Fruit quality of "Manfalouty" pomegranate as affected by putrescine and chitosan treatments

Alaa, M. Gomaa*[1] and Heba F.S. Ibrahim[2]

Department of Horticulture Faculty of Agriculture, Suez Canal University, Ismailia–Egypt[1]. Department of Horticulture Faculty of Agriculture, Minia University, Minia – Egypt[2]

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Abstract: The purpose of this study was to examine the impact of spraying putrescine (at concentrations of 10, 20, and 30 ppm) and/or chitosan (at concentrations of 0.1%, 0.2%, and 0.3%) three times per year on the yield, physical properties, and chemical properties of Manfalouty pomegranate trees that are grown in sandy loam soil. Spraying putrescine and chitosan on pomegranate trees leads to improved fruit production and better fruit physicochemical properties. This positive effect is gradual and parallel to the increase in the concentration of each material used. When both materials were used in combination, regardless of the concentration, they were more effective in enhancing yield and improving fruit quality as compared to trees treated with either material alone or untreated trees. Moreover, trees that received the highest concentration of both materials in combination produced the highest yield and best quality fruit during the two experimental seasons. Additionally, there were no significant differences between the two highest concentrations during the two seasons of the experiment.

Key Words: Manfalouty Pomegranate; Putrescine; Chitosan; Physical and Chemical Properties

INTRODUCTION

The Pomegranate tree (*Punica granatum* L.) belongs to the Punicaceae family and is one of the most popular tropical and sub-tropical fruits. It is native to Iran and is widely grown in Mediterranean regions like Spain, Italy, Morocco, and Egypt (Mercure, 2007). Pomegranate cultivation is widespread in almost all governorates of Egypt, both in the Nile Valley and desert regions. Pomegranates have become an important commercially grown fruit crop due to their wide adaptability to sandy soil, ability to tolerate drought and heat, relatively low cultivation cost, good yields, excellent shelf life, and export potential (Jalikop, 2004). Pomegranate production has expanded, especially in newly reclaimed lands in Egypt, where poor-quality water is available, including recycled or saline water (Franck *et al.,* 2012). As a result, new pomegranate cultivation is now concentrated in the sand decimated soils, in desert regions of Egypt, such as the Western Sama lot region of El-Minia (Teleb, 2023). The 'Manfalouty' pomegranate is a popular Egyptian cultivar due to its good fruit quality. However, it has a major issue of fruit cracking, especially under desert conditions (Ahmed *et al.,* 2014). This problem affects the fruit's market value. To address this issue, researchers have studied the use of salicylic acid and nutrients to control fruit splitting and improve the productivity of Manfalouty pomegranate trees. Proper water management and mineral nutrient supplementation may also help prevent fruit cracking (Teleb, 2023).

Chitosan is a semi-synthetic commercial amino polysaccharide that has received a lot of attention in fundamental science and applied research due to its remarkable macromolecular structure, physical and chemical properties, and bioactivities. It is derived through the deacetylation of the naturally occurring biopolymer chitin (Suseno *et al.,* 2014). The main sources used for chitin and chitosan production are the shells of shrimps and crabs, the bone plate of squids, and other marine crustaceans (El-Sayed *et al.,* 2017; Nechita, 2017 and Philibert *et al.,* 2017). Putericin ($C_4H_{12}N_2$. HCl) is a polyamine that is considered a growth substance and an important factor in improving plant growth and productivity, especially under stress conditions. It is a secondary metabolite and a small aliphatic low molecular weight polycationic nitrogenous compound (Khorshidi and Hamedi, 2014). Putericin is involved in several processes related to fruit tree growth and development. Its role as an anti-oxidant, anti-senescence, and anti-stress agent has been previously reported by Lin *et al.* (2008); Khorshidi and Hamedi (2014); and Ahmed *et al.* (2016). In this study, the impact of spraying putrescine and chitosan on the physicochemical and chemical properties of 'Manfalouty' pomegranates was tested. The focus was on how pre-harvest spraying of these substances affects the productivity and physicochemical characteristics of the fruit.

