	B_1	54.69	60.62	**57.66**
	B_2	60.23	66.02	**63.13**
	B_3	63.68	53.93	**58.83**
LSD $_{0.05}$		**2.827**		**2.122**
		C_1	C_2	A
A x C	A_1	34.54	40.37	**37.46**
	A_2	55.30	56.11	**55.70**
	A_3	59.53	60.21	**59.87**
LSD $_{0.05}$		**2.061**		**2.902**
		C_1	C_2	B
B x C	B_1	41.87	49.86	**45.87**
	B_2	52.15	55.36	**53.76**
	B_3	55.35	51.47	**53.41**
LSD 0.05		**2.801**		**1.812**
		C_1	C_2	C
1.647		49.79	52.23	

It is noted from the same table that spraying with boric acid had a significant effect on increasing the concentration of the IAA hormone. The highest concentration of the hormone was recorded at 54.50 µmol.g^{-1} fresh weight for the first season when treated with 100 mg. l^{-1} of boron and 50 mg. l^{-1}of boron it reached 53.76 µmol.g^{-1} fresh weight for the second season, even if the lowest concentration was 45.87 and 45.78 µmol.g^{-1} fresh weight for the first and second seasons, respectively, in the boron-free treatment.

The results show that the addition of ascorbic acid significantly affected the increase in the concentration of the hormone IAA in grape leaves, as the concentration of IAA reached 52.48 and 52.23 µmol.g^{-1} fresh weight for the first and second seasons, respectively. As for the dual interaction of spraying with the agents potassium silicate and boron, it had a significant effect on increasing the concentration of the IAA hormone. The highest concentration of indole acetic acid was recorded at 62.53 and 63.13 µmol.g^{-1} fresh weight for the first and second seasons, respectively, at of 500 mg. l^{-1}of

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potassium silicate mixed with 50 mg. $l^{-1}$of boron, while the lowest concentration of IAA in the comparison treatment of the two workers reached 26.77 and 25.76 µmol.g^{-1} fresh weight for the first and second seasons, respectively.

Increasing the concentration of potassium silicate when mixed with ascorbic acid had a significant effect on increasing the concentration of the IAA hormone in fresh grape leaves. Its highest concentration was recorded when potassium silicate at of 500 mg. l^{-1} was mixed with ascorbic acid at a concentration of 50 mg. l^{-1}, reaching 62.20 and 60.21 µmol.g^{-1} fresh weight for the first and second season, respectively, while the lowest concentration was 34.92 and 34.54 µmol.g^{-1} fresh weight for the first and second seasons, respectively, when the comparison treatment of the two workers was overlapped.

The results of the dual interaction of the two spraying agents with boric and ascorbic acid indicate that there is a significant effect on the concentration of the IAA hormone. The highest concentration of the hormone was recorded when spraying with boron at a concentration of 100 mg. l^{-1} overlapping with the comparison treatment of ascorbic acid amounted to 55.48 µmol.g^{-1} fresh weight for the first season, which exceeded the interaction of the comparison treatment for workers in the same season was 42.36 µmol.g^{-1} fresh weight, while the highest concentration of the hormone was 55.36 µmol.g^{-1} fresh weight when the two acids were interacted with of 50 mg. l^{-1} of boron and ascorbic, which was significantly superior to the lowest concentration when the comparison treatment of the two acids was 41.87 µmol.g^{-1}fresh weight in the second season.

The results of the statistical analysis show that the triple interaction of factors has a significant effect on the concentration of the IAA hormone. The highest concentration of the hormone was recorded in the spray treatment with 500 mg. l^{-1} of potassium silicate, a concentration of 50 mg. l^{-1} of boron and of 50 mg. l^{-1} of ascorbic acid, reaching 65.28 and 66.02. µmol.g^{-1}fresh weight for the first season and the second season, respectively. The lowest concentration of the hormone recorded when the comparison treatment of the three factors was overlapped was 20.51 and 17.75 µmol.g^{-1}fresh weight for the first and second seasons, respectively.

4- GA_3 concentration (µmol.g^{-1} fresh weight).

