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BIOSTIMULANTS EFFECT ON THE ANTIOXIDANTS OF DATE PALM (*PHOENIX DACTYLIFERA* L.)

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SUMMARY

This study, conducted at the College of Agriculture and Marshlands, Thi-Qar University, commenced during the 2019–2020 season on 36 three-year-old offshoots of date palm (*Phoenix dactylifera* L.) cultivar Lulu, produced from tissue culture. The research aimed to know the impact of Calmax and Stimplex and their interactions on the leaf contents of flavonoids, glutathione, vitamin C, and the superoxide dismutase enzyme. The results showed the significant superiority of the Calmax treatment at a concentration of 4 ml L^{-1}, recording the highest averages (11.61 mg g^{-1}, 12.42 µmol g^{-1}, 1.50 mg g^{-1}, and 14.06 unit g^{-1} f.w.), respectively, compared with the control treatment. The Stimplex treatment at a concentration of 12 ml L^{-1} showed notable superiority, with maximum averages of 13.30 mg g^{-1}, 13.02 µmol g-1, 1.55 mg g^{-1}, and 16.61 unit g^{-1} f.w., respectively, versus the control treatment. The interaction treatment between Calmax at a concentration of 4 ml L^{-1} and Stimplex at a concentration of 12 ml L^{-1} revealed remarkable differences. The highest averages for flavonoids, glutathione, vitamin C, and the superoxide dismutase enzyme emerged at 13.94 mg g^{-1}, 13.56 µmol g^{-1}, 1.66 mg g^{-1}, and 18.45 unit g^{-1} f.w., respectively, compared with the control treatment.

Keywords: Date palm (*P. dactylifera* L.), Calmax, flavonoids, glutathione, Stimplex, vitamin C

Key findings: A significant increase was evident in the leaf content (antioxidants flavonoids, glutathione, vitamin C, and superoxide dismutase enzyme) of the date palm (*P. dactylifera* L.) cultivar Lulu due to foliar application of Calmax (4 ml L^{-1}) and Stimplex (12 ml L^{-1}) and their interaction.

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INTRODUCTION

The date palm (*Phoenix dactylifera* L.) is one of the most valued fruit trees, belonging to the Arecaceae family, whose cultivation is widespread in Iraq and some regions of the Middle East (Barreveld, 1993). It is the first tree in Iraq and the Arabian Gulf Region, with the most widespread palm area in the world, and from there, it has spread to other regions with a suitable atmosphere (Al-Jubouri, 2002). The Lulu date palm cultivar is one of the rare cultivars in the Qatif Region in the Kingdom of Saudi Arabia. The color of its fruits during the Khalal stage is light yellow, their shape is cylindrical and swollen near the funnel, and the tail is short and conical. They are small and eaten in the Rutab stage and late in maturity (Al-Bakr, 1972). It is one of the cultivars sensitive to pests and moderately sensitive to lack of water and salinity; the location of the hilum in the seed is closer to the base, and the groove in it is superficial (Saleh and Ibrahim, 2018).

Date palm culture in Southern Iraq faces many problems, the most critical, of which, are environmental stress, such as, high temperatures, drought, and soil and water salinity. These are determining factors for growth, development, and production in plants, in general, including the date palm, because of their negative effects, especially on new offshoots resulting from tissue culture (Munns and Tester, 2008). Therefore, it is necessary to follow the foliar feeding system by spraying some compounds that act as anti-stress agents. This is in addition to providing the plant with nutrients and organic materials necessary for growth, development, and production to overcome or reduce harmful environmental influences, and thus, improve the plant's ability to withstand undesirable environmental conditions (Taiz and Zeiger, 2006). Appropriateness, as the foliar feeding method is usually one of the most modern and efficient means of treating the deficiency of mineral elements compared to traditional fertilization methods. Its efficiency increases by 8–20 times compared to ground fertilization, especially with trace mineral elements. Among these compounds used in the foliar feeding method are the Calmax nutrient solution and the Stimplex biological growth stimulant (Kuepper, 2003). The Calmax nutrient solution is an emulsifying liquid, which is completely soluble in water. It is a typical foliar nutrient with a distinctive technology consisting of many essential nutrients for the plant, including nitrogen (29%), calcium (22.5%), magnesium (3%), and chelated microelements (manganese–0.15%, iron–0.75%, zinc–0.03%, boron–0.075%, and copper–0.06%). As for the Stimplex biostimulant, it is an extract from the *Ascophyllum nodosum* marine algae, which serves as a plant biostimulant by spraying on the leaves. It is unique by its composition containing various elements, carbohydrates, amino acids, and growth stimulants, such as, auxins and cytokinins, which are all organic components (Crouch and Van-Stadan, 2010). In addition, it contains alginic acid, which is a polysaccharide consisting of units of mannuronic acid and guluronic acid derived from brown seaweeds. Alginic acids are a major component of the cell wall in algae and constitute about 15%–30% of dry weight, in addition to being a typical natural chelating substance for the elements, i.e., Mg, Zn, Fe, Cu, and Mn (Khan *et al*., 2009).