MATERIALS AND METHODS

This study was conducted on thirty uniform and self-rooted 'Manfalouty' pomegranate trees (*Punica granatum L.*) grown in a commercial orchard located at Shosha, in the Western Samalot district of El-Minia Governorate, Egypt, during the 2022 and 2023 seasons. The soil texture of the orchard is sandy loam, and the water table depth is not less than two meters. The trees that were chosen for the study are sixteen years old and were planted at a

*Corresponding author e-mail: dralaamgomaa@gmail.com

distance of 3 X 4 meters apart. The chosen trees have a multi-trunk formation (3 trunks/tree), and an open vase system was followed. Orchard irrigation was carried out using an underground well with pressure and volume controllers, followed by a drip irrigation system with an irrigation capacity of 7 liters/hour/dripper. The chosen pomegranate trees were subjected to conventional horticulture practices that are commonly applied in pomegranate orchards. Composite samples of soil and irrigation water from the orchard were collected and analyzed for physical and chemical properties according to the methods described by Walsh and Beaton (1986) and Wilde *et al.* (1985). The results of the analysis are presented in Table (1).

Table (1): Physicochemical analysis of experiment orchard soil and water used in irrigation

Soil analysis		Water analysis	
Constituents	Values	Constituents	Values
Sand%	69	E.C (m.mhos / ml)	1. 8
Silt%	18.2	Hardness	12.2
Clay%	12.8	pH	7.25
Texture	Sandy Loam	Ca (mg/L)	29.4
EC (1:2.5 extract) m.mhos/cm	3.2	Mg (mg/L)	18.3
Organic matter %	0.21	K (mg/L)	4.1
pH (1 : 2.5 extract)	7.21	Na (mg/L)	20.4
Active lime %	9% ($CaCO_3$)	Sum of Cations (mg/L)	7.26
N (mg/kg)	199	Alkalinity (mg/L)	149
Phosphorus (ppm)	7.82 ppm	Chlorides (mg/L)	101
Available Ca (meq/100g)	19.3	Nitrate (mg/L)	9.0
Available Mg (meq/100g)	3.19	Sulphates (mg/L)	29.3
Available K (meq/100g)	0.44	Sum of anions (mg/L)	6.79

Experimental work: During the 2022 and 2023 seasons, an experiment was conducted to see how spraying putrescine (at 10, 20, and 30 ppm) and chitosan (at 0.1%, 0.2%, and 0.3%) or a combination of both affect the physical and chemical properties of 'Manfalouty' pomegranates. The experiment included eleven treatments: Control (untreated trees), spraying putrescine at 10 ppm, spraying putrescine at 20 ppm, spraying putrescine at 30 ppm, spraying chitosan at 0.1%, spraying chitosan at 0.2%, spraying chitosan at 0.3%, spraying putrescine at 10 ppm + chitosan at 0.1%, spraying putrescine at 20 ppm + chitosan at 0.2%, spraying putrescine at 30 ppm + chitosan at 0.3%. Each concentration was sprayed four times during fruit growth stages, with the first spraying taking place just after fruit setting and the rest at one-month intervals. The experiment was replicated three times, using one tree for each replication. As a result, the study included thirty 'Manfalouty' pomegranate trees.

Experimental design and statistical analyses: The experiment was conducted with 10 treatments and three replications, using a complete randomized block design as described in Snedecor and Cochran's book from 1990. The data obtained were analyzed using proper statistical methods, including analysis of variance (ANOVA) with the MSTATC statistical analysis program. The means were then compared using the New LSD test at 0.05, as recommended by Gomez and Gomez (1984).

Measurement and determination: The following parameters were determined during the two experimental seasons (2022 and 2023):

1- **Yield and its component:** The 'Manfalouty' pomegranate fruits were harvested when they were fully colored, and the TSS/Acid ratio was 3 to 3.5 (according to Hegazi *et al.,* 2014). The number of fruits per tree was counted, and the yield per tree (kg/tree) was recorded. The average fruit weight was determined for each tree, and the total yield per tree was calculated mathematically by multiplying the number of fruits per tree by the average fruit weight.

