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EFFECT OF SPRAYING CHITOSAN ON PRODUCTIVITY AND FRUIT QUALITY OF MANFALOUTY POMEGRANATE TREES

Hamdy I.M. Ibrahim, Ahmed M. K. Abd El-Aal and Mohamed F. Mohamed

Horticulture department Fac. of Agric. Minia Univ. – Egypt

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ABSTRACT:

In order to investigate the effect of spraying Manfalouty pomegranate trees with chitosan at 0.0 (control), 50, 100, 200 and 400 ppm on yield (kg), fruit weight (g), number of fruit/tree as well as fruit physical and chemical properties of Manfalouty pomegranate (*Punica granatium* L), a field trial was conducted in two successive seasons (2018 and 2019) at a private farm located at El-Qusiya district (350 km southern Cairo city), Assiut Governorate. The obtained results confirmed that spraying Manfalouty pomegranate trees with chitosan at 50 ppm to 400 ppm significantly improved pomegranate fruits physical and chemical properties as well as yield (kg)/tree, fruit number/tree and fruit weight (g). This promotion was parallel with increasing chitosan concentration. However, non-significant differences were observed between the two highest concentrations used (200 and 400 ppm), during the two experimental seasons.

INTRODUCTION

The Pomegranate tree (*Punica granatum L.*) belongs to *Myrtales* order and *Punicaceae* family.. Pomegranate is an ancient beloved plant and fruit. The name ‘‘pomegranate’’ follows the Latin name of the fruit Malum granatum, which means ‘‘grainy apple.’’ The generic name *Punica* refers to Phoenicia (Carthage) as a result of mistaken assumption regarding its origin (Shulman *et al.*, 1984; Morton, 1986; Holland *et al.*, 2009, and Ampem, 2017). 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; Fakhour 2012 and Franck *et al.,* 2012).

Over the past three decades, there has been a growing interest in developing natural alternatives to synthetic polymers, namely, biopolymers. Chitosan is produced by deacetylation of chitin, which is the structural element in the exoskeleton of crustaceans, such as crabs and shrimp, and cell walls of fungi. Chitin is the second most prominent biopolymer after cellulose found in nature (Rinaudo, 2006 and Kim & Kim2011), due to their remarkable macromolecular structure, physical and chemical properties, and bioactivities, chitosan have received much attention in fundamental science, applied research, and industrial biotechnology (Dima et al., 2017; Philibert et al., 2017).

This investigation aimed to study the effect of spraying chitosan (at 50. 100, 200, and 400 ppm) and frequencies of applications (once, twice and thrice) on yield and its components as well as fruit quality of Manfalouty pomegranate grown under Assiut Governorate conditions.

MATERIALS AND METHODS

The field work of this investigation was conducted during two successive seasons (2018 and 2019) at private orchard located at El-Qusiya district, Assiut Governorate-Egypt, where the soil texture is heavy clay and water table depth is not less than two meters, surface irrigation system was used. Ten Years old, planted at 4 X 4 meters, uniforms in vigor, Manfalouty pomegranate trees were used in this investigation. Winter pruning was followed at the first week of January.

1- Soil analysis: A composite sample of soil was collected and subjected to physical and chemical analysis according to the procedures outlined by Walsh & Beaton (1986) and Buurman *et al.*, (1996). The data of soil analyses are shown in Table (1).

Table (1): Physical and chemical analysis of experimental orchard soil.

Soil analysis	
Constituents	**Values**
Sand %	9.6
Silt %	22.4
Clay %	68
Texture	Clay
EC (1:2.5 extract) mmhos/cm/ 25 C	6.2
Organic matter %	0.85
pH (1 : 2.5 extract)	7.82
N (mg/kg)	285
Phosphorus (ppm)	15.1 ppm
Available Ca (meq/100g)	7.9
Available Mg (meq/100g)	13.3
Available K (meq/100g)	11.16
C/N Ratio	**9.12**

2- Experimental work: Chitosan was sprayed at five concentrations namely: 0.0 (control), 50 ppm, 100 ppm, 200 ppm, and 400 ppm. Each concentration was sprayed three times (at the beginning of growth, just after fruit setting, and one month later). However, each treatment was replicated four times, one tree per each was used. The treatments were arranged in a complete randomized block design (CRBD).

