

YIELD AND FRUIT QUALITY OF WONDERFUL POMEGRANATE TREES (*Punica granatum* L.) AS INFLUENCED BY SPRAYING ROYAL JELLY

Abdel-Rahman, M.A. Mohamed¹, Aly M. Ibrahim², Hamdy I.M. Ibrahim^{1*} and Ahmed G. Hassan¹

¹Hort. Dept. Fac. of Agric. Minia Univ. El-Minia, Egypt.

²Citrus Dept., Horticultural institute research, ARC, Egypt.

*Corresponding author: hamdy_franc@yahoo.com Received: 25 August 2019 ; Accepted: 14 Oct. 2019

ABSTRACT: Nutritional status, yield and fruit quality of Wonderful pomegranate trees grown in sandy calcareous soil in response to spraying Royal jelly at 0.0125, 0.0250 and 0.050% and frequencies, once (at the end May) twice (at the end of May and again at mid-June) and three applications (at the end May, mid-June and the end of August) were investigated during 2017 and 2018 seasons. spraying Royal jelly was very effective in improving the leaf area, leaf chlorophylls contents and total carotenoids, N, P, and K, yield and fruit quality rather than non- application. The gradual Increasing in Royal jelly concentration and frequencies was remarkably enhancing yield and fruit quality. However, non-significant differences were observed between the two heights concentrations. The best results with regard to vegetative growth, yield and fruit quality of Wonderful pomegranate trees grown under sandy calcareous soil were obtained when the trees received three sprays of Royal jelly at 0.05%. However non-significant differences were obtained between the two highest concentrations of Royal Jelly namely; 0.0250 and 0.050%.

Key words: Pomegranate, royal jelly, yield, growth, quality, chlorophylls.

INTRODUCTION

The Pomegranate tree (*Punica granatum* L.) belongs to Myrtales order and the Punicaceae family. The pomegranate has gained high economic value in recent years due to the large volume of in vivo and in vitro studies attributing numerous health benefits to the fruit and its products extensively reviewed in (Holland *et al.*, 2001; Fadavi *et al.*, 2005; Holland *et al.*, 2009; Jaiswal *et al.*, 2010; Drogoudi *et al.*, 2012; Fakhour, 2012; Franck, 2012 and Franck *et al.*, 2012). Consequently, pomegranate production has expanded to new regions where the available water is poor quality; including recycled or saline water (Holland *et al.*, 2009). Many new orchards are now planted in the reclamation desert regions in Egypt such the Western desert - Egypt. Some investigators classified the pomegranate under salinity and calcareous resistant plants (Grattan and Grieve 1999; Pessaraki, 1999; Yilmaz *et al.*, 2007; Halilova and Yildiz, 2009; Franck, 2012; Franck

et al., 2012 and Gozlekci *et al.*, 2012). Wonderful is a newly introduced pomegranate cultivar successfully grown under Egyptian condition, but still needs additional studies about growth, fruit ripening and quality under desert cultivation.

In order to improve trees growth and production under stress conditions, recently there have been many efforts to find safety alternatives natural materials useable in horticulture sector. Royal Jelly is one of these safety natural products with a high nutritional value (Table 1) is one of these attempts. Royal jelly is considered an essential bio-nutrient containing higher amounts of proteins, amino acids, lipids, fructose, glucose and sucrose, as well as minerals nutrients such as K, Mg, Ca, Fe, P, S & Mn, and vitamins B5 & B6 & B9, B12, & E (Hyel, 1951; Townsend and Lucas, 1966; Nation and Robinson, 1991; Ramadan & Al-Ghamdi, 2012; Buttstedt *et al.*, 2014; Wang, 2016 and Balkanska *et al.*, 2017). However, a remarkable promotion on growth, yield and fruit quality of some

fruit trees was observed due to spraying Royal jelly (Al-Wasfy, 2013; Abada & Ahmed, 2015; Ibrahim *et al.*, 2015 and El-Sayed *et al.*, 2017).

The main object of this study was elucidating the effect of different concentrations and frequencies of application of Royal Jelly on some vegetative growth characteristics, leaf chemical composition, as well as yield and fruit quality of Wonderful pomegranate trees grown under sandy calcareous soil conditions.

MATERIALS AND METHODS

The present investigation was conducted during two seasons 2017 and 2018 on thirty own rooted uniform in vigor Wonderful pomegranate trees, grown in private orchard located at the Cairo-Assiut Western Desert Road, Minia Distract, El-Minia Governorate (250 km southern Cairo city), where the soil texture is sandy, since water table depth is not less than two meters. The chosen pomegranate trees are five-years old, and planted at 4 X 4 meters apart. The formation of the chosen

trees is two-trunk/tree and an open vase system with 4 to 6 principal branches. Winter pruning was followed at the second week of January. Drip irrigation system was adopted. However, irrigation carried by used water supply from underground well with pressure and volume controllers

The chosen trees are subjected to regular horticulture practices that were commonly applied in the orchard including fertilization, (namely: 80 g/tree nitrogen applied in the form of ammonium nitrate "33% N", 245 kg/feddan, calcium superphosphate "15.5% P₂O₅" 200 kg/feddan and 150 kg/feddan potassium sulphate, as well as irrigation, hoeing and pest management.

Soil and irrigation water analysis: The orchard soil where the present experiment carried out was sandy texture (Table 1). A composite sample of soil and irrigation water were collected and subjected to Physical and chemical analysis according to the procedures outlined by Walsh and Beaton (1986) and Buurman *et al.* (1996). The data of soil and water sample analyses are shown in Table (1).