2- **Fruit physical properties:**

2-1- **Percentage of cracked and sunburned fruits**: the number of cracked and sunburned fruits was counted, and the percentage of each one was calculated according to the following equations:

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

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

2-2- **fruit and peel physical determinations**: From each tree, four pomegranate fruits were randomly picked at the maturation date (when they were fully colored, and the TSS/Acid ratio was 3 to 3.5), the following physical parameters were achieved: Average fruit weight (g), by using sensitivity balance with 0.1g accuracy. Fruit dimensions (fruit height without calyx and fruit diameter, cm) were achieved by using a vernier caliper. Fruit shape index was determined according to the following equation:

$$\boxed{\text{Shape index} = \frac{fruit\ height}{fruit\ diameter}}$$

Peel weight plus capillary membranes weight (g), achieved by using sensitivity balance with 0.01g accuracy. Peel thickness (mm), was determined by using vernier caliper with 0.01cm accuracy. Arils weight (0.01 g) and epicarp weight, included capillary membranes (0.01g). Then, the edible to non-edible portion was calculated. Juice percent mathematically calculated. Fruit chemical properties: 200 grams of arils were taken randomly from each replicate fruit sample. Then, pressed by an Electric Extractor to extract the juice. The following chemical characteristics were measured: Total soluble solids (TSS %) using a hand refractometer (as per Ranganna, 1990). Titratable acidity (TA), expressed in grams of citric acid per 100 grams of juice, using titration (with 0.1 N NaOH) in the presence of Phenol-Phthalein indicator (as per A.O.A.C, 2000). Reducing sugars and total sugars percentages were determined by Lane and Eynone volumetric method (Ranganna,1990). Then the non-reducing sugars was mathematically calculated by subtracting the value of reducing sugars from the value of total sugars. So, the total anthocyanins of fruit peel and juice were extracted and determined using the method outlined by Fulcki and Frabcis (1968).

RESULTS AND DISCUSSION

Table (2) presents data regarding the impact of spraying putrescine and chitosan, separately or in combination, on the yield (kg/tree), average weight of fruit, and fruit number per tree of ‘Manfalouty’ pomegranate grown in sandy loam soil in El-Minia conditions during 2022 and 2023. The data shows a significant effect of the two compounds (individually or in combination) on fruit number per tree, average fruit weight, and yield (kg/tree) in both experimental seasons. The data collected from the 2022 and 2023 seasons showed that spraying putrescine at concentrations of 10, 20, and 30 ppm, and chitosan at concentrations of 0.1%, 0.2%, and 0.3% individually or in combination, significantly increased the number of fruits per tree, average fruit weight in grams, and yield in kilograms per tree of the 'Manfalouty' pomegranate. However, the individual application of chitosan resulted in higher fruit numbers per tree, average fruit weight, and yield compared to putrescine in both seasons. Moreover, the combined application of both compounds was found to be more effective than using each one alone. Trees treated with putrescine at 30 ppm and chitosan at 0.3% showed the best results in terms of fruit number per tree, average fruit weight, and yield. In contrast, untreated trees had the lowest values. These results were consistent in both seasons, except for the fruit number per tree, where no significant differences were observed. Several hypotheses have been proposed to explain the role of putrescine (butane-1,4-diamine) in improving the productivity of fruit trees. It is suggested that putrescine plays several roles under stress conditions such as cold, salinity, and potassium deficiency stress. It also has important roles in higher plants, serving as a starting point of the most common polyamines pathway, balancing cations, providing antioxidant activity, controlling cellular K+ and Ca2+, and regulating the bioenergetics of mitochondria and chloroplasts, as well as pH levels. However, its role in the aired soil and salinity tolerances of fruit trees remains poorly understood (Gonzalez-Hernandez *et al.,* 2022). Furthermore, Cui *et al.* (2020) and Gonzalez-Hernandez *et al.* (2022) suggested that putrescine has a regulatory effect on plant cell development through its influence on the biosynthesis of gibberellins, tryptophan, and methionine, which are building blocks of IAA and ethylene, respectively. These previous findings can explain the positive effect of putrescine on enhancing fruit weight and yield/tree of Manfalouty pomegranate trees, as found in the current study. The positive effects of chitosan on enhancing the number of fruit clusters per tree, average fruit weight, and yield (in kg per tree) can be attributed to its essential content in mineral nutrients such as nitrogen, phosphorus, and potassium. Additionally, it has a favorable effect on nutrient and water uptake, and a positive effect on the bio-stimulation of photosynthesis (Ahmed et al., 2016 and Ayed 2018). Chitosan also improves the transportation of nitrogen in functional leaves.