3- **Different measurement and determinations:** Manfalouty pomegranate fruits were harvested when fruits become fully colored and the T.S.S/Acid ratio in the juice of the untreated trees reached 3 to 3.5 in the two experimental seasons. The yield per tree was recorded in terms of average fruit weight (g) and number of fruits per tree. Then, the fruit yield (kg) per tree was calculated, the physical and chemical characteristics of fruit were determined.

- Fruit weight (g), by using sensitivity balance with 0.1g accuracy.
- Fruit length without calyx (cm), by using vernier caliper.
- Fruit diameter (cm), using vernier caliper with 0.01cm accuracy.
- Fruit shape index, mathematically calculated as follows:

$$shape\ Index = \frac{Fruit\ Length\ (cm)}{Fruit\ Diameter\ (cm)}$$

- The percentages of cracked fruits and sunburned fruits per tree were recorded as follow:

$$\text{Cracking fruits } \% = \frac{Number\ of\ creacked\ fruit}{Total\ number\ of\ fruit} X\ 100$$

$$\text{Sunburned fruits } \% = \frac{Number\ of\ sunburn\ fruit}{Total\ number\ of\ fruit} X\ 100$$

- Peel thickness (mm), using vernier caliper with 0.01cm accuracy.

After extracting the arils by hand, 200 g of each replicate were randomly chosen from homogenized sample, pressed by Electric Extractor for extracting the juice, the following chemical characteristics were determined:

- Percentage of total soluble solids (T.S.S %) were determined by using a hand refractometer, according to **Ranganna (1977)**.
- Percentage of total titratable acidity (TA), expressed as grams citric acid per 100 grams of juice, by titration with 0.1 N NaOH, **(**according to **A.O.A.C, 2000)**.
- Percentages of reducing and non-reducing sugars were determined, according to **Ranganna (1977)**. However, the total sugars were mathematically calculated (as the sum of reducing and non-reducing sugars %).
- Total anthocyanins in fruit peel and juice were determined, according to Fulcki & Francis (1968).

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, according to **Snedecor and Cochran, (1990).**

RESULTS AND DISCUSSIONS

1- Effect on Yield and its component:

Data concerning the effect of spraying chitosan, at different concentrations, on fruit numbers/tree, fruit weight (g), and yield per tree (kg) of Manfalouty pomegranate trees during 2018 and 2019 seasons are shown in Table (2). It's clear that the results took similar trend during the two experimental seasons. It is obvious from the obtained data that, subjected Manfalouty pomegranate trees to chitosan (at 50. 100, 200, and 400 ppm) was significantly accompanied with enhancing yield and its components namely: yield/tree (kg), fruit weight (g), and fruit numbers/tree. Regardless the chitosan concentration, during the first season the fruit number/tree didn't vary significantly. However, remarkable and significant increase in yield/tree was observed in the same season, especially with the higher concentration of chitosan, thus due to the significant promotion in fruit weight. Regarding the results of the second season, each one of the four chitosan examined concentrations (50, 100, 200, and 400 ppm) was capable to significantly enhance fruit numbers/tree, fruit weight (g), and yield/tree (kg), rather than control treatment.

It's clear from the obtained data that the trees received chitosan at the highest concentration (400 ppm) produced the highest number of fruits/tree during the second season only (69.0), the highest fruit weight (551.3 & 565.2 g), and highest yield (kg)/ tree (34.46 & 39.00 kg/tree), during the two experimental seasons respectively. Contrary, untreated trees produced the lowest number of fruits/tree (62.1 & 62.3 fruits/tree), the lowest average fruit weight (439.2 & 440.1 g), and the lowest yield (kg)/tree (27.27 & 27.42 kg/tree), during the two experimental seasons respectively.

