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Role of treatment with licorice extract , garlic extract and calcium chloride in controlling the sensitivity of tomato fruits for chilling injuries (*Lycopersicon esculentum* Mill.)

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Abstract

The experiment was conducted in one of the greenhouses of the Agricultural Research Station , College of Agriculture , University of Basrah ,Karmat Ali site during the growing season of 2015-2016in order to improve the storage behavior of tomato fruits hybrid Nuton . The seedlings were planted in the plastic house on 20/10/2015 and all the processes using in the production of this crop were conducted. water extract of licorice at three concentrations (zero, 2, 4 g $.L^{-1}$) was prepared and the plants were sprayed three times. Fruits were harvested at mature green stage and brought to the laboratory of storage technology, then cleaned and soaked in water extract of garlic with concentration of 4% , calcium chloride solution with concentration of 4% in addition to control treatment (distilled water only) for a period of 10 minutes and left to dry at room temperature. A portion of harvested fruits that sprayed with the water extract of licorice and at the concentrations of (zero, 2, 4 g $.L^{-1}$) left without soaking . All the fruits packed in perforated polyethylene bags (16 hole with a diameter of 5 mm per bag and weighed 2 kg per bag).Then stored at the temperature of (5°C) for four weeks. Results indicated that the decay percentage, the percentage of weight loss and the percentage of total soluble solids increased while the amount of vitamin C decreased with an increment of storage periods. The lowest percentages of the decay and weight loss and the highest percentage of total soluble solids were in fruits treated with licorice extract as compared with untreated fruits. Fruits treated with garlic extract recorded the lowest percentage of decay, while fruits soaked in 4% calcium chloride solution gave the highest percentage of total soluble solids. Chilling injuries represented in the glass appearance of the fruit and the softness increment of the tissues as well as small patches turn deep yellow in the skin which were clearly appeared at control fruits some hours after storage.

Key Words: Tomato, chilling injuries ,plant extracts, , total soluble solids.

Introduction

Tomato (Lycopersicon esculentum Mill.) is considered as one of the most important vegetable crops in the world due to the highest nutritive value of fruits such as vitamins (as each 100 g containing 900 IU of vitamin A , 0.06 mg vitamin B 1 , 0.04 mg vitamin B 2 ,0 0.7 mg vitamin b 3 and 10-26 mg of vitamin c) also contains lycopene pigment, protein, carbohydrates, calcium , phosphorus , potassium and iron in addition to 22 calories [1] .

The new orientation in agriculture is to move away from the use of chemical fertilizers, chemical growth regulators and pesticides of different kinds and composition, because of their toxic effects on human and animal life. Therefore, researchers in agriculture have tended to find safer materials such as plant and herb extracts [2].

It is the fact that tomato fruits are sensitive to chilling injury when stored at temperatures less than 10 º C. The glass appearance of the fruit and the softness increment of the tissues as well as small patches turn deep yellow in the skin of fruit as a result of the destruction of galactolipid in the fruit. (Nguyen and Mazliak, 1991) . The infection of the fruit and loss of activity of formation lycopene pigment, these symptoms showed when fruits stored at 0 º C for five days or stored at 4 º C for seven days and 8 c for ten days [3].

The water extract of licorice (*Glycyrrhiza glabra* L.) is composed of the roots and dried rhizomes of the plant. The most important components of the roots are glycyrrhizin, a sweet-tasting substance found in the form of calcium and potassium salts for Glycyrrhizic acid, which is 19.08%. The roots contain glucose by 2.8%, Sucrose 3-6% and unrefined sugar 10.71% [4]. The water extract of licorice is similar to that of the gibberellin in stimulating the flowering as a result of containing the medium compound Mevalonic acid and also improves the vegetative growth as a result of stimulating the enzymes needed to convert the complex compounds into simple compounds and to use them in processing the plant with the energy necessary for its growth. [5].

In study carried out by [6] used the extract of garlic (*Allium sativum* L.) at a concentration of 77 ppm. that reduced fungal infections significantly to potato yields. Garlic extract contains a high percentage of amino acids containing sulfur such as Cystein and Methionin. Alliin is responsible for the release of active compounds in garlic. Alliin is converted to Allicin by the enzyme Alliinase, then transformed into other compounds such as Diallyl disulphide [7].

