



ORIGINAL ARTICLE

EFFECT OF SPRAYING WITH VEGEAMINO SOLUTION ON GROWTH INDICATORS CHEMICAL AND ENZYME CONTENT OF DATE PALM FRUITS (*PHOENIX DACTYLIFERA* L.) CV. HILLAWI

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Abstract: The current experiment was carried out at the Palm Research Center of the Presidency of Basra University, for the 2015-2016 growing season, to study the effect of different concentrations of Vegeamino solution with concentrations 2 and 4 ml.L⁻¹ and a comparison treatment (distilled water) to determine the appropriate concentration of the Vegeamin solution on the physiology of ripening enzymes and the chemical content of fruit. The experiment was carried out as a factorial experiment according to the R.C.B.D. design with three replications and the results showed that the concentrations of Vegeamino solution led to a decrease in the effectiveness of the ripening enzymes in the date palm fruits of the Hillawi variety, as the results indicated an increase in the effectiveness of enzymes in the untreated fruits and a decrease in the fruit content of dissolved phenols as a result of spraying with a concentration of Vegeamino in concentrated solution 4 ml.L⁻¹ at the rate of twice spray. The results also showed that the spray factor significantly increased both total soluble carbohydrates and phenols. There were no significant differences for the concentration factor in the fruit content of total soluble phenols and the enzyme polyphenol oxidase in the fruits.

Key word: Vegeamino, Invertase, Cellulase, Polyphenol oxidase, Chemical composition.

Cite this article

Muntaha Abdul Zahra Ati, Murtadha Shanana Auda and Khawla Hamza Mohammed (2021). Effect of spraying with Vegeamino solution on growth indicators chemical and enzyme content of date palm fruits (*Phoenix dactylifera* L.) cv. Hillawi. *International Journal of Agricultural and Statistical Sciences*. DocID: <https://connectjournals.com/03899.2021.17.1779>

1. Introduction

Date palm (*Phoenix dactylifera* L). belongs to the Arecaceae family. It is one of the most essential fruit trees cultivated in Iraq and some Middle East regions. The Hillawi variety is considered one of the world-famous varieties and comes at the forefront of the early ripening commercial varieties exported by Iraq [Matar (1991)]. Date palm cultivation is spread in most dry areas with high temperatures and rains throughout the Arab world. Date palm occupies great importance in the Arab world in economic and social terms, as it is considered an important element in preserving the environment and combating desertification. The date palm fruits go through several stages during their growth and development, the most important of which are the last three which are Khalal, Al-Rutab and dates. The

Khalal stage is considered the maturation stage. The fruits in them take their natural size and distinctive shape according to the genetic factors of the variety and their interactions with the environmental conditions in which the date palm lives, in addition to their acquisition of color, which is one of the main signs that distinguish the varieties from each other. Especially those characterized by a sweet taste and a taste free of astringent substances, in addition to the fragility of their texture, including Barhi, Barim, Hillawi *etc.* Enzymes are protein molecules with a high degree of specialization and high molecular weight produced by cells in small quantities, as each type of enzyme helps to accelerate one specialized type of chemical reactions, in palm fruits many enzymes are related to transformations ripening. These include (Invertase, Pectinesterase, Pectinase,

Polyphenoloxidase, Cellulase and Polygalacturonase). One of the most important enzymes in the date palm fruits that greatly influence the quality and consistency factor is invertase. This enzyme, as it is known, turns sucrose into monosaccharides (glucose and fructose). And the cellulase enzyme is known for its activity in dissolving the cellulose wall, it this action helps the softness and ripeness of the fruits in addition to the enzyme polyphenol oxidase, which plays a vital role in the distinctive color of the fruit [Murata *et al.* (1995a)].

