

## Evaluation of adding the aqueous and alcoholic extract of Garviola (*Annona muricata*) leaves to drinking water on lipid profile and oxidation parameters in eggs

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### Abstract

The research was conducted in one of the farms of Babylon/Iraq. and the field experiment lasted 16 weeks (4 periods of 4 weeks ) that began on November 20, 2023 and ended on March 10, 2024. The research aimed to know the effect of aqueous and alcoholic extracts of Garviola (*Annona muricata*) leaves in drinking water on the performance of laying hens. (105) laying hens were raised at 65 weeks of age, and (66-69) weeks old, (70-73) weeks old, (74-77) weeks old, and (78-78) weeks. old were distributed 21 groups 7 experimental treatments consisting of 15 birds .treatment included three replicates, each containing five birds. T1: control.( T2: 15 ml/leter, T3: 30 ml/leter, and T4: 45 ml/leter of Graviola leaves aqueous extract 1%). (T5:15 ml/leter . T6: 30 ml/leter,and T7: 45 ml/leter of Alcoholic extract of graviola leaves 1%) . The main results of the study are as follows : The second, third, fourth, fifth, sixth, and seventh treatments showed a significant decrease ( $p \leq 0.05$ ) in the concentration of cholesterol, triglycerides, low-density lipoproteins, and very low-density lipoproteins in egg yolks when stored for different periods (0, 15, 30) days compared to the first treatment with A significant improvement ( $p \leq 0.05$ ) in all addition treatments in the concentration of high-density lipoproteins. The treatments of the aqueous and alcoholic extract of Garviola leaves recorded a significant improvement ( $P \leq 0.05$ ) compared to the first treatment (control) in the concentration of Glutathione ( $\mu\text{mol/mol}$ ) and the concentration of the enzyme catalase and superoxide dismutase ( $\mu\text{mol/mol}$ ) in egg yolks when stored for three periods ( 0, 15, 30) days, while a significant decrease ( $P \leq 0.05$ ) was recorded in the concentration of malondialdehyde in egg yolks ( $\mu\text{mol/mol}$ ) compared to the first treatment (control.)

**Keywords:** *Annona muricata*, lipid profile, oxidation parameters, laying hens.

**\*Research paper from PHD thesis for the first author .**

### Introduction

Laying hens are exposed to a decrease in egg production with age as a result of their exposure to stress as a result of an increase in free radicals and active oxygen species in the body's tissues, which affects the decrease in egg production .medicinal plants nowadays have a great place in industrial production as they are a major source of medicinal drugs of plant origin, which are used in preparing

medicine in the form of extracts or active ingredients, or used as raw materials. Production of some primary chemical compounds used in the pharmaceutical industry, which give their medicinal effect, such as flavonoids, glycosides, and polyphenols. and Medicinal plants are currently widely used in industrial of plant-based medicinal medications, which are either

utilized as raw materials or as extracts or active ingredients in medical preparations. to create several basic chemical compounds, such as flavonoids, glycosides, and polyphenols, and tannins that are employed in the pharmaceutical industry and have a therapeutic impact[23,12 ]. The World Health Organization has determined that 80% of medicinal plants are of medical benefit, and most of these benefits are from the use of plant extracts or the activity of their components as growth stimulants[1,10] antibacterial and antifungal agents. Antifungal agents [30,26] and antioxidants [18,25]. It also helps lower blood serum fat levels and boost immunity in addition to stimulating the functions of the digestive system by increasing the production of digestive enzymes, improving the efficiency of the liver, pancreas, and small intestine, forming bile, and stimulating its secretion [13,24] In light of this, trends have increased significantly towards adding medicinal plants to animal diets, whether in the form of alcoholic or aqueous extracts or feed, in order to improve the human dietary pattern by increasing production and improving the health of agricultural animals [29,22]. Among these medicinal plants is the graviola tree, also called the soursop tree, belonging to the Annonaceae family and the *Annona* genus[5,15]. It is native to South America and is widely cultivated in tropical regions throughout the world [17,2]. The Garviola tree is an important resource. It contains many active compounds, such as flavonoids, tannins, and phenolic compounds, in addition to acetogenins, which are the main components of Garviola, in addition to its good content of amino acids and minerals[6,18]. Garviola is an important source of antioxidants and as an antimicrobial, antitumor and antiparasitic agent [23] Recent