Table 1. Physical and chemical analysis of experiment soil and irrigation used

Soil analysis		Water analysis	
Constituents	Values	Constituents	Values
Sand %	82.4	E.C (mmhos/cm/25C)	1.5
Silt %	10.2	Hardness	21.1
Clay %	7.4	pH	7.81
Texture	Sandy	Ca (mg/L)	39.9
EC (1:2.5 extract) mmhos/cm/25 C	2.8	Mg (mg/L)	28.1
Organic matter %	0.41	K (mg/L)	4.87
pH (1 : 2.5 extract)	8.59	Na (mg/L)	98.9
Active lime %	17.2% (CaCO ₃)	Sum of Cations (mg/L)	8.16
N (mg/kg)	171	Alkalinity (mg/L)	192
Phosphorus (ppm)	7.80 ppm	Chlorides (mg/L)	135
Available Ca (meq/100g)	22.9	Nitrate (mg/L)	11.0
Available Mg (meq/100g)	3.13	Sulphates (mg/L)	49.7
Available K (meq/100g)	0.44	Sum of anions (mg/L)	7.29
C/N Ratio	16.8	SAR	2.97

Experimental work: This study included the following ten treatments from two factors (A & B). The **first factor (A)** comprised from the following four Royall jelly concentrations:

a1- 0.0 ppm Royall jelly. **a2-** 0.0125% Royall jelly.
a3- 0.0250% Royall jelly. **a4-** 0.050% Royall jelly.

The second factor (B) contained the following three frequencies of application of Royall Jelly: **b1-** spraying Royall jelly one time (at the end of May); **b2-** spraying Royall jelly two times (at the end of April and again at the end of May); **b3-** spraying Royall jelly three times (at the end of April, at the end of May and again at the end of July).

The experiment involved the following tenth treatments from the Royal jelly concentration and frequencies. Arranged as follow:

- 1- Spraying Royall jelly at 0.0% (control trees).
- 2- Spraying Royall jelly at 0.0125% one time.
- 3- Spraying Royall jelly at 0.0125% two times.
- 4- Spraying Royall jelly at 0.0125% three times.
- 5- Spraying Royall jelly at 0.0250% one time.
- 6- Spraying Royall jelly at 0.0250% two times.
- 7- Spraying Royall jelly at 0.0250% three times.
- 8- Spraying Royall jelly at 0.050% one time.

9- Spraying Royall jelly at 0.050% two times.

10- Spraying Royall jelly at 0.050% three times.

Each treatment was replicated three times, one tree per each. Then, the present study included thirty Wonderful pomegranate trees. Triton B (at 0.05 g/liter) as a wetting agent was added to all spraying solutions, even control trees. Treatments were arranged in a complete randomized block design (RCBD).

For evaluating the effect of Royal jelly, the following parameters were measured:

Vegetative growth characters

At the second week of August during both seasons, twenty mature leaves from the medial part on the non-productive shoots were picked from each replicate according to **Martin-Préval *et al.* (1984)**, Leaf area (cm²) was estimated by using an area meter (Area Meter CI, 202). The average main shoot length (cm) was recorded as a result of measuring the length of eight shoots per tree (from main geographic directions), two shoots for each direction. The average shoots lengths were recorded at the end of August, during both experimental seasons.

The average leaves number/shoot were recorded as a result of counting the number of leaves located at eight shoots, two shoots for each direction on each tree, and the average leaves number/shoot were recorded at the end of August during both experimental seasons.

Determination of macro and micronutrients in leaves

16 leaves picked from the medial part of 8 main shoots as described by **Martin-Préval *et al.* (1984)** for each tree were taken during the two seasons. The leaves washed with distilled water and dried at air and oven dried and grounded, then 0.5 g weight was digested using H₂SO₄ and H₂O₂ until clear solution was obtained (**Martin-Préval *et al.*, 1984**). The digested solution was quantitatively transferred to 100 ml volumetric flask and completed to 100 ml by distilled water. Thereafter, contents of N, P, K for each sample were determined as follows:

Nitrogen was determined by the modified microkjeldahl (**Walsh and Beaton, 1986**). Phosphorus was determined by using colorimetric method, described by **Walsh and Beaton (1986)**, by measuring the optical density of phosphor-molibdo-vanadate complex by Spectro-photometrically at wave length 430 nm. Potassium was flame-photometrically determined by using the method outlined by **Martin-Préval *et al.* (1984)**.

Measurement of yield as well as physical properties of fruit: The fruits were harvested when

fruits become fully colored and the T.S.S./Acid ratio in the juice of the check treatment reached 3 to 3.5 in the two experimental seasons according to **Hegazi *et al.* (2014)**. The yield per tree was recorded in terms of weight (kg) and number of fruits per tree, and then fruit yield (kg) per tree was calculated. Also the percentage of cracking fruits and sunburned fruits per tree as well as marketable fruit were recorded as follow:

$$\text{Cracking fruits \%} = \frac{\text{No. of cracked fruits/tree}}{\text{Total no. of fruits/tree}} \times 100$$

$$\text{Sunburned fruits \%} = \frac{\text{No. of sunburned fruits/tree}}{\text{Total no. of fruits/tree}} \times 100$$

From each tree, four fruits were randomly picked tree at maturation date (Last week of September).

The following physical and fruit characteristics were studied:

- Average fruit weight (g), by using sensitivity balance with 0.1g accuracy.
- Average fruit length without calyx (cm), by using vernier caliper with 0.01cm accuracy.
- Average fruit diameter (cm), by using vernier caliper with 0.01cm accuracy.

$$\text{Shape Index} = \frac{\text{Fruit length (cm)}}{\text{Fruit Diameter (cm)}}$$

- Average peel weight plus weight of carpellary membranes (g), by using sensitivity balance with 0.01g accuracy.
- Average peel thickness (mm), by using vernier caliper with 0.01cm accuracy (**Ranganna, 1977**).
- Average weight of grains (0.01 g), mathematically calculated by discount the Epicarp weight (included carpellary membranes) from the fruit weight.
- Juice weight %, mathematically calculated according the following equation:

$$\text{Juice weight \%} = \frac{\text{Fruit juice weight (g)}}{\text{Fruit grains weight (g)}} \times 100$$

Statistical analysis of data: All the obtained data were tabulated and subjected for the proper statistical analysis; by analysis of variance (ANOVA) using the statistical package MSTATC Program. Comparisons between means were made by the F-test and least significant differences (New LSD) at p = 0.05 (**Snedecor and Cochran, 1990**).