Many studies have been conducted on invertase and cellulase activity changes during the ripening of palm fruits for different varieties. Khalaf (2003) extensively studied invertase and cellulase enzyme changes during the growth and development of the seed and parthenocarpic date palm fruits and their development for the Barhi variety and found that there is no effect of the invertase enzyme for the Barhi variety in the first growth phase (Kimri phase) until the seventeenth week after pollination. When the fruits entered the coloration phase (Khalal phase), the enzyme activity began to increase until it reached its maximum value of 13141.2 units.Kg⁻¹ min⁻¹ fresh weight at the end of the Khalal stage and then the enzyme activity decreased when the fruits entered the Rutab stage until they reached 984.4 units.Kg⁻¹. minute⁻¹ fresh weight of seed fruits at the Tamer stage. The enzyme invertase activity follows the speed of accumulation of sucrose and the maximum activity reached by the enzyme coincided with the highest level of sucrose [Khalaf (2003)]. Taain *et al.* (2013) also studied the activity of the enzymes of invertase and Cellulase in the fruits of the date palm of the Hillawi variety for three stages of ripening, which are kimri, Khalal and Rutab and they noted that the highest enzyme activity was at the end of the Khalal stage, which reached 7956.42 and 1289.35 units. Kg⁻¹ minute⁻¹. Mustafa *et al.* (2006) during their study of the invertase enzyme in three varieties of Sudanese date palms (Tawa, Bentamoda and Mishrig wad laggai) observed that there were high levels of invertase during the physiological maturity stage (Khalal) for the three varieties, then the levels of invertase started. Soluble enzymes increased when fruits enter the final (wet) ripening stage. Its intensity increased until the end of the Khalal stage (the fourteenth week after pollination) and in the seventeenth week after inoculation did not record any activity of this enzyme and other studies showed that the effectiveness of the enzyme is influenced by the variety of pollen. The study carried

out by Ati *et al.* (2019), when using different treatments of Drin solution and ascorbic acid to be sprayed on date palm trees in the Hillawi variety, showed that the efficacy of the enzymes of invertase and cellulase in the Khalal stage reached 6211 and 6446 units.kg⁻¹ min⁻¹ in the comparison treatment as found. The same researcher reported that the enzymes activity invertase and cellulase in the fruits of the date palm variety Lelwi inoculated with the green Ghanami pollen recorded the highest increase at the end of the Kimri stage (the twelfth week after pollination), reaching 274.22 and 600.84 units.Kg⁻¹.minute⁻¹ [Ati (2019)] indicates the difference in enzyme rates according to the types and treatments used. Given the negative impact of some chemical fertilizers when sprayed on plants and their indirect effect on human health, thinking of new methods that increase growth and one of these methods is the use of spraying with amino acids because they have a major significant role in most physiological processes. In the plant, Vegeamino solution was used because it is a compound rich in free amino acids, nitrogen, organic matter and other compounds.

2. Materials and Methods

This study was conducted in one of the private orchards in the Abu -Al-Khasseb district for the 2015-2016 growing season, where several date palms were selected from the Hillawi variety. They were close in age and growing strength as much as possible, which were sprayed with a Vegeamino solution at two concentrations of 2 and 4 mL.L⁻¹ in addition to the control treatment. Which was sprayed with distilled water and the Tween 20 only and for two sprays, the first in the Hababouk stage and the second in the Kimri stage. Samples were taken in the Khalal stage for use in subsequent experimental measurements. Vegeamino is produced by the Spanish company ARTAL. It contains organic materials and amino acid with 20% free amino acid, 24% total organic matter, 3.9% total nitrogen, 0.03 ammonium nitrogen, 3.85 organic nitrogen. It is a fast-absorbing organic stimulant that stimulates plant growth from as it moves in the vegetable sap and encourages entry of other elements.

2.1 Changes in enzymatic activity

Preparation of extraction solutions: Extraction solution No. (1) 0.06 molar ascorbic acid (pH = 7.5) was prepared by dissolving 10.5678 g of ascorbic acid in a specific distilled water volume and completed the volume to a liter after adjusting the pH value to 7.5.

Extraction solution No. (2) phosphate regulator: 0.25 M potassium phosphate + 0.06 M ascorbic acid (pH = 7.5).

The solution was prepared by dissolving 34.0225 g of di-hydrogen potassium phosphate (KH_2PO_4) with 10.5678 g of ascorbic acid in a specific distilled water volume and completed the volume to a liter after adjusting the pH value to 7.5.

Preparation of enzymatic activity test solutions: 1- Test solution No. (1) 2 M phosphate buffer solution (pH = 4.7) Prepare the solution according to the method described by Christian (1980) by dissolving 11.66 g of potassium di-hydrogen phosphate (KH_2PO_4) with 2.58 g of sodium phosphate ($\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$) in a specific volume of distilled water and complete the volume to a liter of distilled water after adjusting the pH value to 4.7 using HCl (0.01 N).