research has indicated that there are medical therapeutic benefits for many cancer diseases as a result of aqueous and alcoholic extraction of all parts of this tree (fruits, trunk, roots, leaves) [14,16], but international studies are still very rare in using parts of this tree to feed poultry, and most of them are limited to feeding laboratory animals [32,20]. Given the importance of garviola leaves, the aim of study was the effect of garviola leaves on the lipid profile and oxidation parameters in egg yolks after storage. And its effect on the study indicators, whether the aqueous or alcoholic extract .

#### Materials and methods

This study was conducted in one of the farms belonging to Babil Governorate in the Wardiya region, where the field experiment lasted 16 weeks (four periods of four weeks each) starting from 20/11/2023 to 10/3/2024 105 Lohmann white layer hens were raised at the age of 65 weeks, and the rearing period was divided into 4 experimental periods (66-69) weeks, (70-73) weeks. (74 – 77) weeks and (78 – 81) weeks of the chicken's life. The feed was provided according to the standard needs mentioned in the Lohmann white guide and was distributed randomly among 21 groups, with 7 experimental treatments for each treatment, 15 birds. Each treatment included three replicates, each with 5 birds. The experimental treatments were as follows: First treatment: a control group free of any addition, whether aqueous or alcoholic extract. The second treatment: Adding 15 ml of the aqueous extract of Graviola leaves at a concentration of 1%/liter of drinking water. The third treatment: Adding 30 ml of the aqueous extract of Graviola leaves at a concentration of 1%/liter of drinking water.

Fourth treatment: Adding 45 ml of aqueous extract of Garviola leaves at a concentration of 1% per liter of drinking water. Fifth treatment: Adding 15 ml of alcoholic extract of garviola leaves at a concentration of 1%/liter of drinking water. Sixth treatment: Adding 30 ml of the alcoholic extract of Graviola leaves at a concentration of 1% / liter of drinking water. Seventh treatment: Adding 45 ml of the alcoholic extract of Graviola leaves at a concentration of 1% / liter of drinking water.

The experiment included studying the following characteristics: cholesterol, triglycerides, high-density lipoproteins, low-density lipoproteins, very low-density lipoproteins, glutathione GSH, catalase enzyme CAT, superoxide dismutase (SOD) enzyme, and MDA lipid peroxidation. The lipid profile and oxidation parameters were measured. From yolk to eggs stored over three periods (0, 15 and 30) days.. A completely randomized design was used to study the effect of different treatments on the studied traits. The significant differences between the means were compared using the Duncan multinomial test [8], and the ready-made statistical prog SAS [31] was used to analyze the data

**Table 1. Production feed used in the experiment**

Feed material	(%)
corn yellow	36.5
Wheat	12
barley	12.83
Soybean (44% protein)	25.92
Protein center	2.5
Limestone	9.25
The Vegetable oil	1.0
The Total	100
the Chemical analysis **	
The Representative energy (kilocalorie/kg feed)	2700

Crude protein (%)	17
Crude fiber (%)	3.68
Calcium (%)	4.13
Available phosphorus(%)	0.42
methionine + cysteine( %)	0.71
Lysine (%)	0.92
(DCAB) Dietary Cation-Anion Balance (mg/kg)	202.43
Choline(%)	0.17
Folic acid (mg/kg)	0.54
glycine(%)	0.73
Glycine+serine (%)	1.58
Histidine(%)	0.45
Isoleucine(%)	0.71
Leucine(%)	1.41
Lysine(%)	0.92
Methionine(%)	0.42
Cysteine(%)	0.29
Phenylalanine(%)	0.82
Tyrosine(%)	0.70
Phenylalanine + Tyrosine (%)	1.52
Threonine (%)	0.64
Tryptophan(%)	0.25
Valine(%)	0.80
Arginine(%)	1.07