RESULTS AND DISCUSSION

Effect on vegetative growth characteristics

Data in Table (2) show the effect of spraying Royal jelly at different concentration and frequencies on average shoot lengths (cm), numbers of leaves/shoot, number of new shoots/tree and leaf area (cm²) during 2017 and 2018 seasons. It is clear from the obtained data that treating Wonderful pomegranate once, twice, and thrice with Royal jelly at 0.0125% ppm to 0.05% significantly was followed by stimulating all studied parameters of vegetative growth, rather than control trees. This stimulation was related to the increase in concentrations from 0.0125% to 0.05%, and frequencies of application from one to three times. However, increasing Royal jelly concentration from

0.0250% to 0.05% and its frequencies from two to three times have non-significant effects on the shoot length, number of leaves/shoot, number of new shoots/tree and leaf area (cm²) during the two experimental seasons, except those of numbers of new shoot/tree during 2018 where, increasing the frequencies from two to three times with the higher concentration of Royal jelly (0.050%) lead to significant increase in the number of new shoots/tree. The maximum values of main shoot lengths (74.1 & 84.9 cm), number of leaves/shoot (47.3 & 48.6), number of new shoots/tree (70.6 & 76.9) and leaf area (7.91 & 7.93 cm²) during the two experimental season respectively, were recorded on the trees received three sprays of Royal jelly at 0.05% during 2017 and 2018 seasons.

Table 2. Effect of Royal Jelly concentration and frequencies on some vegetative growth characteristics of Wonderful pomegranate during 2017 and 2018 seasons

Royal Jelly (RJ) treatments	Shoot length (cm)		Number of leaves/shoot		Number of new shoots/tree		Leaf area Cm ²	
	2017	2018	2017	2018	2017	2018	2017	2018
Control (0.0 % RJ)	51.2	53.7	29.1	28.9	44.3	45.8	6.01	6.11
RJ 0.0125% one time	59.3	59.2	32.8	40.7	45.8	49.2	7.02	7.10
RJ 0.0125% two times	61.2	66.4	35.5	35.8	55.7	61.3	7.34	7.45
RJ 0.0125% three times	63.1	66.9	37.1	39.3	59.8	64.3	7.57	7.70
RJ 0.0250% one time	62.4	65.9	36.3	37.4	57.1	61.6	7.44	7.69
RJ 0.0250% two times	71.5	75.3	40.1	42.7	66.4	66.1	7.86	7.79
RJ 0.0250% three times	72.5	78.0	44.3	45.1	68.1	70.2	7.92	7.88
RJ 0.05% one time	64.9	66.8	39.9	42.2	64.5	68.2	7.45	7.75
RJ 0.050% two times	73.1	79.2	45.6	46.8	69.8	72.7	7.88	7.87
RJ 0.050% three times	74.1	84.9	47.3	48.6	70.6	76.9	7.91	7.93
New LSD 5%	4.21	5.02	3.91	4.20	5.18	3.11	0.215	0.194

However, the untreated trees produced the lowest values of all vegetative growth parameters during the two experimental seasons respectively. This promotion was relatively little when the trees received one spray with the lowest concentration of Royal jelly (0.0125%). While, this promotion was remarkably higher when the trees received Royal jelly at highest concentration (0.050%) three times yearly. These results were true in both seasons.

The obtained results concerning the stimulation of Wonderful pomegranate vegetative growth as a result of spraying Royal jelly during the present study are in harmony with those obtained by **Al-Wasfy (2013)** on Sakkoti date palm, **Ahmed and Habasy (2014)** on Washington navel orange trees, **Abdelaziz *et al.* (2015)** on Ewaise mango trees, **Ibrahim *et al.* (2015)** on Keitte mango trees, **Abada and Ahmed (2015)** on Superior grapevines, **Wassel *et al.* (2015)** on Flame seedless grapevines and **El-Sayed *et al.* (2017)** on Zebda Mango trees.

Effect on leaves mineral nutrients contents

Table (3) show the effect of different concentrations and frequencies of application of Royal jelly on the leaves macro and micro nutrients contents of Wonderful pomegranate trees during 2017 and 2018 seasons.

It is clear from this Table that subjected Wonderful pomegranate trees once, twice or thrice with Royal jelly at 0.0125% to 0.05% significantly was responsible for enhancing the percentage of the five macro nutrients % (N, P, K, Mg and Ca) relative to the control treatment, except these of calcium at the first season, where non-significant differences were observed. Furthermore, increasing concentrations of Royal jelly from 0.025% to 0.050% had non-significant promotion on N, P, K and Mg nutrients. On the other hand, spraying the highest concentration of Royal jelly (0.050%) three times presents highest and significant Ca contents in leaves than those received the same concentration two times yearly, only during the second season.