Fruit physical properties: Cracked and sunburned fruits %: It has been observed that improper water management and deficiency of certain mineral nutrients can cause fruit cracking in pomegranate trees (Galindo *et al.,* 2014). This problem results in decreased market value due to the increase in cracked and sunburned fruit percentages (Singh *et al.,* 2014; Wunsche *et al.,* 2001 and Teleb 2023). However, studies have shown that treating pomegranate trees with putrescine and chitosan at varying concentrations can significantly reduce these undesirable traits, as demonstrated in Table (3) during the two experimental seasons. The data show that increasing the concentrations of either putrescine or chitosan, or combining the two, effectively decreased the percentage of fruit cracking and sunburn in 'Manfalouty' pomegranate fruits during both 2022 and 2023 seasons.

Table (2): Effect of spraying putrescine and chitosan on yield of Manfalouty Pomegranate, during 2022 and 2023.

Treatments	Fruit numbers/tree		Fruit weight (g)		Yield (kg/tree)	
	2022	2023	2022	2023	2022	2023
Control	28.3	31.2	409.3	412.7	11.50	12.87
Putrescine10 ppm	30.7	34.4	420.2	431.3	12.90	14.84
Putrescine 20 ppm	31.2	35.2	429.6	439.5	13.44	15.47
Putrescine 30 ppm	33.1	36.2	432.5	442.7	14.37	16.03
Chitosan 0.1%	31.5	33.6	431.5	441.1	13.65	14.82
Chitosan 0.2%	32.2	35.7	439.2	452.8	14.14	16.16
Chitosan 0.3%	33.7	36.2	446.1	455.3	15.03	16.48
Putrescine 10 ppm + Chitosan 0.1%	32.3	36.3	437.2	478.7	14.07	17.38
Putrescine 20 ppm + Chitosan 0.2%	34.4	38.9	469.8	487.3	16.16	18.95
Putrescine 30 ppm + Chitosan 0.3%	34.5	43.1	474.2	503.4	16.36	21.69
New LSD at 5%	NS	3.2	17.1	15.5	1.74	1.89

It was observed that reducing the criteria led to an increase in putrescine and chitosan concentration, resulting in the highest values when the trees were sprayed with water (control treatment) during the two seasons (as shown in table 4). The table also indicates that the combined treatments with putrescine and chitosan were more effective in reducing cracked and sunburned fruit percentages compared to individual application. Therefore, spraying 'Manfalouty' pomegranate trees with putrescine at 30 ppm combined with chitosan at 0.3% produced the lowest percentages of fruit cracking and sunburn. These findings were consistent during both experimental seasons. Furthermore, there were no significant differences observed between the two highest concentrations, either individually or in combination. Pomegranate trees are susceptible to fruit cracking and sunburn, which can result in significant economic losses. The average, fruit cracking leads to a loss of 10-40% of the crop (Pal *et al.,* 2017). In some sensitive cultivars, this percentage can reach up to 70%. The cracking percentage varies among varieties, with early ripening varieties typically experiencing lower rates than late-maturing ones (Ahmed et al., 2014; Singh *et al.,* 2014; Omar, 2019). In arid regions with dry atmospheres, fruit cracking is more common (Ahmed *et al.,* 2014). The percentage and degree of cracking also vary depending on the fruit's growth stage, with the highest rates occurring during the ripening stage (Kumar *et al.,* 2010; Omar 2019; Teleb, 2023). Pomegranate fruit cracking can occur due to many factors, including cultivar, environmental factors (Singh *et al.,* 1990 and Singh *et al.,* 2014), and various agricultural treatments (Saad *et al.,* 1998; Khadivi-Khub, 2014; Singh *et al.,* 2014; Aydın and Kaptan, 2015 and Telep, 2023). Higher temperatures, higher transpiration, lower humidity of air and soil, as well as sharp fluctuations in temperature between day and night during the fruit growth period are associated with fruit cracking (Abd El-Rahman, 2010). Nutrient deficiencies, particularly boron, calcium, zinc and potassium, have also been linked to fruit cracking (Kumar et al., 2010; Saeedi et al., 2012 and Aydın & Kaptan, 2015) and irregular fruit growth (Singh et al., 2014; Omar, 2019 and Teleb, 2023).