Protein concentrate from the Dutch company Profimi. Each kg contains: 5.9% crude protein, 3600 representative energy calories/kg, 6.4% calcium, 5.7% phosphorus, 6.5% sodium, 4000 mg/kg iron, 2800 mg/kg zinc, mg /kg 600 copper, 8.35 mg cobalt, 60 mg/kg iodine, 10 mg/kg selenium, 5.9% methionine, 1.5% lysine 5.9% methionine with cysteine, 1200 mg/kg niacin, 400,000 IU vitamin A, 140,000 IU vitamin D3, 2000 mg/kg E, 100 K, 90 mg/kg vitamin B1, 160 ppb vitamin B2, 200 mg/kg vitamin B6 and 1000 mg/kg vitamin B12.

\*\*The analysis of the entering feed materials was used to calculate the chemical composition. [9], according to the Lohman Company guide [2020], and according to the American UFFDA program [2018 ].

## Results

Table (2) shows the evaluation of adding different concentrations of aqueous and alcoholic extracts of Garviola leaves (*Annona muricata*) to drinking water in the concentration of cholesterol and triglycerides in the yolk after storing eggs for periods of (0-15-30) days ( mean  $\pm$  standard error). The results of the statistical analysis indicate that the first treatment (the control) recorded the highest concentration of cholesterol (mg/100 g) during the three storage periods (0, 15, and 30) days, with a significant difference

and

## Discussion

( $P \leq 0.05$ ) from the addition treatments (the second, third, fourth, fifth, sixth, and seventh), which recorded the lowest. Concentration of cholesterol. As for the three triglycerides (mg/100g), the addition treatments (second, third, fourth, fifth, sixth, and seventh) recorded the lowest concentration of triglycerides during the egg storage periods (0, 15, and 30) days compared to the first treatment (control), which recorded the highest concentration of triglycerides and reached (76.63, 97.44, and 98.13 mg/100 g), respectively .

**Table (2) Evaluation of adding the aqueous and alcoholic extract of Garviola (*Annona muricata*) leaves to drinking water on the concentration of cholesterol and triglycerides in the yolk after store eggs for periods of (0-15-30) days ( mean  $\pm$  standard error)**

Treatments	Cholesterol concentration (mg/100g)			Triglyceride concentration (mg/100g)		
	first period the 0days	Second period 15days	Third period 30days	first period 0days	Second period 15days	Third period 30days
<b>T1 control</b>	376.96 $\pm$ 2.10 a	391.40 $\pm$ 2.52 a	435.06 $\pm$ 5.35 a	76.63 $\pm$ 14.37 a	97.44 $\pm$ 1.11 a	98.13 $\pm$ 2.23 a
<b>T2</b>	225.96 $\pm$ 7.59 b	262.16 $\pm$ 1.43 b	234.40 $\pm$ 3.55 bc	55.40 $\pm$ 2.61 b	77.72 $\pm$ 2.53 b	65.23 $\pm$ 2.46 b
<b>T3</b>	217.83 $\pm$ 8.95 b	251.30 $\pm$ 4.20 cd	233.20 $\pm$ 4.30 bcd	46.66 $\pm$ 8.46 bc	58.47 $\pm$ 0.56 d	49.30 $\pm$ 0.89 c
<b>T4</b>	191.26 $\pm$ 3.56 c	242.26 $\pm$ 2.07 d	215.13 $\pm$ 3.93 e	33.06 $\pm$ 5.82 c	50.04 $\pm$ 1.76 e	35.36 $\pm$ 2.05 e
<b>T5</b>	234.20 $\pm$ 9.89 b	257.56 $\pm$ 1.54 bc	244.73 $\pm$ 8.90 b	59.89 $\pm$ 2.94 ab	65.93 $\pm$ 2.11 c	62.73 $\pm$ 1.58 b
<b>T6</b>	221.33 $\pm$ 8.70 b	249.00 $\pm$ 5.07 cd	231.76 $\pm$ 6.79 cd	51.21 $\pm$ 7.95 bc	50.83 $\pm$ 2.30 e	52.06 $\pm$ 1.49 c
<b>T7</b>	193.43 $\pm$ 2.62 c	245.30 $\pm$ 1.98 d	221.50 $\pm$ 2.81 de	41.03 $\pm$ 9.50 bc	43.07 $\pm$ 0.77 f	42.40 $\pm$ 3.20 d
<b>Significant level</b>	*	*	*	*	*	*