These compounds provide good nutrition for the plant and are a safe and good alternative to inorganic industrial fertilizers because the compounds produced from algae and seaweed are an essential part of the marine ecosystem. They usually contain nutrients, vitamins, growth stimulants, and antibiotics, and brown seaweed is usually the most widely used in the field of agriculture, including *Ascophyllum nodosum* (Blunden *et al*., 1986).

Therefore, this study came with the aim of knowing the effect of different concentrations of the two compounds Calmax and Stimplex and their interactions in some antioxidants, such as, flavonoids, glutathione, vitamin C, and the superoxide dismutase enzyme. Likewise, determine their effect in improving the growth and tolerance of young date palm offshoots of the Lulu cultivar, resulting from tissue culture under environmental stress conditions in Southern Iraq.

MATERIALS AND METHODS

The study commenced at the College of Agriculture and Marshlands at the University of Thi-Qar during the 2019–2020 growing season on 36 three-year-old offshoots of the Lulu cultivar, produced from tissue culture, with identical length, size, and leaf numbers. The study had a factorial experiment design, according to a randomized complete block design (RCBD) with two factors. The first factor included the use of three concentrations of the Calmax nutrient solution (0, 2, and 4 ml L^{-1}) and sprayed on the offshoot's leaves. The second factor comprised spraying four concentrations of the biostimulant (marine algae extract) Stimplex (0, 4, 8, and 12 mL L^{-1}) on the offshoot's leaves. The study included 12 treatments with three replicates for each, with the averages compared according to the least significant difference ($LSD_{0.05}$) test. The offshoot leaves applied with the concentrations of study factors were six times between one and the other (15 days) alternately until the study's completion. The spraying process utilized a hand pump until completely wet, using drops of Tween 20 to reduce surface tension.

The soil preparation for culturing the offshoots was according to a mixing ratio (1:2:1) of soil, peat moss, and vermiculite, respectively. Then, the treatment of the fungicide Radomil and the insecticide Rivadan continued as a preventive measure to avoid insect and fungal infections, both following the recommendations by the producing companies. The soil's placement in anvils had a diameter of 40 cm, with the offshoots transferred to them. The EC of the soil and irrigation water used in the study was 0.72 and 0.123 dsm^{-1}, respectively. The pH of the soil and irrigation water was 6.99 and 7.53, respectively.

The offshoots' treatment with a fertilization program during the study period consisted of the NPK complex fertilizer at a concentration of 3 g L^{-1} and amino acids at a concentration of 3 ml L^{-1} watered once every 10 days. Likewise, micronutrients at a concentration of 1 ml L^{-1} sprayed on the leaves occurred once every 10 days.

Studied characteristics

Estimation of total flavonoids content of leaves (mg g^{-1})

Total flavonoids estimation employed the $AlCl_3$ method described by Kosalec *et al*. (2004). The ability to form a complex between $AlCl_3$ and the hydroxyl group resulted in the benzene rings of flavonoids. The appearance of the yellow shade is evidence of the formation of this complex. The absorbance measurement used a spectrophotometer at a wavelength of 430 nm, using the standard curve of Carsten.