Fruit dimensions: The following table (4) presents data on the effect of putrescine and chitosan, alone or combined, at varying concentrations on the length and diameter (in centimeters) of 'Manfalouty' pomegranate fruit during the 2022 and 2023 seasons. The data from both seasons indicates that the application of putrescine at 10, 20, and 30 ppm and chitosan at 0.1%, 0.2%, and 0.3% significantly increased the diameter of the fruit, compared to the control treatment. Increasing the concentration of putrescine and chitosan led to gradual and significant increases in both the length and diameter of the fruit. The findings reveal that chitosan treatment is superior to putrescine treatment. Additionally, the data shows that the combined application of putrescine and chitosan significantly enhances fruit length and diameter compared to using each one alone. This was true for both experimental seasons. The trees treated with putrescine at 30 ppm combined with chitosan at 0.3% demonstrated the highest promotion in fruit length and diameter compared to the other treatments or control trees. On the other hand, untreated trees produced the lowest fruit length and diameter in both experimental seasons.

Average shape index: Table (4) displays the impact of spraying various concentrations of putrescine and chitosan on the fruit shape index of ‘Manfalouty’ pomegranate during the 2022 and 2023 seasons. Regardless of the compound sprayed or its concentration, the spraying of trees with putrescine or/and chitosan did not significantly enhance the fruit shape index The analysis of the data indicated that there were no significant differences between the treatments in either the first or second season. 2-4: fruit main portions %:

Peel thickness and peel %: During the 2022 and 2023 seasons, an experiment was conducted on Manfalouty pomegranate trees to study the effect of putrescine and chitosan, both individually and in combination, on the thickness and percentage of pomegranate fruit peel. The data obtained is presented in Table 5. The results showed that treating the trees with putrescine, chitosan, or both, significantly reduced the thickness and percentage of fruit peel compared to untreated trees. An increase in the concentration of each material led to a gradual decline in both characteristics. However, increasing the concentration of putrescine from 20 ppm to 30 ppm or chitosan from 0.2% to 0.3% had a negligible effect on reducing peel thickness and percentage. In combined applications of putrescine and chitosan, all treatments produced lower peel thickness and percentage than individual applications or untreated trees. The most effective treatment was the application of putrescine at 30 ppm combined with chitosan at 0.3%, which resulted in the lowest peel thickness (0.37 & 0.35 cm) and lowest peel percentage (36.8 & 35.5 %) during the two experimental seasons. In contrast, untreated trees produced the highest peel thickness (0.49 & 0.51 cm) and peel percentage (45.0 & 46.1 %) during the two seasons.

Table 3: Effect of spraying putrescine and chitosan on fruit cracked % and fruit sunburned % of Manfalouty Pomegranate, during 2022 and 2023.

Treatments	Fruit cracked (%)		Fruit sunburned (%)	
	2022	2023	2022	2023
Control	22.2	25.7	17.5	16.7
Putrescine 10 ppm	29.9	29.9	19.9	19.2
Putrescine 20 ppm	25.8	24.2	15.7	14.5
Putrescine 30 ppm	23.1	21.3	13.7	12.4
Chitosan 0.1%	27.3	25.6	16.1	15.9
Chitosan 0.2%	21.4	19.2	13.5	13.3
Chitosan 0.3%	19.1	17.6	12.7	12.8
Putrescine 10 ppm + Chitosan 0.1%	20.9	17.7	10.5	10.2
Putrescine 20 ppm + Chitosan 0.2%	13.2	12.2	8.4	8.3
Putrescine 30 ppm + Chitosan 0.3%	9.7	7.5	7.9	7.4
New LSD at 5%	2.7	3.5	1.1	1.2

Table (4): Effect of spraying putrescine and chitosan on fruit length, fruit diameter and shape index of Manfalouty Pomegranate, during 2022 and 2023.