Table 3. Effect of Royal Jelly concentration and frequencies on leaves macro nutrients (%) of Wonderful pomegranate during 2017 and 2018 seasons

Royal Jelly (RJ) treatments	N (%)		P (%)		K (%)		Mg (%)		Ca (%)		Fe (ppm)		Zn (ppm)		Mn (ppm)	
	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
Control	1.49	1.48	0.19	0.20	1.34	1.35	0.49	0.52	1.69	1.64	109	112	48	46	24	25
RJ 0.0125% One time	1.59	1.61	0.25	0.24	1.36	1.38	0.55	0.56	1.67	1.66	119	125	54	55	27	29
RJ 0.0125% Two times	1.62	1.66	0.32	0.31	1.39	1.41	0.59	0.61	1.68	1.70	129	133	61	66	32	33
RJ 0.0125% Three times	1.61	1.65	0.33	0.33	1.41	1.45	0.60	0.63	1.69	1.74	135	137	63	65	34	34
RJ 0.0250% One time	1.67	1.69	0.29	0.30	1.38	1.39	0.53	0.54	1.70	1.71	134	138	59	61	33	32
RJ 0.0250% Two times	1.82	1.82	0.41	0.42	1.59	1.64	0.68	0.70	1.72	1.85	149	151	69	72	44	42
RJ 0.0250% Three times	1.82	1.83	0.43	0.41	1.59	1.65	0.70	0.72	1.71	1.87	152	153	72	73	46	43
RJ 0.05% One time	1.78	1.80	0.29	0.32	1.43	1.41	0.61	0.61	1.69	1.80	133	141	60	62	39	42
RJ 0.050% Two times	1.82	1.84	0.39	0.40	1.60	1.64	0.71	0.73	1.72	1.87	151	153	67	69	50	52
RJ 0.050% Three times	1.83	1.86	0.40	0.41	1.65	1.67	0.74	0.74	1.75	1.95	151	152	70	71	52	53
New LSD 5%	0.07	0.05	0.04	0.03	0.08	0.06	0.09	0.08	NS	0.07	7.5	8.3	4.5	4.3	3.3	3.5

The same Table showed that, the trees received three sprays with Royal jelly at higher concentration (0.05%) produced the highest levels of iron (151 ppm & 152 ppm) and manganese (52 ppm & 53 ppm) in their leaves, during the two experimental seasons respectively. While the trees received Royal jelly three times at 0.0250 % produced the higher level of zinc in its leaves (72 ppm & 73 ppm), during the two seasons respectively. On the other hand the trees of check treatment present the lowest values of Fe (109 ppm & 112 ppm), Mn (24 ppm & 25 ppm) and Zn (48 ppm & 46 ppm) during the two experimental seasons respectively.

It is known that the structure of Royal jelly present high contents of some macro and micro nutrients plus higher contents of amino acids and antioxidants (Wang, 2016), most likely regulating the absorption and accumulation of macro and micro nutrients for fruit trees (Hyel, 1951). The previous findings can be explained the remarkable positive effect of Royal jelly on enhancing macro and micro nutrients which founded in the present study.

Effect of Royal jelly on yield and its components

Data concerning the effect of different concentrations and frequencies of application of Royal jelly on yield expressed in kg/tree, number of fruit/tree as well as fruit weight (g) of Wonderful pomegranate trees during 2016/2017 and 2017/2018 seasons are presented in Tables (4). It is clearly shown from the data in Table (4) that treating Wonderful pomegranate trees once, twice or three times with a Royal jelly at 0.0125% to 0.050% significantly was accompanied with improving yield, number of fruit/tree (at the second season only) and fruit weight relative to the control treatment. This promotion was in proportional to the

increase in frequencies of applications of Royal jelly from once to thrice as well as increase its concentrations of from 0.0125% to 0.050%.

However, non-significant effects on these parameters were observed between the two higher concentrations (0.0250% and 0.050%) as well as between the two higher frequencies of application (twice or thrice). Therefore, from economical point of view, it is suggested to use Royal jelly twice at 0.0250 % ppm concentration. Under such promised treatment, yield per tree reached 17.3 and 25.2 kg/tree during both seasons, respectively. While, untreated trees produced 14.6 & 13.4 kg/tree during both experimental seasons respectively.

It is worth to mention that the present treatments had non-significant effects on the number of fruits per tree in the first season were observed. This result is logic, since fruiting buds were internally formed during the summer of preceding year. Similar trend was noticed during the two experimental seasons.

This impact of enhancing yield per tree as well as fruit weight was associated with the improvement of leaf stimulatory effect on photosynthetic pigment biosynthesis as well as net photosynthetic rate (Hyel, 1951; Albert and Kludiny, 2004 and Wang, 2016). The higher content of Royal jelly of sugars, amino acids, vitamins, proteins and hormones surely reflected on enhancing fruiting aspects. The great balance among carbohydrates and nitrogen in Royal jelly was followed by increasing fruiting buds, this might be reflected the increase in fruit number/tree, as well as the higher content of Royal jelly from water could result in promoting fruit weight in gram (Hyel, 1951 and Wang, 2016). The obtained results were accordance with those of, Al-Wasfy (2013) on

Flame seedless grapevines, **Ahmed and Habasy (2014)** in Washington navel orange trees, **Abada and Ahmed (2015)** on Superior grapevines, **Abd El-Rady (2015)** and **Abdel-Aziz *et al.* (2015)** on

Ewaise mango trees, **Ibrahim *et al.* (2015)** on Keitte mango trees, **Wassel *et al.* (2015)** on Flame seedless grapevines and **Mohamed (2016)** on Zebda mango trees.