The results of Table (5) indicate that there is a significant effect of increasing potassium silicate in increasing the concentration of the GA_3 hormone. The highest average gibberellin concentration was recorded at 28.47 and 29.36 µmol.g^{-1}fresh weight for the first two seasons at a concentration of 500 mg. l^{-1} of potassium silicate, which exceeded the lowest concentration of the gibberellin hormone (22.86 and 23.62) µmol.g^{-1}fresh weight, when treated with spraying with potassium silicate at a concentration of 250 mg. l^{-1} for the first and second seasons, respectively.

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Table (5) Effect of spraying with potassium silicate, boron, and vitamin C and their interactions on the concentration of the hormone GA_3 (µmol g^{-1} fresh weight) for the black French grape variety for both seasons 2020 and 2021

Season 2019 – 2020

A	B	C: C_1	C: C_2	A x B
A_1	B_1	14.95	19.32	**17.14**
	B_2	28.83	30.38	**29.61**
	B_3	22.63	21.79	**22.21**
A_2	B_1	23.82	23.36	**23.59**
	B_2	23.08	22.40	**22.74**
	B_3	20.60	23.87	**22.24**
A_3	B_1	24.03	23.31	**23.67**
	B_2	30.12	32.14	**31.13**
	B_3	30.74	30.45	**30.60**
LSD 0.05		**1.454**		**1.033**
		C_1	**C_2**	**A**
A x C	A_1	22.14	23.83	**22.98**
	A_2	22.50	23.21	**22.86**
	A_3	28.30	28.63	**28.47**
LSD 0.05		**1.351**		**0.962**
		C_1	**C_2**	**B**
B x C	B_1	20.93	22.00	**21.47**
	B_2	27.34	28.31	**27.83**
	B_3	24.66	25.37	**25.01**
LSD 0.05		**1.072**		**0.792**
		C_1	**C_2**	**C**
0.484		24.31	25.22	

Season 2020 -2021

A	B	C: C_1	C: C_2	A x B
A_1	B_1	14.87	19.35	**17.11**
	B_2	29.57	31.07	**30.32**
	B_3	23.51	26.48	**25.00**
A_2	B_1	23.97	24.24	**24.10**
	B_2	23.95	22.93	**23.44**
	B_3	21.69	24.93	**23.31**
A_3	B_1	24.92	24.33	**24.63**
	B_2	31.13	32.74	**31.94**
	B_3	31.57	31.49	**31.53**
LSD 0.05		**1.526**		**1.091**
		C_1	**C_2**	**A**
A x C	A_1	22.65	25.63	**24.14**
	A_2	23.20	24.03	**23.62**
	A_3	29.21	29.52	**29.36**
LSD 0.05		**1.142**		**0.912**
		C_1	**C_2**	**B**
B x C	B1	21.25	22.64	**21.95**
	B2	28.22	28.91	**28.57**
	B3	25.59	27.63	**26.61**
LSD 0.05		**1.086**		**0.809**
		C_1	**C_2**	**C**
0.592		25.02	26.39	

It is noted from the same table that the difference in concentrations of spraying with boric acid significantly affected the difference in the concentration of the GA_3 hormone in the French black variety in grape leaves. The highest concentration of the AG3 hormone was recorded when treating boron with a concentration of 50 mg. l^{-1}(27.83 and 28.56) µmol.g^{-1}fresh weight for the first and second seasons over respectively, which outperformed the other two treatments, while the lowest concentration of gibberellin recorded in the comparison treatment of boric acid amounted to 21.47 and 21.95 µmol.g^{-1}fresh weight for the first and second seasons, respectively.

The addition of ascorbic acid significantly affected the increase in the concentration of the GA_3 hormone, reaching (25.23 and 26.39) µmol.g^{-1}fresh weight for the first and second seasons, respectively.

As for the bilateral interaction between potassium silicate and spraying with boric acid, it significantly affected the concentration of the GA_3 hormone. The highest concentration of gibberellin was recorded at 31.13 and 31.94 µmol.g^{-1}fresh weight when

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treating potassium silicate with a concentration of 500 mg. l^{-1} with boron at a concentration of 50 mg. $l^{-1}$for the first and second seasons, respectively, both of them were significantly superior to most of the two-factor interactions, and the lowest concentration of gibberellic hormone in the comparison treatment for the two agents was 17.14 and 17.11 µmol.g^{-1}fresh weight for the first and second seasons, respectively.