\*•means that there are significant differences between the treatments at the significance level ( $P \leq 0.05$ ).• t1 (control) without any additions. T2, t3 and t4 treatments added the aqueous extract of Garviola leaves at a dose of 15, 30 and 45 ml/liter of drinking water, respectively, at a concentration of 1%. T5, t6 and t7 treatments added the alcoholic extract of Garviola leaves at a dose of 15, 30 and 45 ml/liter of water. Drinking water, respectively, at a concentration of 1%.

And seventh (significantly  $P \leq 0.05$ ) on the first treatment

Table (3) indicates the evaluation of adding the aqueous and alcoholic extract of garviola leaves (*Annona muricata*) to drinking water at different concentrations of aqueous and alcoholic extract (15, 30 and 45 ml/liter) and reached (56.66, 57.30, and 54.30 mg/100 g), respectively, as for low-density lipoproteins and very low-density lipoproteins (mg). The results of the statistical analysis indicated that the first treatment recorded the highest concentration of high-density lipoproteins and very low-density lipoproteins, with a significant difference ( $P \leq 0.05$ ) compared to the addition treatments (the second, third, fourth, fifth, sixth, and seventh), which recorded the lowest concentration of high-density lipoproteins and very low-density lipoproteins, with a significant difference ( $P \leq 0.05$ ) compared to the addition treatments (the second, third, fourth, fifth, and sixth) exceed

lowest concentration of low-density lipoproteins and very low-density lipoproteins.

**Table (3) Evaluation of adding the aqueous and alcoholic extract of Garviola (*Annona muricata*) leaves to drinking water in relation to the concentration of high-density lipoproteins and the concentration of low-density lipoproteins and very low-density lipoproteins (mg/100 g) in the yolk after egg storage. For periods (0, 15,30) days ( mean  $\pm$  standard error.(**