Estimation of the glutathione content of leaves (μmol g^{-1})

The concentration estimation of glutathione followed the method of Moron *et al.* (1979), as glutathione reacts with 5,5-dithiobis nitro benzoic acid (DTNB) and produces a yellow hue. The absorbance measurement using a spectrophotometer had a wavelength of 412 nm and the standard curve for glutathione concentration.

Estimation of the vitamin C content of leaves (mg g^{-1})

Estimating the vitamin C content of the leaves was according to the method (Hussain *et al.*, 2010) where the measuring absorbance used a spectrophotometer at a wavelength of 760 nm and the vitamin C standard curve.

Estimating the activity of the enzyme superoxide dismutase (unit g^{-1} f.w.)

The activity of the enzyme superoxide dismutase bore measuring by the method of Marklund and Marklund (1974) based on the ability of the enzyme to prevent oxidation of pyrogallol at pH (8.2). The absorbance reading was at a wavelength of 421 nm against the equivalent solution at time zero and one minute after adding pyrogallol.

Table 1. The effect of Calmax nutrient solution, Stimplex biostimulant, and their interactions on the leaf content of total flavonoids (mg g^{-1}).

Calmax Con. (ml L^{-1})	Stimplex Con. (ml L^{-1})				Calmax averages
	0	4	8	12	
0	7.17	9.26	11.45	12.61	10.12
2	8.10	10.22	11.92	13.34	10.90
4	8.98	10.95	12.58	13.94	11.61
Stimplex averages	8.08	10.14	11.98	13.30	
$LSD_{0.05}$	Calmax		Stimplex		Interactions
	0.039		0.045		0.079

Statistical analysis

The results of the experiment underwent analysis statistically as a factorial experiment according to a randomized complete block design (RCBD) using the Genstat program, with the differences between the means tested using the least significant difference ($LSD_{0.05}$) test.

RESULTS AND DISCUSSION

Total flavonoids content

The results showed the Calmax nutrient solution caused a significant increase in the leaf content of total flavonoids with an increase in its added concentration (Table 1). The Calmax concentration (4 ml L^{-1}) recorded the highest average of 11.61 mg g^{-1} compared with the control treatment, with the lowest average (10.12 mg g^{-1}). As for the effect of Stimplex, it was noticeable to raise the total flavonoid content of the leaves when increasing its concentration. The concentration treatment 12 ml L^{-1} excelled and recorded the topmost average of 13.30 mg g^{-1} compared with the control treatment, which recorded the lowest at 8.08 mg g^{-1}.

The interaction treatments between the concentrations of the Calmax nutrient solution and the Stimplex biostimulant caused a significant increase in the content of flavonoids in the leaves. The interaction treatment between the Calmax nutrient solution at 4 ml L^{-1} and the stimplex biostimulant at 12 ml L^{-1} outshone, giving the maximum average of 13.94 mg g^{-1} compared with the control treatment, recording the minimum average of 7.17 mg g^{-1}.

The results further revealed a substantial rise in the leaf content of flavonoids with an increase in the concentration of each of the nutrient solutions Calmax and the biostimulant Stimplex by spraying on the leaves (Table 1). This may be because spraying them on the leaves ensures their components of macro- and micronutrients, amino acids, carbohydrates, and hormones enter directly into the plant tissues. Furthermore, this reduces energy consumption, helping the plants meet their nutritional needs during the growth period. In addition to their role in increasing the plant's tolerance to environmental stress conditions, especially since their components include many elements connected to the enzymes responsible for mechanical cellular metabolism within the plant (Al-Ibrahimi, 2009).

Metabolic processes lead to the formation of many compounds, helping the plant improve its growth and development and increase its tolerance to stress conditions. Among these compounds are flavonoids, which are secondary metabolic compounds produced by some types of plants, the most vital are anthocyanins, myricetin-quercetin, tannic acid, and chromogenic acid. These compounds are often the most effective and act as antioxidants to reduce the toxic effects of free radicals and give hydrogen. They can also inhibit the lipoxygenase enzyme and stimulate the glutathione and catalase enzymes when exposed to stress conditions (Vitor *et al*., 2004).