The obtained results were accordance with those of *Van et al.,* (2013) on coffee trees; Ahmed *et al.,* (2016) on Washington navel orange; El-Kenawy, (2017) on grapevines and Ayed, (2018) 0n Zebda mango trees, whereas their results proved that application of chitosan improved yield, fruit weight, and fruit numbers/tree.

Table (2): Effect of different concentration of chitosan on fruits numbers/tree, fruit weight, and yield (kg)/tree of Manfalouty pomegranate during 2018 and 2019 seasons.

Treatments	Fruit numbers/tree		Fruit weight (g)		Yield (kg) per tree	
	2018	2019	2018	2019	2018	2019
Control	62.1	62.3	439.2	440.1	27.27	27.42
Chitosan at 50 ppm	62.1	64.5	489.0	497.9	30.37	32.11
Chitosan at 100 ppm	62.3	66.8	527.5	532.1	32.86	35.54
Chitosan at 200 ppm	62.5	68.1	544.9	563.1	34.06	38.35
Chitosan at 400 ppm	62.5	69.0	551.3	565.2	34.46	39.00
LSD at 5%	NS	1.1	19.1	22.2	0.8	0.7

2- Effect on fruit physical properties:

2-1: Fruit length, fruit diameter, and shape index: Data concerning the effect of chitosan concentrations on Manfalouty pomegranate fruits physical properties during 2018 and 2019 seasons are shown in Table (3). It is obvious that the results took similar trend during the two seasons. There was a gradual promotion on fruit height and fruit diameter of Manfalouty pomegranate trees parallel with increasing the chitosan concentrations from 50 ppm to 400 ppm. While, increasing chitosan concentration from 200 ppm to 400 ppm had non-significant promotion in the two studied characters (fruit length and fruit diameter), during the two experimental seasons. Furthermore, the highest fruit length (10.3 & 10.1 cm) and fruit diameter (9.8 & 9.9 cm) were produced by the trees received chitosan at the highest concentration (400 ppm) followed by those received chitosan at 200 ppm, during the two experimental seasons respectively. On the other hand, untreated trees produced the lowest length (8.6 & 8.8 cm) and the lowest diameter (8.0 & 8.3 cm) of fruits, during 2018 and 2019 seasons, respectively. The gradual improvement in fruit height and diameter was parallel with increasing chitosan concentration, this led to regular effect on fruit shape index, that is why the fruit shape index did not significantly varied during the two experimental seasons.

In accordance with our results Scortichini (2014) found that spraying chitosan was very effective in enhancing fruit physical properties of Kiwi fruit. Also, Zagzog *et al.*, (2017) confirmed that spraying chitosan at different concentration enhanced fruit length and fruit diameter of mango fruits. In the same trend Gayed *et al.*, (2017) observed similar results on peach trees.

Chitosan products are proposed as substrate for controlling the release of agrochemicals (fertilizers and pesticides). The chelating properties of chitosan also make it an excellent source of macro and micronutrients (Rabea et al., 2003; Harfoush et al., 2017; Divya & Jisha 2018 and Rahman et al., 2018). Also, chitosan have been extensively researched as natural antioxidants which are not only inexpensive but also biodegradable. Furthermore, the various antioxidant capacity of chitosan were observed by certain authors such as Kim and Thomas (2006); Liu et al., (2009); El-Sayed et al., (2017); Laokuldilok et al. (2017); Anraku et al., (2018); Chang et al., (2018) and Rahman et al., (2018). The previous lines may be explain the role of chitosan in improving fruit length and diameter of Manfalouty pomegranate that be confirmed in our trial.

2-2: Pell thickness (mm): Data obtained during the two experimental seasons shown in Table (4) displayed that, regardless the concentration used of chitosan, all treatments of chitosan were failed to significantly change the thickness of Manfalouty pomegranate fruit during the two experimental seasons.