Treatments	(HDL)High-density lipoprotein (mg/100g)			(LDL) low-density lipoproteins (mg/100g)			(VLDL)very low-density lipoproteins (mg/100g)		
	0days	15days	30days	0days	15days	30days	0days	15days	30days
<b>T1 control</b>	56.66 <sub>f</sub> $\pm$ 1.24	57.30 <sub>c</sub> $\pm$ 3.76	54.30 <sub>c</sub> $\pm$ 2.55	301.88 <sub>a</sub> $\pm$ 3.22	314.61 <sub>a</sub> $\pm$ 2.80	361.14 <sub>a</sub> $\pm$ 1.82	15.32 <sub>a</sub> $\pm$ 0.29	19.48 <sub>a</sub> $\pm$ 0.22	19.62 <sub>a</sub> $\pm$ 0.44
<b>T2</b>	76.13 <sub>de</sub> $\pm$ 2.24	68.93 <sub>ab</sub> $\pm$ 4.33	65.85 <sub>b</sub> $\pm$ 2.68	138.75 <sub>b</sub> $\pm$ 5.63	177.69 <sub>b</sub> $\pm$ 3.35	155.51 <sub>b</sub> $\pm$ 0.97	11.08 <sub>bc</sub> $\pm$ 0.52	15.54 <sub>b</sub> $\pm$ 0.50	13.04 <sub>b</sub> $\pm$ 0.49
<b>T3</b>	80.35 <sub>cd</sub> $\pm$ 1.19	67.13 <sub>ab</sub> $\pm$ 5.25	69.90 <sub>ab</sub> $\pm$ 6.29	128.15 <sub>b</sub> $\pm$ 4.37	172.48 <sub>bc</sub> $\pm$ 2.39	153.44 <sub>b</sub> $\pm$ 2.56	9.33 <sub>de</sub> $\pm$ 0.89	11.69 <sub>d</sub> $\pm$ 0.11	9.86 <sub>c</sub> $\pm$ 0.17
<b>T4</b>	90.67 <sub>b</sub> $\pm$ 0.99	73.90 <sub>a</sub> $\pm$ 1.20	78.82 <sub>a</sub> $\pm$ 3.84	93.98 <sub>c</sub> $\pm$ 6.29	158.36 <sub>d</sub> $\pm$ 6.35	129.24 <sub>c</sub> $\pm$ 2.67	6.61 <sub>f</sub> $\pm$ 0.56	10.00 <sub>e</sub> $\pm$ 0.35	7.07 <sub>e</sub> $\pm$ 0.41
<b>T5</b>	72.25 <sub>e</sub> $\pm$ 1.68	62.56 <sub>bc</sub> $\pm$ 3.84	74.62 <sub>ab</sub> $\pm$ 0.68	149.98 <sub>b</sub> $\pm$ 19.03	181.82 <sub>b</sub> $\pm$ 5.63	157.57 <sub>b</sub> $\pm$ 1.72	11.97 <sub>b</sub> $\pm$ 0.59	13.18 <sub>c</sub> $\pm$ 0.42	12.54 <sub>b</sub> $\pm$ 0.31
<b>T6</b>	82.63 <sub>c</sub> $\pm$ 0.84	65.50 <sub>b</sub> $\pm$ 1.49	66.66 <sub>b</sub> $\pm$ 2.85	128.46 <sub>b</sub> $\pm$ 4.14	173.34 <sub>bc</sub> $\pm$ 3.13	154.69 <sub>b</sub> $\pm$ 3.79	10.24 <sub>cd</sub> $\pm$ 0.18	10.16 <sub>e</sub> $\pm$ 0.46	10.41 <sub>c</sub> $\pm$ 0.29
<b>T7</b>	100.71 <sub>a</sub> $\pm$ 2.81	73.43 <sub>a</sub> $\pm$ 1.29	78.37 <sub>a</sub> $\pm$ 2.50	84.52 <sub>c</sub> $\pm$ 4.80	163.26 <sub>cd</sub> $\pm$ 2.76	134.65 <sub>c</sub> $\pm$ 3.67	8.20 <sub>ef</sub> $\pm$ 0.30	8.61 <sub>f</sub> $\pm$ 0.15	8.48 <sub>d</sub> $\pm$ 0.64
<b>Significant level</b>	*	*	*	*	*	*	*	*	*

\*•means that there are significant differences between the treatments at the significance level ( $P \leq 0.05$ ). • t1 (control) without any additions. T2, t3 and t4 treatments added the aqueous extract of Garviola leaves at a dose of 15, 30 and 45 ml/liter of drinking water, respectively, at a concentration of 1%. T5, t6 and t7 treatments added the alcoholic extract of Garviola leaves at a dose of 15, 30 and 45 ml/liter of water. Drinking water, respectively, at a concentration of 1%.

The reason for the high concentration of high-density lipoproteins and the low concentration of cholesterol and triglycerides (Table 2) and low-density lipoproteins and very low-density lipoproteins .Table (3) in the addition treatments (second, third, fourth, fifth, sixth, and seventh) compared to the first treatment (the control) may be due to The evaluation of the aqueous and alcoholic extract of Garviola leaves in increasing the activity of the thyroid gland by secreting the hormone thyroxine, which leads to an increase in the representation of cholesterol and the rate of its utilization, and then reduces the concentration of fat [1] This means that the addition treatments have maintained the stability of the fat and its stability in the body and thus It reduces the oxidation and rancidity of fats in egg yolks. As a result, Grviola leaves work to enhance the efficiency of the antioxidant system and thus reduce the evaluation of free radicals in the oxidation and damage of fats inside the body, as they preserve fatty compounds after they are assimilated in the liver and then transported through the blood to the organs. To meet the body's needs and thus reduce the fat in the egg yolk [7] or the reason for the decrease in the concentration of cholesterol, triglycerides, and low-density lipoproteins in the supplement treatments may be due to the evaluation of Garviola leaves in inhibiting the activity of free radicals, especially the negative superoxide radical and hydroxyl radical, and their ability to bind

minerals that stimulate oxidation (bound iron), limit fat decomposition, and reduce acids. Free fatty acids, thus protecting LDL from oxidation [27]. The positive role of vitamin C in reducing egg cholesterol may be through increasing the activity of the thyroid gland and increasing the formation of its hormones [33 ].[