Treatments	Fruit length (cm)		Fruit diameter (cm)		Shape index	
	2022	2023	2022	2023	2022	2023
Control	7.22	7.29	7.41	7.39	0.97	0.98
Putrescine 10 ppm	7.71	7.77	7.90	7.89	0.98	0.98
Putrescine 20 ppm	7.92	7.96	8.01	8.07	0.98	0.98
Putrescine 30 ppm	8.12	8.21	8.29	8.32	0.97	0.99
Chitosan 0.1%	7.88	7.93	8.10	8.18	0.86	0.97
Chitosan 0.2%	8.42	8.43	8.65	8.66	0.97	0.97
Chitosan 0.3%	8.45	8.57	8.69	8.76	0.97	0.98
Putrescine 10 ppm + Chitosan 0.1%	8.60	8.63	8.79	8.83	0.98	0.98
Putrescine 20 ppm + Chitosan 0.2%	8.79	8.83	9.66	9.84	0.91	0.89
Putrescine 30 ppm + Chitosan 0.3%	8.89	8.92	9.02	9.88	0.97	0.90
New LSD at 5%	0.25	0.26	0.23	0.25	NS	NS

Table 5: Effect of spraying putrescine and chitosan on peel thickness (cm), peel %, arils % and juice % of Manfalouty Pomegranate fruit, during 2022 and 2023.

Treatments	Peel thickness (cm)		Peel %		Arils %		Juice %	
	2022	2023	2022	2023	2022	2023	2022	2023
Control	0.49	0.51	45.0	46.1	55.0	53.9	29.5	29.7
Putrescine 10 ppm	0.47	0.46	43.2	42.1	56.8	57.9	30.9	31.3
Putrescine 20 ppm	0.43	0.43	41.5	41.1	58.5	59.9	32.2	32.9
Putrescine 30 ppm	0.41	0.40	40.2	40.0	59.8	60	33.4	33.8
Chitosan 0.1%	0.46	0.47	44.3	44.0	55.7	56.0	29.1	30.3
Chitosan 0.2%	0.44	0.43	42.0	41.7	58.0	58.3	32.2	32.7
Chitosan 0.3%	0.42	0.41	41.5	40.8	58.5	59.2	32.9	33.3
Putrescine 10 ppm + Chitosan 0.1%	0.39	0.39	39.9	40.1	61.1	59.9	31.7	32.2
Putrescine 20 ppm + Chitosan 0.2%	0.38	0.37	38.9	37.7	61.1	62.3	34.9	36.9
Putrescine 30 ppm + Chitosan 0.3%	0.37	0.35	36.8	35.5	63.2	64.5	36.6	37.1
New LSD at 5%	0.04	0.07	3.3	3.2	3.1	3.8	2.1	2.2

Arils % and Juice %: The data in Table 5 demonstrate that varying concentrations of putrescine and/or chitosan had a significant impact on the percentage of fruit arils and juice, in comparison to the control treatment. Increasing the concentration of both compounds had a gradual and positive effect on the percentages of arils and juice. Using both compounds together produced significantly better results than using either compound alone. These findings were consistent across two experimental seasons. The highest values were observed in trees treated with the highest concentrations of both compounds, as there were no significant differences between the two highest concentrations. In contrast, untreated trees had the lowest percentages of arils and juice. These findings were consistent across the two experimental seasons. The positive effect of putrescine and chitosan on improving the physical properties of 'Manfalouty' pomegranate could be explained by their beneficial effects on biosynthesis and translocation of carbohydrates, as well as improving mineral nutrient absorption, assimilation, nitrogen metabolism, and activating important enzymes. This observation was made in the present study and is supported by previous research conducted by Khorshidi and Hamedi (2014), Nechita (2017), and Philibert *et al.* (2017).

Fruit chemical properties: TSS% and Sugar contents: Data on the effects of putrescine and chitosan on Manfalouty pomegranate juice's total soluble solids and sugar content during the 2022 and 2023 seasons are presented in Table 6. Applying putrescine or chitosan alone led to a significant increase in T.S.S% and sugar contents (reducing, non-reducing, and total sugars %) in Manfalouty pomegranate fruit juice compared to the control trees during the two experimental seasons.