3- Cracked and sun burned Fruits %: Data concerning the effect of chitosan spraying on cracked fruit % and sunburned fruit % during 2018 and 2019 seasons which are illustrated in Table (4) declared that, both fruit cracked% and fruit sunburned% of Manfalouty pomegranate were significantly decreased, during the two experimental seasons. Such decrease in both seasons was generally parallel with the gradual increase in chitosan concentration. However, the highest values were produced when the trees were sprayed by water "check treatment" (24.2 & 26.3% for fruit cracked percentage and 20.1 & 20.9% for fruit sunburned percentage), during the two seasons respectively. Contrary, the lowest values of cracked fruits% (10.4 & 9.5 %) and sunburned fruits% (8.1 & 7.9 %) were produced by the trees received the highest concentration of chitosan (400 ppm), during the two experimental seasons respectively. These results were true during both experimental seasons. It's worth to mention that, non-significant differences were observed between the two highest concentrations of chitosan (200 ppm and 400 ppm) neither for cracked fruit nor for sunburned fruits.

The obtained results concerning the effect of chitosan on cracked fruit % and sun burned fruit % are in accordance with those obtained by Abdel-Mawgoud *et al.,* (2010); El Hadrami *et al.,* (2010); Ghasemnezhad *et al.,* (2010); Reglinski *et al.,* (2010); Ferri *et al.,* (2011); Saei *et al.,* (2014); Ahmed *et al.,* (2016); Romanazzi *et al.,* (2017); Zagzog *et al.,* (2017) and Gayed *et al.,* (2017) on different fruit trees. Chitosan products are used as biocides either alone or blended with other products against plant diseases, plant growth promotion, seed-coating, and postharvest (Gilbert *et al.,* 2007; Divya & Jisha 2018; Sharif *et al.,* 2018 and Rahman *et al.,* 2018). Due to its chelating properties, chitosan conceded as excellent source of macro and micronutrients (Hirano, 1989; Rabea *et al.,* 2003; El-Hadrami *et al.,* 2010; Divya & Jisha 2018 and Rahman *et al.,* 2018). Also, chitosan have been extensively researched as natural antioxidants which are not only inexpensive but also biodegradable. The various antioxidant capacity of chitosan were illustrated by certain authors such as Kim and Thomas (2006); Liu *et al.,* (2009); El-Hadrami *et al.,* (2010); El-Sayed *et al.,* (2017); Laokuldilok *et al.* (2017); Anraku *et al.,* (2018); Chang *et al.,* (2018) and Rahman *et al.,* (2018). These favorable properties of chitosan maybe demonstrated its positive effect on the physical properties of Manfalouty pomegranate fruit, that was observed in the present trial.

Effect of chitosan on fruit chemical properties

1- **Effect on juice TSS% and sugars contents%:** It was clear from the obtained data presented in Tables (5 & 6) that treating Manfalouty pomegranate trees with chitosan at 50 ppm to 400 ppm significantly enhance TSS%, reduced sugars% and total sugars% rather than control treatment, during the two experimental seasons. This promotion of reducing sugars% and total sugars% were associated with increasing the total soluble solids %. Furthermore, the enhancement of the three estimated characters was parallel with increasing chitosan concentrations from 50 to 400 ppm, during the two experimental seasons. However, non-significant differences in TSS%, reducing sugars% and total sugars% were observed between the two highest concentrations, during the two experimental seasons.

Table (3): Effect of different concentration of chitosan on fruit length, fruit diameter, and shape index of Manfalouty pomegranate, during 2018 and 2019 seasons.

Treatments	Fruit diameter (cm)		Fruit lengt(cm)		Fruit shape index	
	2018	2019	2018	2019	2018	2019
Control	8.0	8.3	8.6	8.8	1.08	1.06
Chitosan at 50 ppm	8.5	8.7	9.0	9.1	1.06	1.05
Chitosan at 100 ppm	8.9	9.2	9.6	9.5	1.08	1.07
Chitosan at 200 ppm	9.5	9.7	10.0	9.9	1.05	1.02
Chitosan at 400 ppm	9.8	9.9	10.3	10.1	1.05	1.02
LSD at 5%	0.4	0.3	04	0.3	NS	NS

Table (4): Effect of different concentration of chitosan on fruit peel thickness (mm), cracked fruit %, and sun burned fruit % of Manfalouty pomegranate during 2018 and 2019 seasons.