It appears that the interaction of potassium silicate with ascorbic acid significantly affected the concentration of the GA_3 hormone, with the highest concentrations of the hormone recorded at 68.47 and 29.52 µmol.g^{-1}fresh weight when overlapping treatment with potassium silicate spraying at a concentration of 500 mg.l^{-1} with the addition of ascorbic acid, both of them outperformed the control treatment intervention for workers in the first and second seasons, which recorded the lowest concentrations of the hormone, 22.14 and 22.65 µmol.g^{-1}fresh weight for respectively.

It appears that the interaction of boric and ascorbic acids has a significant effect on the concentration of gibberellin. The highest concentration of gibberellin was recorded as 28.31 and 28.91 µmol.g^{-1}fresh weight when treated with boron interference at a concentration of 50 mg.l^{-1} with the addition of ascorbic acid for both the first and second seasons, respectively, while the lowest concentration of gibberellin in the comparison treatment due to the interaction of the two factors in the first and second seasons was 20.93 and 21.25 µmol.g^{-1}fresh weight, respectively.

The triple interaction had a significant effect on the concentration of the GA_3 hormone. The highest rate of hormone concentration was recorded in grape leaves when potassium silicate treatment at a concentration of 500 mg. l^{-1} was combined with boron at a concentration of 50 mg. l^{-1} in the presence of ascorbic acid, reaching (32.14 and 32.74) µmol.g^{-1}fresh weight for the first and second seasons, respectively, both of them were significantly superior to the lowest concentration of gibberellin in the first and second seasons, which recorded (14.95 and 14.87) µmol.g^{-1}fresh weight, respectively.

discussion

Moderate or severe stresses to which the plant is exposed increase the accumulation of reactive oxygen species (ROS), including: superoxide (O_2.-), peroxide hydrogen (H_2O_2), and hydroxyl radical (-OH), whose production increases when Cells are exposed to various stress conditions as a defensive state for the cell, and if the stress continues, these radicals increase in quantity and are highly toxic to cells because they interact directly with cell components (25). They interact with the lipids present in the cell wall, causing damage to the cell wall. Damage to the cell envelope affects the respiratory activity of the mitochondria, and destroys the chlorophyll pigment (20), thus reducing the ability to fix carbon dioxide (CO_2) in the chloroplast. (32), If it interacts with proteins, it causes them to be destroyed or change their nature (10).

Plants have two defense mechanisms to reduce the toxicity of these forms. The first is non-enzymatic systems, such as ascorbate and carotenoids, and the second is enzymatic systems, including superoxide dismutase (SOD), peroxidase (POD) and catalyse (CAT), as superoxide dismutase accelerates the conversion of two oxygen molecules free (O_2.-) turns into hydrogen peroxide (H_2O_2) and bound oxygen (O_2.-), and hydrogen peroxide is removed by catalase and peroxidase enzymes (18).

The above may explain the variation in the effect of potassium silicate treatments, boron and ascorbic acid, and their interactions, on the concentration of the peroxidase enzyme, the decrease in catalase, and the increase of the growth hormones indole acetic acid (IAA) and gibberellin GA_3, with an increase in the concentration of potassium silicate, because potassium has a role in

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regulating the osmotic potential, reducing the osmotic potential of the xylem, and stimulating the process of Photosynthesis and the transfer of its products to plant parts (33) and its activation of a large number of enzymes (35), and therefore the chlorophyll content of the leaves increases (36), in addition to the role of hydrated amorphous silica ($SiO_2 . nH_2O$) which accumulates in the endoplasmic reticulum, cell walls, and in the spaces between cells and acts instead of lignin in strengthening cell walls (15).

As for boron, increasing its concentration reduced the effectiveness of the enzymes peroxidase and catalase and increased the hormones IAA and GA_3, because boron has a role in building nucleic acids, hormonal responses, and the transfer of photosynthesis products, such as sugars, from their places of production to their active places in the plant after combining with boron through membranes cellular cells (9) and its role in auxin synthesis (2).

Adding ascorbic acid increased the effectiveness of the CAT enzyme and the hormones IAA and GA_3 because of its role in protecting cells from the harmful effects of temperatures, toxins and photo-oxidation (14) and its role in preserving chlorophyll from oxidation because it is an antioxidant and a stimulator of respiratory processes (12).

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