Table 4. Effect of Royal Jelly concentration and frequencies on fruits number/tree, average fruit weight (g) and yield (kg)/tree of Wonderful pomegranate during 2017 and 2018 seasons

Royal Jelly (RJ) treatments	Fruit number / tree		Fruit weight (g)		Yield (Kg) / tree	
	2017	2018	2017	2018	2017	2018
Control (0.0 % RJ)	40	37	365	361	14.6	13.4
RJ at 0.0125% One time	39	44	373	389	14.5	17.1
RJ at 0.0125% Two times	40	53	403	412	16.1	21.8
RJ at 0.0125% Three times	40	54	409	389	16.4	21.0
RJ at 0.0250% One time	41	45	399	403	16.4	18.1
RJ at 0.0250% Two times	42	60	412	419	17.3	25.2
RJ at 0.0250% Three times	40	63	412	414	16.8	26.1
RJ at 0.05% One time	40	49	429	412	17.2	20.2
RJ at 0.050% Two times	42	63	443	420	18.6	26.5
RJ at 0.050% Three times	42	64	445	420	18.7	26.8
New LSD 5%	NS	5.5	17.1	11.8	1.55	1.32

Effect of Royal jelly on fruit physical properties

Effect on peel thickness (mm) and peel weight %

Data concerning the effect of different concentrations and frequencies of application of Royal jelly on peel thickness (mm) and peel weight % (g) of Wonderful pomegranate during 2017 and 2018 seasons are illustrated in Table (5). It is noticed from the obtained data that peel thickness (mm) and peel weight (%) were decreased significantly as a result of increasing the frequencies and the concentrations of Royal jelly. In relation to Royal jelly concentration and frequencies of application resulted in significant decrease, in both seasons, in peel weight and thickness less than untreated trees as clearly shown in Table (5). It could be seen that, the high Royal jelly concentrations (0.0250% or

0.050%) and the frequencies of application (two or three times) were remarkable effective than the lower Royal jelly concentration (0.0125%) and lower frequencies (one times).

The lowest peel fruit thicknesses (3.4 & 3.3 mm) and the lowest fruit peel weights (32.1 & 31.0g) were obtained from the trees received three sprays of Royal jelly at 0.050%. However, increased Royal jelly concentration from 0.0250% to 0.050% and its frequencies of application from twice to thrice, failed to cause a significant decrease neither in fruit peel weight % nor in fruit peel thickness (mm) during the two experimental seasons. On the other hand, untreated trees produced the higher peel weight (49.6 & 48.9 %) and higher peel thickness (5.9 & 5.8 mm) during the two experimental seasons respectively.

Table 5. Effect of Royal Jelly concentration and frequencies on peel weight (%),and juice weight % of Wonderful pomegranate during 2017 and 2018 seasons

Royal Jelly (RJ) Treatments	Peel weight (%)		Peel thickness (mm)		Cracked fruits (%)		Sunburned fruit %	
	2017	2018	2017	2018	2017	2018	2017	2018
Control	49.6	48.9	5.9	5.8	19.1	19.5	21.6	22.0
RJ 0.0125% One time	43.8	43.1	5.2	4.9	16.9	17.1	18.1	17.4
RJ 0.0125% Two times	39.7	38.5	4.8	4.5	14.3	14.1	16.2	15.3
RJ 0.0125% Three times	38.9	38.2	4.7	4.3	13.9	13.7	16.0	15.3
RJ 0.0250% One time	38.8	37.9	4.5	4.2	12.1	11.8	17.2	15.1
RJ 0.0250% Two times	33.2	33.6	3.8	3.9	10.0	9.0	12.5	10.9
RJ 0.0250% Three times	32.5	32.7	3.8	3.8	10.1	8.3	12.0	10.3
RJ 0.05% One time	37.5	36.6	4.2	4.3	11.2	9.2	14.2	14.0
RJ 0.050% Two times	32.4	31.5	3.6	3.5	10.2	8.9	12.3	10.7
RJ 0.050% Three times	32.1	31.0	3.4	3.3	10.2	8.8	11.6	10.1
New LSD 5%	4.21	5.12	0.33	0.43	1.05	1.11	1.17	1.12

Effect on cracked and sunburned fruits %

Under Egyptian conditions pomegranate losses due to fruit cracking and fruit sunburn are quite high. Fruit cracking problem due to improper water management and deficiency of some mineral nutrients, often commonly in desert new reclamation land. Furthermore, fruit cracked and fruit sunburned percentages are two of the most important factors limiting marketable value of pomegranate fruits. These two studied characters were significantly reduced rather than the control trees as a result of treating the Wonderful pomegranate trees with the Royal jelly, during the two experimental seasons, as clearly shown in Table (5).

Increasing the concentrations of Royal jelly from 0.0125% to 0.050% had considerable effect on the cracked fruits % as well as sunburned fruits %, rather than untreated trees. Furthermore, increasing the frequencies of application from once to thrice remarkable and significant decreased these two undesirable characteristics. However, non-significant differences were observed neither between the two highest concentrations (0.0250% and 0.050%) nor between the two highest frequencies (twice and thrice). During the two experimental seasons, the trees received two sprays of Royal jelly at 0.0250% produced the lowest percentage of fruits cracked (10.0% & 8.3%). While, the trees received three sprays of Royal jelly

produced the lowest sunburned fruit percentage (11.65 & 10.1%). However, non-significant differences were observed neither between the two highest concentrations nor between the two highest frequencies of application, during the two experimental seasons.

The obtained results concerning the positive effect of Royal jelly concentrations and frequencies on fruit physical properties during the present study are in harmony with those obtained by **Abada and Ahmed (2015)**, **Abdel-Aziz *et al.* (2015)**, **Ibrahim *et al.* (2015)**, **Wassel *et al.* (2015)** and **Mohamed (2016)**. For improving physical characteristics of Wonderful pomegranate fruit, with regard to the economic costs, it was preferable to use Royal jelly at 0.0250% two times during the vegetative growth cycle.

Effect of Royal jelly on fruit chemical properties

Effect on TSS%, sugar contents % and anthocyanin contents

Data concerning the effect of Royal jelly concentration and frequencies on TSS%, reducing sugars %, non-reducing sugars and total sugars % in fruit juice of Wonderful pomegranate trees during 2017 and 2018 seasons are shown in Tables (6).