The results of this study agreed with Kondi and Shanna (2019), when they studied the detection of some antioxidants in the fruits of four cultivars of local dates, namely, Al-Bakrari, Al-Ami, Al-Tabouni and Al-Fazzani. The four cultivars contained flavonoids, which are important antioxidants. The findings are also consistent with those of Al-Tawati *et al*. (2021) during their study of the chemical composition and detection of active ingredients in the fruits of two cultivars, Al-Ami and Taghiat, of the Libyan date palm cultivars. They found flavonoid compounds in the fruits of both cultivars. Similarly, this study results are analogous to the findings of Shareef (2015) and Faisal (2019) during their studies of spraying date palm leaves with nutrients, as they found a significant increase in the concentrations of non-enzymatic antioxidants.

Glutathione content

The results revealed the Calmax nutrient solution had a significant effect on increasing the leaf content of glutathione when raising its added concentration (Table 2). The Calmax concentration (4 ml L^{-1}) excelled and recorded the highest average (12.42 μmol g^{-1}) compared with the control treatment, which recorded the lowest average (11.62 μmol g^{-1}). As for the Stimplex biostimulant treatments, they caused a considerable increase in the glutathione content of the leaves with an increase in its added concentration. The concentration treatment 12 ml L^{-1} recorded the premier average (13.02 μmol g^{-1}) compared with the control treatment, with the lowest average (10.89 μmol g^{-1}). The interaction treatments between Calmax and Stimplex concentrations significantly affected an enhancement in the leaves' glutathione content. The interaction treatment between Calmax at 4 mL L^{-1} and Stimplex at 12 mL L^{-1} recorded the utmost average of 13.56 μmol g^{-1} versus the control treatment, which recorded the lowest average of 10.38 μmol g^{-1}.

The outcomes enunciated a remarkable increase in the glutathione content of the leaves due to the effect of both the Calmax nutrient solution and the Stimplex biostimulant (Table 2). This may refer to their addition as a spray to the leaves, ensuring their components, including macro- and micronutrients, amino acids, carbohydrates, and hormones, go straight into plant tissues. It increases plants' tolerance to environmental stress conditions, improves their growth in meeting the nutritional needs of the plants during the growth period. Moreover, reduced energy consumption occurs, especially since its components include many elements related to enzymes responsible for mechanical cellular metabolism within the plant (Al-Ibrahimi, 2009). Metabolic processes produce many compounds making up the plant. They work to improve its growth and development and increase its tolerance to stress conditions, with glutathione as one of these compounds, which is a tripeptide consisting of three amino acids: glutamic, cysteine, and glycine (Balavandy *et al.*, 2014).

Table 2. The effect of Calmax nutrient solution, Stimplex biostimulant, and their interactions on the leaf content of glutathione (μ mol g^{-1}).

Calmax Con. (ml L^{-1})	Stimplex Con. (ml L^{-1})				Calmax averages
	0	4	8	12	
0	10.38	11.25	12.19	12.68	11.62
2	10.95	11.92	12.45	12.82	12.03
4	11.35	11.95	12.84	13.56	12.42
Stimplex averages	10.89	11.71	12.49	13.02	
$LSD_{0.05}$	Calmax		Stimplex		Interactions
	0.072		0.084		0.145

It is a common antioxidant found in plant cells. It is of low molecular weight and works to remove free radicals and reduce or eliminate stress by binding to it with the molecules. Then, enzymes bind to the outer surface of glutathione, or some enzymes use glutathione as an auxiliary substance in the glutathione process. Glutathione is a small reduced and oxidized molecule crucial in the growth and defense signals in plants (Rouhier *et al.*, 2008). Additionally, it has a role in the pathways for building glutathione, ascorbate, jasmonic acid, and plant hormones (Foyer and Noctor, 2005; Noctor, 2006). It also participates in the metabolism of hydrogen peroxide in chloroplasts, as well as, regulate the cell cycle and protect it from oxidative stress, and the level of glutathione fluctuates in the cell (Noctor *et al.*, 2011).