Calcium salts are used to increase the firmness of fruits and to treat many of the physiological disorders by controlling decay because the role of calcium in building the cell wall , activating the process of cell division and enzymes [8].

In a study conducted by [9] on the storage of tomato fruits cv. Super Maramond packed with polyethylene bags for 21 days at 5 ° C. The results showed that decay percentage increased with the continuation of the storage periods. The fruits also showed a gradual increment in total soluble solids with decreasing in weight loss , vitamin C and the content of organic acids

This work was conducted to study some qualitative characteristics and sensitivity for chilling injuries of tomato fruits under low temperature storage in additions, study the role of pre-harvest water extract of licorice, as well as the post-harvest treatment of the water extract of garlic and calcium chloride on storage ability of fruits hybrid Nuton.

Material and Method

The experiment was conducted in one of the greenhouses of the Agricultural Research Station , College of Agriculture , University of Basrah ,Karmat Ali site during the growing season of 2015-2016.

The soil was prepared by planting with a deep plowing of two times perpendicularly. The decomposed organic fertilizer by 20 m3 and NPK fertilizer by 5 kg per square meter were added.

The seedlings were planted in the plastic house on 20/10/2015 and all the processes using in the production of this crop were conducted. water extract of licorice at three concentrations (zero, 2, 4 g $.L^{-1}$) was prepared

and the plants were sprayed In the early morning three times starting from 5/11 /2015 With a time interval of two weeks from the date of planting for the first spray and the operation returned after 10 days.

Fruits were harvested at mature green stage in the early morning and brought to the laboratory of storage technology. then cleaned and soaked in the following solutions for a period of ten minutes and left to dry at room temperature

1. Water extract of garlic with concentration of 4%
2. Calcium chloride solution with concentration of 4%
3. Distilled water only (control)

A portion of harvested fruits that sprayed with the water extract of licorice and at the concentrations of (zero, 2, 4 g $.L^{-1}$) left without soaking . All the fruits packed in perforated polyethylene bags (16 hole with a diameter of 5 mm per bag and weighed 2 kg per bag).Then stored at the temperature of (5°C) for four weeks. The following parameters were studied weekly:

1. Vitamin C (mg / 100g) determined according to [10].
2. Total soluble solids (T.S.S.) were measured by hand refractometer and the results were corrected to 20 ºC.
3. Weight loss (%): was measured via the changes in fresh weight of fruits during storage.

4. Decay percentage:-It was measured according to the following formula :-

$$\text{1. Decay percentage} = \frac{\text{Weight of damaged fruits per package}}{\text{Weigh of total fruits per package}} \times 100$$

The experiment included six treatments came from the interaction among pre harvest spraying of licorice water extract at the concentrations of (0, 2, 4 g $.L^{-1}$), post harvest soaking with (4% w/v) water extract of garlic and calcium chloride for both of them in addition to control treatment (distill water only). Complete Randomized Design was used with three replicates . The results were analyzed by the analysis of variance

and mean values were compared using the Revised Least Significant Difference Test at 0.05 probability level. [11].

Results and discussion

1. Vitamin C (mg . 100 g-¹)

Table (1) showed that the amount of vitamin C decreased with the continuation of storage period reached to (19.58 mg . 100 g-¹) after four weeks of storage. The highest value of vitamin C was in fruits sprayed with 2 g. L-¹ licorice extract, which amounted to (21.78 mg . 100 g-¹) .Regarding to the postharvest treatments, the highest value of vitamin C (21.64 mg . 100 g-¹) was in fruits soaked in 4% calcium chloride with no significant differences with control fruits.

In regard to Binary interactions ,there were significant differences between factorial treatments , the highest value of vitamin C was in the fruits sprayed with 2 g. L-¹ licorice extract and soaked in 4% garlic extract (22.34 mg . 100 g-¹). The lowest amount of vitamin C was in fruits of the spraying with 0 g. L-¹ licorice extract and soaked in 4% garlic extract, which reached to (20.32 mg . 100 g-¹). The table also showed the significance of the interaction between the spray treatments with the licorice extract and the storage period. The highest vitamin C value was in the fruits of the 4 g. L-¹ licorice extract spray after a week of storage, which was (23.63 mg . 100 g-¹). The lowest value of vitamin C Was in fruits of 0 g. L-¹ licorice extract spray after four weeks of storage, which was (18.54 mg . 100 g-¹). Regarding to the interaction between postharvest treatments and storage period , The highest value of vitamin C in control fruits after a week of storage which was (23.30 mg . 100 g-¹). The lowest value of vitamin C was in comparison treatment (soaking in distilled water only) after four weeks of storage, which amounted to (18.44 mg . 100 g-¹).