Table (4) indicate the evaluation of adding different concentrations of the aqueous and alcoholic extract of garviola leaves (*Annona muricata*) to drinking water in terms of oxidation standards for the concentration of glutathione, the enzyme catalase, the concentration of the enzyme superoxide dismutase, and the concentration of malondialdehyde in the yolk after storing the eggs for periods ( 0 - 15 - 30) days ( mean  $\pm$  standard error), where the results of the statistical analysis indicated during the three periods of egg storage (0, 15, 30) days, recording the addition parameters of (the second, third, fourth, yesterday, sixth, and seventh) a significant improvement in the concentration Glutathione in egg yolk (micro mol/mol), with a significant difference ( $P \leq 0.05$ ) from the first treatment, which recorded the lowest concentration (19.26, 22.28, and 12.73 micro mol/mol), respectively, while the two treatments (fourth and seventh) recorded the highest concentration. For glutathione. As for the concentration of the catalase enzyme and the concentration of the enzyme superoxide dismutase in the egg yolk ( $\mu\text{mol/mol}$ ), the



addition treatments of (the second, third, fourth, fifth, sixth, and seventh) continued to have a significant improvement ( $P \leq 0.05$ ) compared to the first control treatment, which recorded the lowest concentration of the catalase enzyme. In egg yolk ( $\mu\text{mol/mol}$ ). While in the concentration of malondialdehyde in the egg yolk

( $\mu\text{mol/mol}$ ), the results of the statistical analysis showed indications during the periods (0, 15, and 30) days after storage, where the first control treatment recorded the highest concentration of the enzyme malondialdehyde in the egg yolk ( $\mu\text{mol/mol}$ ), with a significant difference  $P \leq 0.05$  for all addition coefficients.

**Table (4) Evaluation of adding the aqueous and alcoholic extract of Garviola (*Annona muricata*) leaves to drinking water in the oxidation parameters of glutathione concentration, catalase enzyme, malonide dehyde concentration, and superoxide dismutase enzyme concentration in the yolk after storing eggs for periods (0 - 15 - 30) days ( mean  $\pm$  standard error.)**

Treatments	Glutathione concentration in egg yolk ( $\mu\text{mol/mol}$ )			Catalase enzyme concentration in egg yolk ( $\mu\text{mol/mol}$ )			Concentration of superoxide dismutase in egg yolk ( $\mu\text{mol/mol}$ )			Concentration of malondialdehyde in egg yolk ( $\mu\text{mol/mol}$ )		
	The first period (0) days	The second period (15) days	The third period (30) days	The first period (0) days	The second period (15) days	The third period (30) days	The first period (0) days	The second period (15) days	The third period (30) days	The first period (0) days	The second period (15) days	The third period (30) days
<b>T1 control</b>	19.26 <sub>d</sub> $\pm 1.82$	22.28 <sub>d</sub> $\pm 1.40$	12.73 <sub>e</sub> $\pm 1.13$	24.63 <sub>d</sub> $\pm 3.00$	22.06 <sub>e</sub> $\pm 0.58$	17.36 <sub>d</sub> $\pm 0.96$	34.47 <sub>d</sub> $\pm 2.56$	31.13 <sub>d</sub> $\pm 1.37$	26.83 <sub>e</sub> $\pm 1.82$	0.042 <sup>a</sup> $\pm 0.002$	0.060 <sup>a</sup> $\pm 0.001$	0.074 <sup>a</sup> $\pm 0.003$
<b>T2</b>	34.5 <sub>c</sub> $\pm 3.52$	31.51 <sub>c</sub> $\pm 1.53$	26.20 <sub>d</sub> $\pm 1.32$	45.33 <sub>c</sub> $\pm 1.09$	41.63 <sub>d</sub> $\pm 0.64$	44.60 <sub>c</sub> $\pm 0.60$	48.58 <sup>c</sup> $\pm 5.04$	45.91 <sup>c</sup> $\pm 1.47$	39.06 <sub>d</sub> $\pm 1.28$	0.030 <sub>bc</sub> $\pm 0.001$	0.042 <sub>b</sub> $\pm 0.002$	0.042 <sub>b</sub> $\pm 0.003$
<b>T3</b>	48.54 <sub>b</sub> $\pm 1.1$	40.73 <sub>b</sub> $\pm 1.16$	35.76 <sub>c</sub> $\pm 1.44$	64.86 <sub>b</sub> $\pm 1.69$	57.53 <sub>c</sub> $\pm 1.21$	52.00 <sub>b</sub> $\pm 3.17$	59.12 <sub>b</sub> $\pm 2.78$	53.75 <sub>b</sub> $\pm 1.21$	47.56 <sub>c</sub> $\pm 1.24$	0.023 <sub>cde</sub> $\pm 0.003$	0.035 <sub>c</sub> $\pm 0.001$	0.043 <sub>b</sub> $\pm 0.002$