The results of this study agreed with the findings of Sakre and El-Metwally (2009), who found a significant increase in the glutathione content of wheat leaves as a result of sprinkling with some nutrients, such as, ascorbic acid, glutathione, and tocopherol. The outcomes were also consistent with the findings of Al-Hayani (2015) when she studied the effect of glutathione and hydrogen peroxide and their interactions on some qualitative and quantitative traits of the *Vigna radiata* L. plant. She found a significant increase in the concentration of glutathione in the leaves. Glutathione improves plant growth and increases its tolerance to environmental stress conditions as an antioxidant, as it interacts with atomic oxygen, superoxide, and hydroxyl, and thus, works directly to reduce or eliminate free radicals and their harmful effects. Glutathione also enhances plant membranes’ stability by removing Acycleperoxide, consisting of a lipid peroxidation reaction. Additionally, it serves as a reducing agent to restore the ascorbic cycle from the oxidized form to a reduced form by the enzyme ascorbate reductase (Saqr, 2006).

Vitamin C content

The results showed the Calmax nutrient solution treatments caused a noteworthy rise in the vitamin C content of the leaves (Table 3). The Calmax concentration (4 ml L^{-1}) recorded with the highest average (1.50 mg g^{-1}) compared with the control treatment, which gave the lowest average (1.35 mg g^{-1}). The Stimplex biostimulant treatments also significantly boosted the vitamin C content of the leaves, with an increase in its added concentration. The concentration treatment (12 ml L^{-1}) recorded the supreme average (1.55 mg g^{-1}) compared with the control treatment, with the least average (1.25 mg g^{-1}). The interaction treatments between Calmax and Stimplex concentrations caused a pronounced increase in the vitamin C content of the leaves. The interaction between Calmax concentration (4 ml L^{-1}) and Stimplex concentration (12 ml L^{-1}) provided the highest average (1.66 mg g^{-1}) versus the control treatment, with the lowest average (1.16 mg g^{-1}).

The findings expressed a meaningful increase in the vitamin C content of leaves resulting from the effect of both Calmax and Stimplex (Table 3). The reason may be due to their components directly entering into the plant tissues and their linkage to the enzymes

Table 3. The effect of Calmax nutrient solution, Stimplex biostimulant, and their interactions on the vitamin C content of leaves (mg g^{-1}).

Calmax Con. (ml L^{-1})	Stimplex Con. (ml L^{-1})				Calmax averages
	0	4	8	12	
0	1.16	1.35	1.43	1.46	1.35
2	1.27	1.41	1.48	1.52	1.42
4	1.33	1.45	1.56	1.66	1.50
Stimplex averages	1.25	1.40	1.49	1.55	
$LSD_{0.05}$	Calmax		Stimplex		Interactions
	0.018		0.021		0.036

responsible for the mechanics of cellular metabolism within the plant. It further results from processes metabolizing many compounds produced by the plant, including vitamin C, improving its growth and tolerance to environmental stress conditions (Al-Ibrahimi, 2009). Ascorbic acid (vitamin C) is one of the water-soluble vitamins widespread in many plants and has multiple functions. It works to resist oxidation, as it can take oxygen from aqueous solutions and oxidize it easily, forming the compound dehydroascorbic acid. This reaction reached catalyzing by metal ions. Moreover, it works through a mechanism relying on the acid's ability to donate hydrogen to stop the chain reaction of free radicals by creating its own inactive free radicals (Shigeoka *et al.*, 2002).

The outcomes of this study agreed with the results of Shareef (2015) and Faisal (2019) during their studies on some date palm cultivars. They found a significant increase in the concentrations of non-enzymatic antioxidants, including vitamin C, in date palm leaves when sprayed with some nutrient solutions. Ascorbic acid (vitamin C) has many physiological roles in plants, including stimulating the formation of nucleic acids and proteins, acting as a strong electron donor, and serving as a cofactor in many plant enzymes (Mahalingam and Fedoroff, 2003). It also contributes to controlling cell growth, as it has an effect on cell elongation and division, and it reduces many free radicals, thus, lessening the damage caused by oxidative stress (Smirnoff and Wheerler, 2000). Another physiological role of ascorbic acid is to protect plants from harmful effects of high and low temperatures (Walker and Mckersie, 1993), salt stress (Khan *et al*., 2006), freezing stress (Lie *et al*., 2007), and drought stress (Amin *et al*., 2011).