In regard to triple interaction, the highest value of vitamin C was in fruits sprayed with 4 g. L-¹ licorice extract and soaked in 4% garlic extract after a week of storage, which amounted to (23.82 mg . 100 g-¹).

The reason for decreasing the vitamin C with the continuation of storage period may be due to the continuation of vital processes and increased the activity of ascorbase and oxidase with the continuation of storage period and exposure to light which caused the oxidation of vitamin C to dehydro ascorbic acid. This is in agreement with [8] for jujube fruits cv.Tufahi.

Table(1) Effect of spraying with licorice extract, postharvest treatments and storage period on vit. C (mg 100 g^{-1}) of tomato fruits hybrid Nuton stored at 5 ° C

licorice extract g L-¹	Postharvest treatments(%)	Storage period (week)				licorice extract × postharvest treatments
		1	2	3	4	
0	control 0	22.77	21.59	19.04	17.95	20.34
	garlic extract 4	21.81	20.74	19.88	18.86	20.32
	calcium chloride 4	22.19	20.90	19.68	18.81	20.39
2	control 0	23.55	21.27	19.60	18.73	20.79
	garlic extract 4	23.73	22.47	22.25	20.92	22.34
	calcium chloride 4	23.57	22.57	21.77	20.92	22.21
4	control 0	23.57	20.57	19.81	18.64	20.65
	garlic extract	23.82	22.33	21.22	20.22	21.90

	4					
	calcium chloride 4	**23.50**	**22.63**	**21.99**	**21.15**	**22.32**
						Means of licorice extract
licorice × storage period	**0**	**22.26**	**21.07**	**19.53**	**18.54**	**20.35**
	2	**23.61**	**22.11**	**21.20**	**20.19**	**21.78**
	4	**23.63**	**21.84**	**21.01**	**20.00**	**21.62**
						Means of postharvest treatments
Postharvest treatments× storage period	**Control 0**	**23.30**	**21.14**	**19.48**	**18.44**	**20.59**
	garlic extract 4	**23.12**	**21.85**	**21.12**	**20.00**	**21.52**
	calcium chloride 4	**23.09**	**22.03**	**21.15**	**20.29**	**21.64**
Means of storage period		**23.17**	**21.67**	**20.58**	**19.58**	
			RLSD 0.05			

licorice extract	**Postharvest Treatments**	**Storage period**	**licorice× postharvest treatments**	**licorice× storage period**	**Postharvest treatments× storage period**	**licorice× postharvest treatments× storage period**
0.3847	**0.3847**	**0.4442**	**0.6663**	**0.7694**	**0.7694**	**1.3327**

2. Percentage of total soluble solids (T.S.S)

Results presented in table (2) showed the effect of spray treatment with licorice extract, garlic extract, calcium chloride and storage period on total soluble solids of tomato fruits stored at 13 ° C. The table showed that the percentage of total soluble solids increased up to (10.1%) after four weeks of storage. The increment in the percentage of total soluble solids may be due to the reduction of moisture content of fruits with the continuation of storage period. , as the storage period progresses, the lower moisture content of the

fruit increases the concentration of the cell juice of the fruit and thus increases the percentage of soluble solids [12].

The highest percentage of TSS was in fruits sprayed with 4 g. L-¹ licorice extract and fruits soaked in 4% calcium chloride solution which were (9.1% , 9.5%) respectively with significant difference with the rest of treatments.

In regard to Binary interactions ,there were significant differences between factorial treatments , the highest percentage of TSS was in the fruits sprayed with 4 g. L-¹ licorice extract and soaked in 4% garlic extract (10.1%) with no significant differences from 2 g. L-¹ licorice extract spraying and 4%calcium chloride solution soaking. the highest percentage of TSS was in fruits of 4 g. L-¹ licorice extract spray after four weeks of storage , with no significant differences from 2 g. L-¹ after four weeks . The highest percentage of total soluble solids was in the fruits of the treatment with calcium chloride solution (4%), which reached (11.2%) after four weeks of storage with no significant differences with 4% garlic extract soaking after four weeks of storage .