Test solution No. (2) Sucrose solution (0.1 M Sucrose): The solution was prepared by dissolving 34.2 g of sucrose in a liter of phosphate buffer solution Test Solution No. (1). This solution was used to measure the effectiveness of the invertase enzyme after adjusting the pH value to 4.0.

Test solution No. (3) solution (DNSA) 3.5 dinitro silicylic acid: The solution was prepared according to the method described by Taya *et al.* (1985).

DNSA was used as the reagent in the determination of reducing sugars resulting from the degradation of sucrose by invertase.

Extraction method: The extraction process was conducted according to the method mentioned in AL-Bakir and Whitaker (1978).

Estimating the effectiveness of the enzyme invertase: The effectiveness of the invertase enzyme was determined by taking 5 ml of test solution No. (2) (sucrose), which is the substance subject to the enzyme in the test tube and incubated for 5 minutes at a temperature of 35°C. Then 0.5 ml of the enzymatic solution was added to each tube (this is the beginning of the reaction). After the tubes were shaken well, they were placed in a water bath at a temperature of 35°C for 20 minutes. Then 0.5 ml of test solution No. (3) was added to each tube and the tubes were cooled with cold water then calculate the absorption spectrum of each sample in a Spectrophotometer with a wavelength of 540 nm. Similarly, the control solution or the blank solution was prepared, by adding 0.5 ml of

test solution No. (1) (phosphate buffer solution) instead of the enzyme solution.

Extraction of the enzyme polyphenol oxidase (PPO): Hamza (2007) method was adopted for estimation by following the increase in absorption at a wavelength of 420 nm in a spectrometer resulting from oxidation of the material subject to the enzyme substrate.

2.2 Change in Total Soluble Phenols

The total soluble phenols in the leaves were estimated according to the method described in Melo *et al.* (2005).

Estimation of the leaves' total dissolved carbohydrates content: The soluble carbohydrate content of the leaves was estimated according to the phenol-sulfuric acid method based on Dobois *et al.* (1956).

2.3 Statistical analysis

The experiment was designed according to the R.C.B.D. As factorial experiment of two factors, the first represents the concentrations and the second factor represents the number of sprays and three replications (Dates) for each treatment. The results were analyzed using the GenStat statistical program and the averages were compared according to the modified mean difference test (R.L.S.D.) at a probability level of 0.05.

3. Results and Discussion

3.1 The effect of foliar spray on the effectiveness of the invertase enzyme

Table 1 shows the effect of foliar spraying with Vegeamino solution on the efficacy of the invertase enzyme in the Khalal stage of the date palm fruits of the Hillawi variety, as it is noticed through the table that the foliar spray caused a decrease in the effectiveness of the invertase enzyme. Still, the comparison treatment recorded a significant increase in this trait amounting to 6225 units.Kg⁻¹. min⁻¹. This result may indicate that spraying with a solution of Vegeamino at the mentioned concentrations led to a delay in ripening due to the decrease in the activity of the enzymes responsible for the ripening of the fruits. As for the concentration factor, the concentration was recorded at 2 ml.L⁻¹ significant increase of 3175 units. Kg⁻¹.min⁻¹ fresh weight, which was significantly superior to the treatment of spraying at a concentration of 4 ml.L⁻¹, indicating that the lower concentrations led to

Table 1: The effect of foliar spraying with Vegeamino solutions on the efficacy of the invertase enzyme of the date palm fruits of the Hillawi variety (unit. Kg⁻¹.minute⁻¹) in the Khalal stage.