Treatments	Peel thickness (mm)		Cracked fruit %		Sun burned fruit %	
	2018	2019	2018	2019	2018	2019
Control	5.1	5.2	24.2	26.3	20.1	20.9
Chitosan at 50 ppm	5.2	5.3	17.7	16.3	17.5	16.1
Chitosan at 100 ppm	5.0	5.1	14.3	13.1	11.4	11.2
Chitosan at 200 ppm	4.9	5.1	10.8	10.4	8.9	8.2
Chitosan at 400 ppm	4.9	4.9	10.4	9.5	8.1	7.9
LSD at 5%	NS	NS	2.1	2.3	0.9	0.8

The trees received chitosan at 400 ppm produced the higher TSS (17.8% & 18.3%), reducing sugars (15.8% & 15.9%) and total sugars (16.7% & 16.8%) in its fruits, during the two experimental seasons. on the other hand, untreated trees produced fruits with the lowest TSS% (16.5% & 16.6%), reducing sugars % (14.3% & 14.6%), and total sugars (15.0% & 15.3%), during the two experimental seasons. Regarding the effect of spraying chitosan at different concentration on non-reducing sugars of Manfalouty pomegranate, the data illustrated in Table (5) confirmed that all chitosan concentration used failed to increasing non-reducing sugars content during the two seasons, except the highest concentration (400 ppm) which produced higher and significant non-reducing sugars% than the control treatment, during the two experimental seasons.

2- **Effect on total acidity%:** Data presented in Table (6) showed the effect of different concentrations of chitosan on fruit acidity confirmed that, increasing the chitosan concentration from 50 ppm to 400 ppm proved to cause gradual decrement in total acidity of Manfalouty pomegranate fruits than control treatment, during the two experimental seasons. However, non-significant differences were observed between the two highest concentrations (200 ppm and 400 ppm), during the two seasons respectively. Its worth to mention that, the trees received the highest concentration (400 ppm) produced the lowest total acidity % in their fruits (0.887% & 0.885%). Hover, untreated trees produced the highest total acidity % in fruit (1.184% & 1.199%), during the two experimental seasons, respectively. Furthermore, no-significant promotion was attributed to increasing chitosan concentration from 200 ppm to 400 ppm, during the two experimental seasons. So, in order to improve the chemical properties of Manfalouty pomegranate we recommend treated the application of chitosan at 200 ppm.

3- Effect of chitosan on total anthocyanins content:

Data concerning the effect of different concentrations of chitosan on total anthocyanin contents of Manfalouty pomegranate fruits during 2018 and 2019 seasons are illustrated in Table (5). This Table shows that, all chitosan concentrations were capable of causing significant promotion in total anthocyanin's (mg/100g F.W.) in Manfalouty pomegranate fruits over the control trees, during the two experimental seasons respectively. It's clear from Table (5) that the trees received the highest chitosan concentration produced the highest total anthocyanin's in their fruits (85.1 & 85.4 mg/100g F.W.). However, untreated trees produced the lowest anthocyanin's in their fruits (77.2 & 76.9 mg/100g F.W), during the two experimental seasons respectively.

Positive effects of chitosan application on enhancing TSS%, sugar contents (%) and total anthocyanins (100g/100g F.W.), as well as decreasing total acidity% of pomegranate fruits were reviewed by Romanazzi *et al.,* (2017) and Candir *et al.,* (2018). Moreover, Extensive studies have been carried out on some fruit trees, their final results confirmed the positive effect of chitosan on fruit chemical properties Abdel-Mawgoud *et al.,* (2010); Reglinski *et al.,* (2010); Samra *et al.,* (2012); El-Miniawy et al., (2013); Ghasemnezhad *et al.,* (2010); Ferri *et al.,* (2011); Ahmed *et al.,* (2016); Zagzog *et al.,* (2017) and Gayed *et al.,* (2017).