Table 5: Effect of spraying putrescine and chitosan on TSS% and sugars contents (%) of Manfalouty Pomegranate fruit, during 2022 and 2023.

Treatments	TSS (%)		Reducing sugars %		Non reducing sugars %		Total sugars %	
	2022	2023	2022	2023	2022	2023	2022	2023
Control	10.1	10.3	9.8	9.5	1.4	1.5	11.3	11.0
Putrescine 10 ppm	11.2	11.8	10.4	10.5	1.7	1.7	12.1	12.2
Putrescine 20 ppm	12.8	12.9	10.7	10.7	1.9	2.2	12.6	12.9
Putrescine 30 ppm	13.1	13.4	10.8	10.9	2.1	2.3	12.9	13.2
Chitosan 0.1%	11.0	11.3	10.1	10.7	1.6	1.7	11.7	12.4
Chitosan 0.2%	12.9	13.2	10.9	11.2	1.9	2.1	12.8	13.3
Chitosan 0.3%	13.3	13.7	11.2	11.5	2.0	2.2	12.9	13.5
Putrescine 10 ppm + Chitosan 0.1%	12.1	12.1	11.0	11.4	1.9	1.8	12.9	13.2
Putrescine 20 ppm + Chitosan 0.2%	13.9	14.2	11.9	12.1	2.1	2.3	14.0	14.4
Putrescine 30 ppm + Chitosan 0.3%	14.4	14.7	12.1	12.5	2.7	2.9	14.7	15.4
New LSD at 5%	0.7	0.6	0.5	0.5	0.7	0.6	0.8	0.7

This increase was proportional to the concentration of each material. It is worth noting that the combined application of putrescine and chitosan was more effective in enhancing the juice's TSS% and sugar content in both seasons. The analyzed data indicated that the maximum fruit total soluble solids and sugar contents were obtained from the trees treated with higher concentrations of putrescine and chitosan, while the minimum values were obtained from untreated trees. These results were consistent during both experimental seasons (2022 and 2023).

Total acidity % and anthocyanins contents: Data on the effect of putrescine, at concentrations of 10, 20 and 30 ppm, and chitosan, at concentrations of 0.1%, 0.2% and 0.3%, on the total acidity% and total soluble tannins% of ‘Manfalouty’ pomegranate fruits during the 2022 and 2023 seasons can be found in Table 6. The data shows that spraying putrescine or chitosan, either alone or in combination, resulted in a significant decrease in the total acidity% and an increase in the total anthocyanins (mg/100g F.W.) in both the peel and juice of ‘Manfalouty’ pomegranate fruits. This trend was observed during both experimental seasons and was consistent across all concentrations of putrescine and chitosan. The combined application of both materials was found to be more effective than using each material alone, regardless of the concentration used. In addition, the trees that received putrescine at 30 ppm combined with chitosan at a higher concentration (0.3%) produced the lowest total acidity % (2.69% & 2.51%) and the highest total anthocyanins in the peel and juice during both experimental seasons.

Table 6: Effect of spraying putrescine and chitosan on TSS% and sugars contents (%) of Manfalouty Pomegranate fruit, during 2022 and 2023.

Treatments	Total acidity (%)		Peel total anthocyanins (mg/100g F.W.)		Juice total anthocyanins (mg/100g F.W.)	
	2022	2023	2022	2023	2022	2023
Control	2.17	2.14	67	69	89	92
Putrescine 10 ppm	2.01	1.99	78	77	95	99
Putrescine 20 ppm	1.80	1.81	85	88	101	107
Putrescine 30 ppm	1.83	1.74	87	90	107	110
Chitosan 0.1%	1.69	1.65	77	79	99	103
Chitosan 0.2%	1.49	1.30	88	89	111	117
Chitosan 0.3%	1.43	1.35	91	94	115	119
Putrescine 10 ppm + Chitosan 0.1%	1.33	1.29	87	90	119	118
Putrescine 20 ppm + Chitosan 0.2%	1.29	1.10	98	99	122	127
Putrescine 30 ppm + Chitosan 0.3%	1.23	1.05	99	102	125	129
LSD at 5%	0.08	0.09	8	7	10	8