Treatment	Number of spray		concentration rate
	twice	trice	
Control	6225	6225	6225
2ml.L ⁻¹	2898	3452	3175
4ml.L ⁻¹	2870	2413	2642
Average number of sprays	3998	4030	interfering
R.L.S.D.	concentration	Number of spray	357.8
0.05	253.0	206.6	

an increase in the activity of the enzyme and that increase was associated with an increase in the ripeness of the fruits. The same table also showed that the number of sprays did not record any significant differences in this characteristic. In contrast, the interaction factor showed significant differences, as the interaction factor for spraying with Vegeamino solution recorded a concentration of 2 ml.L⁻¹ and for three sprays a significant increase in the enzyme activity reached 3452 units.Kg⁻¹.min⁻¹ while the interfering agent was recorded to spray with 4ml.L⁻¹ Vegeamino solution and three sprays of less enzymatic activity amounted to 2413 units. Kg⁻¹.min⁻¹ fresh weight. The decrease in the efficacy of the ripening enzymes as a result of spraying with a solution of Vegeamino is attributed to the increase in the levels of plant hormones (auxins) in the fruits during the stage of the Khalal as a result of spraying with this solution because it contains the nitrogen component that works to stimulate and increase the production of the hormone IAA, as nitrogen is a necessary component to build the amino acid Tryptophan which it forms the basis for building acid indole acetic acid that promotes cell division and cell elongation [Noaema *et al.* (2020)].

3.2 Effect of foliar spray on the activity of the

cellulase enzyme

Table 2 shows the effect of foliar spray with Vegeamino solution on the effectiveness of the cellulase enzyme, as it is also noticed through the table the superiority of the comparison treatment in increasing the effectiveness of the cellulase enzyme in the date palm fruits of the Hillawi variety in the Khalal stage, which amounted to 6433 units. Kg⁻¹.min⁻¹. The table also showed no significant differences in spraying with Vegeamino solution at concentrations of 2 and 4 ml.L⁻¹ for two sprays and three sprays, while the factor of the number of sprays did not record any significant difference except that the interaction factor for spraying with Vegeamino solution concentration of 4 ml.L⁻¹ and two sprays achieved a significant increase in enzyme activity that reached 3245 unit.kg⁻¹.minute⁻¹, which was significantly superior to the interaction treatment of 2 ml.L⁻¹ Vegeamino solution and two sprays and Vegeamino 4 ml.L⁻¹ treatment for three sprays, which I amounted to 2274 and 2330 units. Kg⁻¹ minute⁻¹.

3.3 Effect of the spray on the effectiveness of the enzyme polyphenol oxidase

The results in Table 3 showed that spraying with Vegeamino solution at the concentrations used (2, 4 ml.L⁻¹) did not show any significant differences with

Table 2: The effect of foliar spraying with Vegeamino solutions on the efficacy of the Cellulase enzyme of the date palm fruits of the Hillawi variety (unit. Kg⁻¹.minute⁻¹) in the Khalal stage.

Treatment	Number of spray		concentration rate
	twice	trice	
Control	6433	6433	6433
2ml.L ⁻¹	2274	3106	2690
4ml.L ⁻¹	3245	2330	2787
Average number of sprays	3984	3956	interfering
R.L.S.D.	concentration	Number of spray	341.9
0.05	241.7	197.4	

the comparison treatment free from Vegeamino solution. The results also showed that the factor of the number of sprays did not show significant differences in the activity of the enzyme Polyphenol oxidase in fruits and the interaction treatment was shown to spray with Vegeamino solution at a concentration of 4 ml.L⁻¹ and for three sprays of less enzymatic activity. This may be due to the increase of the abscisic acid as a result of spraying with a solution of Vegeamino at a concentration of 4 ml.L⁻¹ and for three sprays [Ati (2016)]. Polyphenol oxidase (PPO) is one of the enzymes containing copper in its active site. This mineral ion can oxidize the phenolic group into a reaction group known as the quinone. These quinones can interact with each other and surrounding proteins to form dark spots in plant tissues and it is considered one of the responsible enzymes for the appearance of browning when cells are injured during transport [Olewi *et al.* (2020)]. Most of the fruits of fruit trees show a decrease in the activity of the enzyme polyphenol oxidase during ripening, especially in the early stages of ripening. A positive increase occurs as the fruits enter the stage of final maturity and browning of the fruit. As for date palm trees, Ati (2017) showed that the activity of the enzyme Phenol oxidase decreased during the ripening stages of the Hillawi variety of date palm fruits, until it reached its lowest value in the Rutab stage.