In regard to triple interaction, the highest percentage of TSS was in fruits sprayed with 2 g. L-¹ licorice extract and soaked in 4% calcium chloride after four weeks of storage, which amounted to ()%11.7) with no significant differences with fruits sprayed with 2 g. L-¹ licorice extract and soaked in 4% garlic extract after four weeks of storage and with fruits sprayed with 4 g. L-¹ licorice extract and soaked in 4% calcium chloride after four weeks of storage.

Table(2) Effect of spraying with licorice extract, postharvest treatments and storage period on total soluble solids percentage of tomato fruits hybrid Nuton stored at 5 ° C

licorice extract g L-¹	Postharvest treatments(%)	Storage period (week)				licorice extract × postharvest treatments
		1	2	3	4	
0	control 0	4.8	6.3	7.3	8.3	6.6
	garlic extract 4	5.7	7.1	8.2	9.3	7.5
	calcium chloride	6.7	7.8	9.4	11.3	8.8

	4					
2	control 0	5.7	5.5	6.7	7.5	6.3
	garlic extract 4	7.3	9.0	10.2	11.6	9.5
	calcium chloride 4	6.6	8.3	9.6	10.8	8.8
4	control 0	5.7	7.2	8.5	8.9	7.5
	garlic extract 4	8.8	10.1	11.3	12.3	10.6
	calcium chloride 4	8.2	9.2	10.4	11.4	9.8
						Means of licorice extract
licorice × storage period	0	5.7	7.1	8.3	9.6	7.6
	2	6.5	7.6	8.8	10.0	8.2
	4	7.6	8.8	10.1	10.9	9.3
						Means of postharvest treatments
Postharvest treatments× storage period	Control 0	5.4	6.3	7.5	8.2	6.9
	garlic extract 4	7.2	8.7	9.9	11.1	9.2
	calcium chloride 4	7.1	8.4	9.8	11.2	9.17
Means of storage period		6.6	7.8	9.0	10.1	

RLSD 0.05

licorice extract	Postharvest Treatments	Storage period	licorice× postharvest treatments	licorice× storage period	Postharvest treatments× storage period	licorice× postharvest treatments× storage period
0.1891	0.1891	0.2183	0.3275	0.3782	0.3782	0.6550

3. weight loss (%)

Table (3) showed the effect of spraying licorice extract ,treating with garlic extract and calcium chloride and the duration of storage in the percentage of weight loss of tomato fruits hybrid Nuton stored at 5 ° C. The results indicate that the percentage of weight loss increased by increasing the storage period reached to (0.891%) after four weeks of storage. As for the effect of spraying treatments with licorice extract, the lowest percentage of weight loss was in fruits treated with 4 g $.L^{-1}$ licorice extract which reached (0.213%). The effect of post-harvest treatments on the percentage of weight loss was significant. Fruits treated with 4% calcium chloride recorded the lowest percentage of weight loss (0.426%) with significant differences with the rest treatments.

The results of the same table showed a significant difference between the spray treatments with licorice extract and post-harvest treatments. The lowest percentage of weight loss was in fruits sprayed with licorice extract 4 g. L^{-1} and treated with 4%garlic extract , which reached (0.170%).

Regarding to the interaction between the spray treatments and storage period and the interaction between postharvest treatments and storage period , the lowest percentages of weight loss (0.125% , 0.134%) were in fruits treated with 4 g $.L^{-1}$ licorice extract and fruits treated with 4%garlic extract after a week of storage respectively.

The interaction between spraying with licorice extract and post-harvest treatments with garlic extract and calcium chloride and storage period was significant. The t lowest percentages of weight loss was in the fruit sprayed with 4 g $.L^{-1}$ licorice extract and soaked in 4%garlic extract after a week of storage, which amounted to (0.107%).