Table 6. Effect of Royal Jelly concentration and frequencies on TSS%, reducing sugars% and non-reducing sugars% of Wonderful pomegranate during 2017 and 2018 seasons

Royal Jelly (RJ) treatments	TSS (%)		Reducing sugars %		Non-reducing sugars %		Total sugars %	
	2017	2018	2017	2018	2017	2018	2017	2018
Control (0.0 % RJ)	11.1	11.0	9.3	9.2	1.42	1.38	10.72	10.58
RJ 0.0125% one time	11.9	11.8	9.7	9.8	1.55	1.63	11.25	11.43
RJ 0.0125% two times	12.4	12.6	10.1	10.4	1.73	1.78	11.83	12.18
RJ 0.0125% three times	12.5	12.9	10.4	10.4	1.81	1.83	12.21	12.23
RJ 0.0250% one time	12.5	12.4	10.5	10.6	1.72	1.69	12.47	12.29
RJ 0.0250% two times	13.3	13.4	11.4	11.5	1.80	1.79	13.20	13.29
RJ 0.0250% three times	13.7	13.9	11.6	11.8	1.83	1.83	13.43	13.63
RJ 0.05% one time	13.0	13.5	11.1	11.3	1.76	1.77	12.96	13.07
RJ 0.050% two times	13.8	13.9	11.6	11.7	1.81	1.82	13.51	13.52
RJ 0.050% three times	13.8	14.1	11.7	12.0	1.83	1.86	13.53	13.86
New LSD 5%	0.49	0.51	0.47	0.49	0.045	0.033	0.611	0.451

It is clear from this table that spraying Royal jelly once, twice and three times at 0.0125% to 0.050% significantly was responsible for improving T.S.S%, reducing sugars %, non-reducing sugars% and total sugars% of Wonderful pomegranate fruits rather than non-application. This promotion was associated with increasing the concentration of Royal jelly from 0.0125% to 0.050% and its frequencies of applications from one to three times. However, three sprays of Royal jelly at 0.050% give the best results with regard to T.S.S % (13.8 &

14.1%), reducing sugars % (12.4% & 12.5%), non-reducing sugars (1.83% & 1.86%) and total sugars (13.53% & 13.86%) during the two seasons respectively. While, non-significant differences were observed neither between the two higher concentrations (0.0250% and 0.050%) nor between the two higher frequencies of RJ applications (two and three times).

The same Table (7) declared that, both peel and juice anthocyanin contents (mg/100g F.W.) of

Wonderful pomegranate fruits were significantly increased during the two experimental seasons as a result of spraying Royal Jelly. Such increment of both parameters was generally parallel to the gradual increase in the concentration used, with the lowest

values being given due to sprayed the trees by water "control treatment" 62 & 60 mg/100g F.W. for peel anthocyanin and 77 & 77 mg/100g F.W. for juice anthocyanin, during the two seasons respectively.

Table 7. Effect of Royal Jelly concentration and frequencies on juice total acidity%, peel total anthocyanin (mg/100g F.W.) and juice total anthocyanin (mg/100g F.W.) of Wonderful pomegranate during 2017 and 2018 seasons

Royal Jelly (RJ) treatments	Total acidity (%)		Peel anthocyanin (mg/100g F.W.)		Juice anthocyanin (mg/100g F.W.)	
	2017	2018	2017	2018	2017	2018
Control (0.0 % RJ)	3.12	3.19	62	60	77	77
RJ at 0.0125% One time	3.00	3.01	64	65	81	82
RJ at 0.0125% Two times	2.91	2.88	70	72	89	90
RJ at 0.0125% Three times	2.89	2.86	72	74	91	93
RJ at 0.0250% One time	2.82	2.79	71	72	88	90
RJ at 0.0250% Two times	2.58	2.58	79	82	99	103
RJ 0.0250% Three times	2.55	2.56	81	84	101	106
RJ at 0.05% One time	2.61	2.55	80	83	95	97
RJ at 0.050% Two times	2.52	2.51	86	87	105	110
RJ at 0.050% Three times	2.49	2.48	87	89	107	112
New LSD 5%	0.063	0.054	5.3	6.7	6.4	6.2

Regarding the concentration used, applied three sprays of Royal jelly at higher concentration 0.050% given the highest value of anthocyanin contents in peel (87 and 89 mg/100g F.W.) and juice (107 and 112 mg/100g F.W.) during the two experimental season respectively. This increment was generally slight and non-significant when the trees received one spray of Royal jelly at 0.0125% (3.2 & 8.3 % for peel and 5.2% & 6.5% for juice), but this increment in peel and juice anthocyanin contents was sharp for the trees treated three times with Royal jelly at 0.050% (40.3% & 48.3 % for peel and 38.9% & 45.5% for juice) during the two experimental seasons respectively. Furthermore, increasing the frequencies of application of Royal jelly from one to three times was also capable to significantly promoted the peel and juice total anthocyanin contents.

Effect of Royal jelly on total acidity%

Data obtained during the two experimental seasons as shown in Table (7) displayed that regardless the concentration used or its frequencies, all treatments with Royal jelly caused a significant decrease of total acidity in Wonderful pomegranate fruits, rather than untreated trees. This remarkable decrement in total acidity was related to increase Royal jelly concentrations from 0.0125% to 0.050%. Significant differences in the total acidity were recorded among all concentrations of Royal jelly, except those between the two higher concentrations namely 0.0250% and 0.050%. The lowest values (2.49% & 2.48% respectively) of total acidity were recorded on the trees received three sprays of Royal

jelly at 0.050%. While, untreated trees produced the highest values of total acidity (3.12% & 3.19% respectively). These resulted were true during both seasons.