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<b>T4</b>	59.96 <sup>a</sup> ± 4.13	47.30 <sup>a</sup> ± 1.97	46.13 <sup>ab</sup> ± 1.92	74.10 <sup>a</sup> ± 0.96	69.33 <sup>a</sup> ± 1.38	63.80 <sup>a</sup> ± 2.75	71.70 <sup>a</sup> ± 1.14	68.27 <sup>a</sup> ± 1.18	61.50 <sup>a</sup> ± 1.41	0.017 <sup>e</sup> ± 0.001	0.031 <sup>c</sup> ± 0.003	0.025 <sup>d</sup> ± 0.001
<b>T5</b>	47.45 <sup>b</sup> ± 3.00	52.50 <sup>a</sup> ± 3.83	42.33 <sup>b</sup> ± 2.36	48.30 <sup>c</sup> ± 1.58	68.96 <sup>ab</sup> ± 2.14	56.63 <sup>b</sup> ± 1.32	47.14 <sup>c</sup> ± 5.74	67.28 <sup>a</sup> ± 0.64	53.80 <sup>b</sup> ± 3.65	0.032 <sup>b</sup> ± 0.004	0.029 <sup>c</sup> ± 0.001	0.043 <sup>b</sup> ± 0.006
<b>T6</b>	53.73 <sup>ab</sup> ± 4.54	52.40 <sup>a</sup> ± 4.57	36.40 <sup>c</sup> ± 0.35	62.73 <sup>b</sup> ± 3.99	62.56 <sup>b</sup> ± 4.06	50.43 <sup>bc</sup> ± 5.17	55.48 <sup>bc</sup> ± 2.62	67.19 <sup>a</sup> ± 1.16	55.00 <sup>b</sup> ± 1.50	0.026 <sup>bcd</sup> ± 0.002	0.032 <sup>c</sup> ± 0.003	0.032 <sup>c</sup> ± 0.005
<b>T7</b>	61.51 <sup>a</sup> ± 4.20	52.61 <sup>a</sup> ± 3.68	49.73 <sup>a</sup> ± 3.55	75.43 <sup>a</sup> ± 2.60	69.76 <sup>a</sup> ± 1.57	64.30 <sup>a</sup> ± 1.61	71.72 <sup>a</sup> ± 3.46	67.45 <sup>a</sup> ± 0.91	62.70 <sup>a</sup> ± 2.45	0.019 <sup>de</sup> ± 0.001	0.023 <sup>d</sup> ± 0.005	0.029 <sup>cd</sup> ± 0.002
<b>Significant level</b>	*	*	*	*	*	*	*	*	*	*	*	*

\*•means that there are significant differences between the treatments at the significance level ( $P \leq 0.05$ ). • t1 (control) without any additions. T2, t3 and t4 treatments added the aqueous extract of Garviola leaves at a dose of 15, 30 and 45 ml/liter of drinking water, respectively, at a concentration of 1%. T5, t6 and t7 treatments added the alcoholic extract of Garviola leaves at a dose of 15, 30 and 45 ml/liter of water. Drinking water, respectively, at a concentration of 1%.