The increment of the percentage of weight loss by increasing storage period due to the reduction of fruits weight as the storage period progresses, resulting in loss of the water content of the fruits while the storage period continues, as well as the consumption of the food stored in the fruit as a result of breathing. These finding are in the accordance with those previously reported by [9] for Super Maramond tomato cultivar. Spraying licorice extract and soaking in garlic extract and calcium chloride solution , reduced the weight loss percentage of tomatoes. The effect of plant extracts may be due to the fact that plant extracts have formed a

layer of insulation covering stomata and act as anti-transpirations because they contain substances of a similar effect to wax or vegetable oils [13]

Table(3) Effect of spraying with licorice extract, postharvest treatments and storage period on weight loss percentage of tomato fruits hybrid Nuton stored at 5 ° C

licorice extract g L-1	Postharvest treatments(%)	Storage period (week)				licorice extract × postharvest treatments
		1	2	3	4	
0	control 0	1.208	1.435	1.520	1.750	1.478
	garlic extract 4	0.155	0.425	1.503	1.580	0.915
	calcium chloride 4	0.177	0.225	0.995	1.655	0.763
2	control 0	0.122	0.310	0.328	1.202	0.490
	garlic extract 4	0.142	0.282	0.327	0.468	0.304
	calcium chloride 4	0.173	0.240	0.255	0.517	0.296
4	control 0	0.112	0.250	0.300	0.343	0.251
	garlic extract 4	0.107	0.140	0.193	0.242	0.170
	calcium chloride 4	0.158	0.213	0.240	0.270	0.220
						Means of licorice extract
licorice × storage period	0	0.513	0.695	1.339	1.661	1.052
	2	0.145	0.277	0.303	0.729	0.363
	4	0.125	0.201	0.244	0.258	0.213
						Means of postharvest treatments
Postharvest treatments×	Control 0	0.480	0.665	0.716	1.098	0.739

storage period	**garlic extract 4**	**0.134**	**0.282**	**0.674**	**0.763**	**0.463**
	calcium chloride 4	**0.169**	**0.226**	**0.496**	**0.814**	**0.426**
Means of storage period		**0.261**	**0.391**	**0.628**	**0.891**	
			RLSD 0.05			

licorice extract	**Postharvest Treatments**	**Storage period**	**licorice× postharvest treatments**	**licorice× storage period**	**Postharvest treatments× storage period**	**licorice× postharvest treatments× storage period**
0.1191	**0.1191**	**0.1375**	**0.2062**	**0.2381**	**0.2381**	**0.2411**

4.Decay percentage (%)

Results presented in table (4) showed the effect of spraying licorice extract ,treating with garlic extract and calcium chloride and the duration of storage in the decay percentage of tomato fruits hybrid Nuton stored at 5 ° C. As shown in the table (1) , the decay percentage increased with an increment of storage periods reached to (16.28%) after four weeks of storage. Spraying treatments with licorice extract showed that the lowest percentage of the decay(7.25%) was in fruits treated with licorice extract 4 g $.L^{-1}$ with a significant difference with the rest treatments. The spraying treatment of 2 g $.L^{-1}$ licorice extract was also superior to the control treatment. The effect of post-harvest treatments on the percentage of decay was significant, with the fruits treated with garlic extract in recording the lowest percentage of decay which was (8.09%) , while the control fruits gave the highest percentage of decay (20.18%), Fruits soaked in 4% calcium chloride solution significantly differed on the control treatment.

The interaction between spraying with licorice extract and post-harvest treatments was significant. The lowest percentage of decay was in the fruits sprayed with 4 g. L^{-1} licorice extract and soaked in 4% garlic extract, which was(6.87 %).

The table also showed the significant interaction between the spraying with licorice extract and the storage period. The lowest percentage of decay was in the fruits sprayed with 2 g. L^{-1} licorice extract after a week of storage which was (7.00%). The highest percentage of decay (20.60%) was in untreated fruits after four weeks of storage.

The interaction between the post-harvest and storage period was significant. The fruits that soaked in 4% garlic extract after one week of storage gave the lowest percentage of decay(4.66%) ,while the highest percentage of decay was in untreated fruits after four weeks of storage.