The positive effect of chitosan on chemical properties of Manflouty pomegranate fruits might be explained by its content of some macro and micro nutrients, its role as bio-catalytic of some important enzymes and enhancement of some hormones syntheses. Furthermore, chitosan have been extensively researched as natural antioxidants. The various antioxidant capacity assays along with their principle determination have been noted by Hirano (1989); Van *et al.,* (2013) and Crimi & Lichtfouse (2019).

Table (5): Effect of different concentration of chitosan on TSS%, total acidity%, and reducing sugars % of Manfalouty pomegranate during 2018 and 2019 seasons.

Treatments	TSS %		Total acidity %		Reducing sugars %	
	2018	2019	2018	2019	2018	2019
Control	16.5	16.6	1.184	1.199	14.3	14.6
Chitosan at 50 ppm	16.8	17.2	1.020	1.023	14.7	15.0
Chitosan at 100 ppm	17.1	17.7	0.905	0.901	15.1	15.3
Chitosan at 200 ppm	17.5	18.2	0. 890	0.888	15.6	15.7
Chitosan at 400 ppm	17.6	18.3	0.887	0.885	15.8	15.9
LSD at 5%	0.2	0.3	0.04	0.03	0.3	0.3

Table (6): Effect of different concentration of chitosan on non-reducing sugars%, total sugars%, and total anthocyanins (mg/100g F.W.) of Manfalouty pomegranate during 2018 and 2019 seasons.

Treatments	Non-reducing sugars %		Total sugars %		Total anthocyanins mg/100g F.W.	
	2018	2019	2018	2019	2018	2019
Control	0.7	0.7	15.0	15.3	77.2	76.9
Chitosan at 50 ppm	0.8	0.7	15.5	15.7	80.2	80.9
Chitosan at 100 ppm	0.8	0.7	15.9	16.0	82.3	83.3
Chitosan at 200 ppm	0.8	0.8	16.4	16.5	84.9	85.2
Chitosan at 400 ppm	0.9	0.9	16.7	16.8	85.1	85.4
LSD at 5%	0.2	0.2	0.3	0.4	0.4	0.4

CONCLUSION: The results of this investigation confirmed that , in order to improve the vegetative growth and production as well as fruit physical and chemical properties of Manfalouty pomegranate trees growing under Assiut governorate conditions, it is strongly recommended to spray Manfalouty pomegranate three times yearly with chitosan at 200 ppm.

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تأثير الرش بالشيتوسان على الأنتاجية وجودة الثمار فى أشجار الرمان المنفلوطى

حمدى إبراهيم محمود إبراهيم، أحمد محمد كمال، و محمد فتحى محمد

قسم البساتين – كلية الزراعة – جامعة المنيا

من أجل دراسة تأثير رش الشيتوسان بتركيزات خمسة وهى: الصفر (كنترول)، 50 جزء فى المليون، 100 جزء فى المليون، 200 جزء فى المليون و400 جزء فى المليون على كمية المحصول والموصفات الفيزيائية والكيميائية لثمار الرمان صنف المنفلوطى، تم أجراء هذه التجربة الحقلية خلال موسمين متتاليين (2017 و 2018) فى مزرعة خاصة بمركز القوصية بمحافظة أسيوط (350 كم جنوب القاهرة). وقد أكدت النتائج المتحصل عليها خلال موسمي التجربة ان زيادة تركيز الشيتوسان من 50 إلى 400 جزء فى المليون أدى إلى حدوث تحسن معنوى فى مواصفات الجودة الفيزيائية والكيميائية للثمار كما ادى إلى زيادة كمية المحصول على الشجرة مقدرة بالكجم، وزن الثمرة بالجرام وكذلك عدد الثمار على الشجرة. وكان هذا التحسن متوازى مع زيادة تركيز الشيتوsان. فى حين لم تسجل أى فروق معنوية بين التركيزين المرتفعين من الشيتوسان (200 جزء فى المليون و 400 جزء فى المليون)، خلال موسمي الدراسة.