On the line with our results, concerning the effect of Royal jelly on fruit chemical properties were the findings of **Al-Wasfy (2013)** on Sakkoti date palm, **Ahmed and Habasy (2014)** on Washington Neval Orange, **Abdel-Aziz et al., (2015)** on Ewaise mango, **El-Sayed et al. (2015) & Ibrahim et al. (2015)** on Keitte mango and **Mohamed (2016)** on Zebda mango trees.

It is well known that Royal jelly is a natural material rich in vitamins, amino acids, antioxidants and higher energy phosphorus compounds (such as ADP and ATP) as well as macro and micro elements (**Lercker et al., 1992; Tamura et al., 2009; Barnutiu et al., 2011 Ramadan & Al-Ghamdi, 2012 and Wang, 2016**), these can be stimulate the photothynsis, thus may be produced more sugars can conserve in fruits tissues and stimulate some important plant enzymes such as invertase, as well as increasing the total soluble solids and reduced the total acidity in pomegranate fruit juice. Furthermore, **Popescu et al., 2009; Wu, et al., 2009 and Cruz et al., 2011 and Wu et al., 2015** mentioned that Royal jelly also rich in antioxidants and higher energy phosphorus compounds such as AMP, ADP and ATP that might be lead to increase the carbohydrate metabolism (**Wu et al., 2009 and 2015**).

Conclusion: In Order to enhancing vegetative growth, improving nutritional status of trees,

improving yield quantitatively and qualitatively of Wonderful pomegranate under new reclamation sandy soils in Egypt, it is recommended spraying the trees with Royal jelly at 0.0250% two times on the end of April and again on the end of May.

REFERENCES

- Abada, M.A.M. and Ahmed, B.R. (2015).** The beneficial effects of using royal jelly, arginine and tryptophane on fruiting of superior grapevines. *Egypt. J. Hort.*, 42(1): 345- 354.
- Abdelaziz, F.H.; Mohamed, M.A. and Abd El-Rady, S.E.M. (2015).** Relation of fruiting in Ewaise mango trees to foliar application of Royal Jelly, Magnesium and Boron. *World Rural Observ.*, 7(2): 85-92.
- Abd El-Rady, S.E.M. (2015).** Fruiting of Ewaise mango tree in relation to spraying Royall Jelly, magnesium and boron. M.Sc. Thesis Fac. of Agric. Minia Univ. Egypt.
- Ahmed, F.F. and Habasy, R.E.Y. (2014).** Productivity performance of Washington Navel orange trees in relation to foliar application of Balady seed sprout and Royal Jelly. *World Rural Observations* 6(4): 109-114.
- Albert, S. and Klaudiny, J. (2004).** The MRJP/YELLOW protein family of *Apis mellifera*: identification of new members in the EST library. *J. Insect Physiol.*, 50(1):51–59.
- Al-Wasfy, M.M. (2013).** Response of Sakkoti date palms to foliar application of Royal Jelly, Silicon and vitamins B. *J. American Sci.*, 9(5): 315-319.
- Balkanska, R.; Marghitas, L.A. and Pavel, C.I. (2017).** Antioxidant Activity and Total Polyphenol Content of Royal Jelly from Bulgaria Ralitsa. *Intentional J. Current Microbiology App. Sci.*, 6(10): 578-585.
- Barnutiu, L.I.; Marghitas, D.D.S.; Mihai. C.M.; Bobis, O. (2011).** Chemical composition and antimicrobial activity of royal jelly–review. *J. Anim. Sci. Biotechnol.*, 44(2):67–72.
- Buttstedt, A.; Moritz, R.F.A. and Erler, S. (2014).** Origin and function of the major royal jelly proteins of the honeybee (*Apis mellifera*) as members of the yellow gene family. *Biol. Rev.*, 89 (2): 255-269.
- Buurman, P.; Van Lagen, B. and Velthorst, E.J. (1996).** *Soil and Water Analysis.* Bachuys Publishers Leiden. pp 122-217.
- Cruz, G.C.N.; Garcia, L.; Silva, A.J.; Barbosa, J.A.R.G.; Ricart, C.A.O.; Freitas, S.M. and Sousa, M.V. (2011).** Calcium effect and pH-dependence on self-association and structural stability of the *Apis mellifera* major royal jelly protein. *Apidologie* 42:252–269.
- Drogoudi, P.; Pantelidis, G. and Manganaris, A. (2012).** Morphological and physiological characteristics in pomegranate cultivars with different yields. *Optios Mediterraneenes, A*, no. 103, 2012. II Intern. Sympos. On the pomegranate.
- El- Sayed, M.A.; Mohamed, A.Y. and Mohamed, F.E.A. (2017).** The effect of foliar spraying Royall Jelly on growth and fruiting of Zibda mango trees grown under Aswan rejoin conditions. *J. Product. & Dev.*, 22(2): 267- 285.
- Fadavi, A.; Barzegar, M.; Azizi, M.H. and Bayat, M. (2005).** Physicochemical composition of ten pomegranate cultivars (*Punica granatum* L.) grown in Iran. *Food Science & Technology International*, 11(2): 113-119.
- Fakhour, S. (2012).** La Culture du grenadier dans la rigion du Tadla (Maroc). *Option Méditerranéennes. A*, no. 103, 2012. II International Symposium on the Pomegranate. Pages 145-150.
- Franck, N. (2012).** The cultivation of pomegranate cv. Wonderful in Chile. *Option Méditerranéennes, A*, no. 103, 2012. II International Symposium on the Pomegranate. Pages 97-99.
- Franck, N.; Alfaro, F.; Castillo, M.; Kremer, C.; Opazo, I. and Mundaca, P. (2012).** Effect of different periods and levels of water deficit on physiological, productive and quality parameters of Pomegranate cv. Wonderful fruit. *Optios Mediterraneenes, A*, no. 103, 2012. II Intern. Sympos. On the pomegranate: Pages 137-140.
- Grattan, S.R. and Grieve, C.M. (1999).** Salinity-mineral relations in horticulture crops. *Scientia Horticulturae*, 78: 127-157.
- Gozlekci, S.; Kafkas, E. and Ercisli, S. (2012).** Changes in some free sugars and phenolic contents of pomegranate fruits (*Punica granatum* L.) in three development stages. *Option Méditerranéennes, A*, no. 103, 2012. II International Symposium on the Pomegranate. Pages 205-208.
- Halilova, H. and Yildiz, N. (2009).** Does climate change have an effect on proline accumulation in pomegranate (*Punica granatum* L.) fruits?. *Scientific Research and Essay*, 4 (12): 1543-1546.
- Hegazi, A.; Samra, N.R.; El-Baz, E.E.T.; Khalil, B.K. and Gawish, M.S. (2014).** Improving fruit quality of Manfalouty and Wonderful pomegranates by using bagging and Calcium spray treatments with gibberellic acid, calcium chloride and kaolin. *J. Plant Production, Mansoura Univ.*, 5(5): 779-792.
- Holland, D.; Hatib, K. and Yaakov, I. (2001).** *Pomegranate: Botany, Horticulture, Breeding.* Horticulture Reviews, 35 Edited by Jules Janick.