The positive impact of putrescine and chitosan on improving the chemical properties of Manfalouty pomegranate fruits has been previously noted by other authors who have conducted research on pomegranate and other fruit trees. Romanazzi *et al.* (2017), Mohamed *et al.* (2018), and Mohamed (2021) have all reported similar findings. In addition, some studies have shown that other fruit trees, such as those examined by Ghasemnezhad *et al.* (2010), Gayed *et al.* (2017), and Gonzalez-Hernandez et al. (2022), also benefited from the use of putrescine and chitosan. The beneficial impact of putrescine and chitosan on the chemical properties of Manfalouty pomegranate fruits can be explained by their higher levels of certain macro and micronutrients, their effect on some crucial enzyme bio-catalysis, and their ability to enhance the synthesis of some plant hormones (Romanazzi *et al.,* 2017 and Mohamed, 2021). Both putrescine and chitosan are considered as sources of biodegradable antioxidants. These compounds can enhance the chemical properties of fruits by increasing carbohydrate production and accumulation. Various assays that determine the antioxidant capacity and their principles have been noted by Hirano (1989), Crini *et al.* (2010), Jelali *et al.* (2014), and Gonzalez-Hernandez *et al.* (2022).

CONCLUSION

Spraying putrescine at 10, 20, and 30 ppm or chitosan at 0.1, 0.2, and 0.3% three times a year results in improving fruit production and physicochemical properties of 'Manfalouty' pomegranates. The improvement is gradual and parallel to the increase in concentration of each material. Using both putrescine and chitosan in combination is more effective in enhancing yield and fruit quality compared to using each material alone or no treatment at all. Additionally, the trees that received the highest concentration of both materials produced the highest yield and best fruit quality during the two experimental seasons.

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تأثير المعاملة بالبوتريسين والشيتوزان على جودة ثمار الرمان المنفلوطى

علاء محمد جمعة[1] و هبة فوزى سيد إبراهيم [2]

[1]فرع الفاكهة – قسم البساتين – كلية الزراعة بالأسماعيلية – جامعة قناة السويس

فرع الفاكهة - قسم البساتين - كلية الزراعة – جامعة المنيا [2]

المستخلص: هدفت الدراسة الحالية إلى اختبار إضافة البتروسين والشيتوزان رشاً على الأشجار (بتركيز 10 و20 و30 جزء في المليون) مع أو بدون رش الشيتوزان (بتركيز 0,1 و0,2 و0,3 ٪) ثلاثة مرات كل عام على المحصول ومواصفات الجودة للثمار سواء الفيزيائية أو الكيميائية لأشجار الرمان صنف المنفلوطي النامي تحت ظروف الأراضي الرملية الطمية بمحافظة المنيا .وقد أوضحت النتائج المتحصل عليها خلال هذه الدراسة أن رش البتروسين والشيتوزان قد أدى إلى تحسين إنتاجية أشجار الرمان المنفلوطي وأيضا إلى تحسين مواصفات الجودة الفيزيائية والكيميائية للثمار. وكان هذا التحسن تدريجيا ومتوازي مع زيادة التركيز المستخدم من كلا المركبين. في حين أن النتائج قد أكدت على أنه بغض النظر عن التركيز المستخدم من البتروسين والشيتوزان فإن الاستخدام المشترك لكلا المركبين (البتروسين والشيتوزان) كان أفضل من حيث تحسين الإنتاجية ومواصفات الجودة في الثمار وذلك بالمقارنة بالاستخدام الفردي لكلاهما أو بأشجار الكنترول. وعلاوة على ما سبق فإن الأشجار التي تم رشها بالتركيز الأعلى من كلا المركبين معا قد أعطت المحصول الأعلى وكذلك أفضل مواصفات لجودة الثمار خلال موسمي الدراسة. كما أكدت النتائج على انه لم تكن هناك فروق معنوية بين التركيزين الأعلى من كلا المركبين خلال موسمي الدراسة، سواء تم رش كلاهما بصورة فردية أو رشها معا بصورة مشتركة.