3.4 Total dissolved phenols

The results in Table 4 showed that the number of sprays factor recorded a significant superiority in this characteristic, as the spray factor for three sprays was significantly superior to the spray factor for two sprays. Still the concentration factor did not record any significant difference in the fruit content of the total dissolved phenols in the fruits. It showed the superiority of the interaction treatment of spraying with a solution of Vegeamino solution 4 ml.L⁻¹ and for three sprays, which recorded the highest value of 573 mg. 100 g⁻¹, while the interaction treatment was recorded for spraying with a solution of Vegeamino at a concentration of 4 ml.L⁻¹ and two sprays of the lowest value for total phenols in fruits was 89 mg. 100 mg⁻¹, which coincided with the enzyme polyphenol oxidases highest activity in the fruits at this stage and there were no significant differences when spraying with Vegeamino solution with concentrations 2 and 4 ml.L⁻¹ for two sprays, which amounted to 156 and 89 mg. 100 g⁻¹. The study [Ati and Faleh (2011)] showed that the phenolic compounds in the Hillawi variety recorded the maximum in the Kimri stage, reaching 0.22% when the fruits were green and then decreased to a large degree when entering the stage of the Khalal. This decline continued until it reached its lowest value in the Tamer stage.

Table 3: The effect of foliar spraying with Vegeamino solutions on the efficacy of the Polyphenol Oxidase enzyme of the date palm fruits of the Hillawi variety (Unit.ml⁻¹) in the Khalal stage.

Treatment	Number of spray		concentration rate
	twice	trice	
Control	257.0	257.0	257.0
2ml.L ⁻¹	222.0	252.0	237.0
4ml.L ⁻¹	272.7	220.7	246.7
Average number of sprays	250.6	243.2	interfering
R.L.S.D.	concentration	Number of spray	61.59
0.05	43.55	35.56	

Table 4: The effect of foliar spraying with Vegeamino solutions on the efficacy of the Total dissolved phenols of the date palm fruits of the Hillawi variety (mg.g⁻¹) in the Khalal stage.

Treatment	Number of spray		concentration rate
	twice	trice	
Control	273	273	273
2ml.L ⁻¹	156	416	286
4ml.L ⁻¹	89	573	331
Average number of sprays	173	420	interfering
R.L.S.D.	concentration	Number of spray	116.7
0.05	82.5	67.4	

Table 5: The effect of foliar spraying with Vegeamino solutions on the efficacy of the Total carbohydrate of the date palm fruits of the Hillawi variety (mg.g⁻¹) in the Khalal stage.

Treatment	Number of spray		concentration rate
	twice	trice	
Control	3.24	3.24	3.24
2ml.L ⁻¹	6.10	18.75	12.43
4ml.L ⁻¹	7.80	18.19	13.00
Average number of sprays	5.71	13.40	interfering
R.L.S.D.	concentration	Number of spray	1.66
0.05	1.17	0.96	

3.5 The effect of spraying on total dissolved carbohydrates

The results in Table 5 showed that the spraying agent for three sprays significantly exceeded the total dissolved carbohydrates in the fruits, which reached 13 mg.g⁻¹ and the concentration factor showed that the concentration exceeded the concentration of 4 ml.L⁻¹ over the concentration factor of 2 ml.L⁻¹ and control treatment, which recorded the lowest value of 3.24 mg.g⁻¹, also showed that the interaction of spraying with a solution of Vegeamino at a concentration of 2 ml.L⁻¹ and three sprays showed significant superiority in increasing the total dissolved carbohydrates in the fruits of 18.75 mg.g⁻¹, but it did not record significant differences with the interaction. For spraying with a solution of Vegeamino at a concentration of 4 ml.L⁻¹ and for three sprays of 18.19 mg.g⁻¹. The accumulation of total soluble carbohydrates in the fruits as a result of spraying with the Vegeamino solution and for trice spray may be due to the role of the amino acids contained in this solution in overcoming the states of stress, which may be ready to absorb when added as a spray on the trees, thus increasing the photosynthesis process, increasing the manufacture of carbohydrates and transferring them to the storage places in the fruits [AL-Shater and AL-Balkhi (2010)]. Besides, the foliar spray with Vegeamino solution caused an increase in the content of the leaves of the potassium element, which plays an essential role in many physiological aspects, chemical and biological processes represented in the absorption of mineral elements such as nitrogen, the effectiveness of enzymes and the assimilation of carbohydrates [Ati (2016)].

Acknowledgements

All thanks and appreciation to the management of the Palm Research Center of Basra University for

facilitating the completion of the research process in the center's technology laboratory.

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