- Holland, D.; Hatib, K. and Bar-Yaakov, I. (2009).** Pomegranate: botany, horticulture, breeding. Hort. Rev. (Amer. Soc. Hort. Sci.) 35: 127-191.
- Hyel, H.I. (1951).** An observation suggesting the presence of gonadotrophic hormone in Royal jelly. Science, 89: 590-591.
- Ibrahim H.I.M.; Mansour, A.E.M. and Merwad M.A. (2015).** Impact of spraying some organic manure tea, seaweed extract and royal jelly on fruiting of Keitte Mango trees. J. of ChemTech Res., 8(4): 2131-2141.
- Jaiswal, V.; Der-Marderosian, A. and Porter, J.R. (2010).** Anthocyanin and polyphenol oxidase from dried arils of pomegranate (*Punica granatum* L.). Food Chemistry, 118(1): 6-11.
- Lercker, G.; Caboni, M.F.; Vecchi, M.A.; Sabatini, A.G. and Nanetti, A. (1992).** Characterization of the main constituents of royal jelly. APIC 410:27–37.
- Martin-Préval, P.; Gagnard, J. and Gautier, P. (1984).** L'analyse végétale dans le contrôle de l'alimentation des plantes tempères et tropicales. 2nd Ed. pp 810. Technique et Documentation – Lavoisier, Paris, France.
- Mohamed, F.E.A. (2016).** Response of Zebda Mango trees grown under Aswan region conditions to spraying Royall jelly. M.Sc Thesis, Fac. of Agric. Minia Univ.
- Nation, J.L. and Robinson, E.A.S. (1991).** Concentration of some major and trace elements in honey bee, Royal jelly and pollen. J. Apic. Res., 10(1): 35-43.
- Popescu, O.; Marghitas, L.A.; Bobis, O.; Stanciu, O.; Bonta, V.; Moise, A. and Dezmirean, D. (2009).** Sugar profile and total proteins content of fresh royal jelly. Bull UASVM Anim. Sci. Biotechnol., 66:265–269.
- Pessarakli, M. (1999).** Handbook of plant and crop stress. Second Edition. Marcel Dekker New York – USA. Pp 660.
- Ramadan, M.F. and Al-Ghamdi, A. (2012).** Bioactive compounds and health-promoting properties of royal jelly: a review. J. Func. Foods, 4: 39–52.
- Ranganna, S. (1977).** Manual analysis of fruit and vegetable products. Edition Tata Mc Grow-Hill Publishing Company, New Delhi India, 634 P.
- Snedecor, G.W. and Cochran, W.G. (1990).** Statistical Methods, 7th Ed. The Iowa State Univ. Press Ames. pp 80-100.
- Tamura, S.; Amano, S.; Kono, T.; Kondoh, J.; Yamaguchi, K.; Kobayashi, S.; Ayabe, A. and Moriyama, T. (2009).** Molecular characteristics and physiological functions of major royal jelly protein 1 oligomer. Proteomics, 9: 5534–5543.
- Townsend, G. and Lucas, C. (1966).** The chemical nature of Royal jelly. Biochemical. J., 34: 1115-1162
- Wang, X. (2016).** Studies of molecular mechanisms of Royall Jelly mediated health span promotion in caenorhabditis elegans. PhD. Biological Science Clemson University.
- Walsh, L.M. and Beaton, J.D. (1986).** Soil testing and plant analysis. 6th Edition. Editor, Soil science society of America, Inc. pp 489.
- Wassel, A.M.; Gobara, A.A. and Hussien, M.A.M. (2015).** Effect of spraying Royal Jelly on productivity of Flame seedless grapevines. World Rural Observations, 7(4): 51-59.
- Wu, L.; Zhou, J.; Xue, X.; Li, Y. and Zhao, J. (2009).** Fast determination of 26 amino acids and their content changes in royal jelly during storage using ultra-performance liquid chromatography. J. Food Comp. Anal., 22: 242–249.
- Wu, L.; Chen, L.; Selvaraj, J. N.; Wei, Y.; Wang, Y.; Li, Y.; Zhao, J. and Xue, X. (2015).** Identification of the distribution of adenosine phosphates, nucleosides and nucleobases in royal jelly. Food Chem., 173:1111–1118.
- Yilmaz, y.; Celik, I. and Isik-Fatma (2007).** Mineral composition and total phenolic content of pomegranate molasses. J. Food Agriculture & Environment, 5(3&4): 102-104.