The results indicate to a significant interaction between spraying with licorice extract and post-harvest treatments with garlic extract and calcium chloride and the storage period. Fruits sprayed with 4 g $.L^{-1}$ licorice extract, soaked in 4% garlic extract gave the lowest percentage of decay(4.25%) after a week of storage. The highest percentage of decay (32.50%) was in the fruits sprayed with 0 g $.L^{-1}$ licorice extract and soaked in distilled water only (control fruits) after four weeks of storage with no significant difference with fruits sprayed with 0 g $.L^{-1}$ licorice extract and soaked in distilled water after three weeks of storage and significant for the rest of the factorial treatments.

The fruits are exposed during the process of packing and storage to the damage, which takes several forms according to its causes. It may be the result of mechanical disorders to the fruits during packing and storage, such as bruises caused by the pressure of the fruits of each other inside the package. The damage is caused as a result of the progress of physiological disorders such as chilling injury, and also due to injuries with pathogens such as bacteria, fungi and yeast. [14] ; [8].

As previously mentioned, spraying with licorice extract and soaking with garlic extract and calcium chloride, reduced the damage rate of tomatoes.

The effect of plant extracts in reducing the incidence of microbial infections may be due to their effect in inhibiting the growth, activity and reproduction of fungi, especially volatile oils and alkaloids that prevent the spread of pathogens and inhibit their growth [15] In addition, the volatile oils and their compounds have the potential to inhibit the growth of bacteria and fungi [16].

The effect of calcium chloride treatment in reducing decay percentage due to the role of calcium in an increment of fruit firmness that leads to delay ripening and increase the shelf life of fruits . As pointed out by [8] postharvest treatment of jujube fruits with calcium chloride and calcium nitrate decreased the decay of fruits particularly caused by fungi, decreased weight loss , total soluble solids and total sugars at the end of storage at 0 º C and 5 ºC.

The storage of fruits at a temperature of 5 º C led to the occurrence of chilling injury in a portion of the fruits after storage which represented in the glass appearance of those fruits with yellow spots on the skin clearly appeared at control fruits some hours after storage as well as the fungal infection . The fungus *Rhizopul stolonifer* was identified which was the main reason for decay of fruits.

Table(4) Effect of spraying with licorice extract, postharvest treatments and storage period on decay percentage of tomato fruits hybrid Nuton stored at 5 ° C

licorice extract g L^{-1}	Postharvest treatments(%)	Storage period (week)				licorice extract × postharvest treatments
		1	2	3	4	
0	control 0	19.23	25.75	28.00	32.50	26.37
	garlic extract 4	5.00	7.25	12.00	13.60	9.46
	calcium chloride 4	9.22	11.61	13.34	15.70	12.46
2	control 0	14.00	18.50	21.00	23.25	19.18
	garlic extract 4	4.75	7.00	9.75	10.33	7.95
	calcium chloride 4	6.50	7.00	9.50	12.50	8.87
4	control 0	12.00	13.50	16.50	18.00	15.00
	garlic extract 4	4.25	5.75	7.75	9.75	6.87

		calcium chloride 4	**4.75**	**5.50**	**8.25**	**11.00**	**7.37**
							Means of licorice extract
licorice	×	**0**	**11.15**	**14.87**	**17.78**	**20.6**	**16.10**
storage		**2**	**8.41**	**10.83**	**13.41**	**15.36**	**12.00**
period		**4**	**7.00**	**8.25**	**10.83**	**12.91**	**9.74**
							Means of postharvest treatments
Postharvest treatments×		**Control 0**	**15.07**	**19.25**	**21.83**	**24.58**	**20.18**
storage period		**garlic extract 4**	**4.66**	**6.66**	**9.83**	**11.22**	**8.09**
		calcium chloride 4	**6.82**	**8.03**	**10.36**	**13.06**	**9.56**
Means of storage period			**8.85**	**11.31**	**14.00**	**16.28**	

RLSD 0.05

licorice extract	**Postharvest Treatments**	**Storage period**	**licorice× postharvest treatments**	**licorice× storage period**	**Postharvest treatments× storage period**	**licorice× postharvest treatments× storage period**
1.322	**1.322**	**1.527**	**2.290**	**2.644**	**2.644**	**4.580**

Conclusions

In conclusion, the results obtained in the present work clearly indicated to the role of spraying icorice and postharvest application garlic extract and calcium chloride solution in keeping qualitative characteristics and reducing chilling injuries of tomato fruits hybrid .Nuton stored at 5°C for four weeks.

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