Superoxide dismutase enzyme activity

Significant differences appeared between the Calmax concentrations in increasing the leaf activity of the enzyme superoxide dismutase, with results shown in Table 4. The Calmax concentration (4 ml L^{-1}) achieved the highest average (14.06 unit g^{-1} f.w.) compared with the control treatment, which recorded the lowest average (10.98 unit g^{-1} f.w.). On the Stimplex biostimulant, it indicated a noteworthy buildup in the leaf activity of the enzyme superoxide dismutase, with an increase in its concentration. The concentration (12 ml L^{-1}) recorded the maximum average (16.61 unit g^{-1} f.w.) compared with the control treatment, providing the lowest average (8.10 unit g^{-1} f.w.). The interaction treatments between Calmax and Stimplex showed prominent differences between them. The interaction treatment between Calmax at a concentration of 4 ml L^{-1} and Stimplex at a concentration of 12 ml L^{-1} obtained the maximum average (18.45 unit g^{-1} f.w.) versus the control treatment, with the lowest average (5.67 unit g^{-1} f.w.).

The results showed a significant increase in the activity of the enzyme superoxide dismutase due to the effect of Calmax and Stimplex and their interactions (Table 4). This may be due to the role of these two compounds in providing many necessary nutrients for the plant. Calmax nutrient

Table 4. The effect of Calmax nutrient solution, Stimplex biostimulant, and their interactions on the leaf content of superoxide dismutase enzyme (unit g^{-1} f.w.).

Calmax Con. (ml L^{-1})	Stimplex Con. (ml L^{-1}) 0	4	8	12	Calmax averages
0	5.67	10.98	12.53	14.75	10.98
2	7.88	11.91	13.78	16.62	12.55
4	10.76	12.08	14.93	18.45	14.06
Stimplex averages	8.10	11.66	13.75	16.61	
$LSD_{0.05}$	Calmax 0.03133		Stimplex 0.03618		Interactions 0.06267

solution contains various nutritional elements in its composition vital for plant growth, especially micronutrients, such as, iron, zinc, boron, copper, and manganese. Meanwhile, the Stimplex biostimulant contains numerous nutritional elements, carbohydrates, amino acids, and growth stimulants, such as, auxins and cytokinins, with all of these components are of organic origin. In addition to containing chelating substances, it contains primary elements, such as, magnesium, manganese, iron, copper, and zinc (Crouch and Van-Stadan, 2010; Khan *et al*., 2009).

Therefore, as a result of the important nutritional components that make up these two compounds, it is highly likely that they play an essential role in increasing the leaves' content of the superoxide dismutase enzyme. Specifically, they provide many crucial mineral elements binding with this enzyme, such as iron, copper, manganese, and zinc. The superoxide dismutase enzyme is a mineral enzyme found in plants in three types called isozymes, depending on the metal used as catalysts, which are MnSOD, FeSOD, and Cu/ZnSOD. A great similarity exists in the functions of these types, which are the detoxification of superoxide ions, and their presence is in different places inside the cell (Morgan *et al.,* 2008). The Cu/Zn SOD is prevalent in the cytosol and MnSOD is often in the mitochondria, with its presence also noted in chloroplasts, while the FeSOD is evident only in chloroplasts (Coratao *et al*., 2006).

The results of this study agreed with the findings of Al-Mayahi (2016) when he studied the treatment of date palm plantlets of a Nersy cultivar with salicylic and ascorbic acids. Findings revealed a significant increase in the activity of the enzyme superoxide dismutase in the leaves. The study findings are also consistent with the results of Al-Badri *et al*. (2021). They stated a marked rise in the activity of the enzyme superoxide dismutase for the leaves of date palm offshoots, a Nabaiti cultivar, due to the effect of spraying the organic nutrient fulvic acid. Enzymes are the key organic contributing effectively in the plant's protective system, thus, improving its growth and increasing its tolerance to environmental stress conditions (Ashraf, 2009).

CONCLUSIONS

From the study results, one can conclude the effect of the Calmax nutrient solution and the Stimplex biostimulant treatments, individually or in interactions, significantly increased the studied content of leaves. These included the antioxidants flavonoids, glutathione, vitamin C, and superoxide dismutase enzyme in the date palm offshoot Lulu cultivar resulting from tissue culture. The treatments help them greatly improve growth and tolerance to their exposure from environmental stress conditions, such as, soil and water salinity.

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