Non-enzymatic antioxidants such as glutathione and enzymatic antioxidants such as catalase and superoxide dismutase, their

activity and concentration in the body decrease due to stress. When the levels of antioxidants in the diet decrease, exogenous

antioxidants must be added to the diets of laying hens, which leads to an increase in the concentration of antioxidants and an increase in their activity in tissues and serum, as their effectiveness increases and decreases. From the activity of oxidative enzymes that stimulate the oxidation of fats, such as It reduces the consumption of antioxidants of bodily origin and thus increases their concentration in the tissues and blood serum of laying hens, which play an important role in reducing and inhibiting lipid peroxidation [28]. Therefore, the reason is the high level of effectiveness of glutathione, catalase enzymes, and superoxide dismutase in yolks. Eggs for the second, third, fourth, fifth, sixth, and seventh treatments in the periods 0, 30, and 60 days after the eggs were stored compared to the first treatment (control) to the evaluation of cerviola leaves in acting as one of the most important natural antioxidants [6] because they contain many antioxidants. Oxidative stress is included in its composition, including glutathione, catalase enzymes, superoxide dismutase, lycopene, vitamin C, and alpha-tocopherol [19] which was reflected in the health status of the herd and reduced stress resulting from free radical oxidation represented by an increase in glutathione, catalase, and superoxide dismutase, as catalase and superoxide enzymes are considered Dismutase is one of the body's lines of defense against oxidative stress, and a high level of their concentrations is considered one of the protective mechanisms that the body takes against free radicals, preventing and neutralizing these radicals from causing damage to the cellular membranes of liver cells and repairing that damage [4] , Malondialdehyde (MDA) is the end product of lipid peroxidation that occurs spontaneously in the body's cells [11]. It is one of the end

products of the peroxidation of polyunsaturated fatty acids in cells and is a sign of oxidative stress [11]. The process of fat peroxidation occurs when the production of free radicals exceeds the ability of antioxidant defense systems to scavenge them or get rid of their products. Lipid hydroperoxide is formed when fatty acids are oxidized, and then fragmentation occurs in these substances to finally form short-chain compounds called MDA [3] , Malondialdehyde plays a major role in the occurrence of mutations as a result of its interaction with deoxyribonucleic acid (deoxyribonucleic acid), leading to the occurrence of cancerous tumors. The method of measuring MDA (Malondialdehyde) is the best way to measure fat peroxidation in the body, and its concentration in egg yolks is lower for the treatment of adding Garviola leaves compared to the treatment. The first (evidence) may be due to the evaluation of the active compounds in Garviola leaves, especially carotenoids and phenols, which prevent exposure to oxidative stress through the biochemical functions they perform in living organisms, as they work to inhibit the oxidation of cell membrane lipids and curb free radicals by cutting off chains of free reactions, and this leads to It slows down the formation of hydroperoxides and then peroxides, and thus it will inhibit the formation of lipid peroxidation, so the concentration of malondialdehyde (MDA) will decrease in the egg yolk [33; 11.]

#### Conclusions

Adding different levels of aqueous extract and alcoholic extract of Garviola leaves to the drinking water of laying hens improved the lipid profile of the resulting egg yolk and protected it from oxidation after storage for three different periods of time. The ability of the Garviola plant as a natural antioxidant

works to enhance the activity of the antioxidants glutathione, catalase, and superoxide dismutase and reduce the level of malonidehyde in the yolk during periods of egg storage.

### Conclusion

According to obtained results the girdling lead to improve all studied parameters compared with non-girdle one. Girdling of Zark cultivar have a significant effect on number of clusters, shoot length, number of leaves per shoots, number of berries per clusters, Total sugar in berries and TSS in berries. Also, the girdling of Kamali cultivar led to enhancement in number of clusters, shoot length, number of shoots per vine, number of leaves per shoots and size of berries. Spraying of phosphorus especially at 10g.L-1 had a significant effect on all studied parameters compared with control.

The combination of girdling and 10 g.L-1 with either cultivar was superior treatment with most of parameters compared to the control of both cultivars.

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