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Foliar Application of Gebbrillic and Salicylic Acids Improves Fruit Quality and Yield and Reduces Aphid Population in Pomegranate (*Punica granatum* L.)

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ABSTRACT

Pomegranate crop suffers many problems during tree growth and fruit production stages. The pomegranate ramp, *Aphis punicae* (Passerini) is serious pests that attack pomegranate in Egypt. This study was carried out to examine the influence of ascorbic acid (ASA), gibbrellic acid (GA_3) and salicylic acid (SA) on yield and fruit quality and aphid population in pomegranate. The application rates were (250, 500 and 1000 ppm) for ASA, 250, 500 and 1000 ppm for GA and 200, 400 and 600 ppm for SA thrice during the growing season. The foliar application of GA_3 and SA improved yield (kg/tree), fruit weight (g) , fruit number/tree, fruit cracking, TSS, acidity %, TSS/acid % ratio , and reduced aphid population.

Keywords: *Aphis punicae*, control, elicitors, fruit quality, pomegranate, yield

INTRODUCTION

Pomegranate (*Punica granatum* L.) is an important fruit species that has been widely cultivated in many parts of the world (Sarkhosh *et al*., 2007; Stover and Mercure, 2007). Recently, pomegranate has drawn attention as a commercial crop because of the benefits of human nutrition provided by these fruits and their derived products associated with high levels of antioxidants in pulp or juice (Davidson *et al.*, 2009). However, pomegranate production suffers many problems during tree growth and fruit production. The most common problems during maturity are the low fruit quality and cracking. In addition, the pomegranate ramp, *Aphis punicae* (Passerini) (Homoptera: Aphididae) is serious pests that attack pomegranate orchards around the world, including Egypt. It is well known for its ability to reduce plant activity, facilitate the growth of mold on leaves, and reduce the quality and quantity of fruit.

A common practice used in enhancing fruit quality and controlling pest in pomegranate is the application of traditional chemical substances which may have harmful effects and serious consequences on human, animals and environment. The alternative approach to improve the quality of pomegranate fruit and to control pomegranate pests effectively to respond to problems associated with the indiscriminate use of chemical substances has become an urgent necessity. Elicitors such as ascorbic acid (ASA), gibberelic acid (GA_3) and salicylic acid (SA) are signalling molecules that play an important role in regulating plant growth and improving plant strength under biotic and abiotic stresses (Hayat *et al.*, 2010 and War *et al.*, 2012).

They can play an essential role in controlling the quality of fruits such as colour, flavour, acidity, size and weight (Kondo, 2006; Elwan and El-Hamahmy, 2009; Marzouk and Kassem, 2011). Moreover, aphid feeding may trigger plant signalling pathways driven by salicylic acid (SA), ascorbic acid (ASA) or gibberelic acid (GA_3), that induce the production of chemical defences (Smith and Boyko, 2007; Goggin, 2007 and Walling, 2008).

These natural elicitors, however, may be produced in plant in low quantities that might not help the plant to overcome the biotic and abiotic stress. Thus, the exogenous applications of these substances enhance plant performance. But no previous study has been conducted on the double effect of these elicitors on improving the fruit quality and reducing the incidence of the aphids on pomegranate orchards. Therefore, the objective of the current study is to investigate the influence of ascorbic acid (ASA), gibberelic acid (GA_3) and salicylic acid (SA) use to improve fruits quality of pomegranate and reduce aphid population in the pomegranate tree.

MATERIALS AND METHODS

Field experimental

The experiment was performed on 19-year-old pomegranate trees (*Punica granatum*L.), cultivar Manfaloty planted in 5X5 meter in sandy soil in the Experimental Farm at Sohag University (El-Kawther, Sohag, Egypt), during 2017 and 2018 seasons. The experiment was laid out in a randomized complete block design (RCBD) with three replications per treatment. Three levels of ascorbic acid (ASA), gibberelic acid (GA_3) and salicylic acid (SA) were used. Knapsack hand spray (20 L capacity) fitted with one nozzle was used for spraying the treatments.

Ascorbic acid (ASA), gibberelic acid (GA_3) and salicylic acid (SA) application

Three rates of each substance were applied thrice in the third week of April, the first week of May and the first week of June. The application rates were 250, 500 and 1000 ppm) for ASA 250, 500 and 1000 ppm for GA_3 and 200, 400 and 600 ppm for SA. Control sprayed with distilled water. Tested trees were received equal amounts of applied solutions. All chemicals were purchased from Sigma-Aldrich Co., Egypt.

Insect counting

The aphid populations were counted from five terminal branches, including leaves, flowers, and fruits before and after foliar application at periods of 1, 3, 6, 9 and 12 days after first spray only due to the high population levels of the aphid. The reduction percentages of aphids were calculated according to Henderson and Tilton's equation (Henderson and Tilton, 1955).

Statistical analysis

Data were analysed using one-way ANOVA and presented as mean of values. Means were compared by New LSD Test at $P < 0.05$ level of probability.

RESULTS AND DISCUSSION

RESULTS

In general, the application of ASA, GA_3 and SA improved plant growth and fruit quality in pomegranates and reduced aphid population. GA_3 surpassed ASA and SA in tested yield and fruit quality traits. There were

significant differences among all treatments, but the lowest rates of ASA did not differ significantly from control.

1–Yield (kg/tree), fruit weight (g) and fruit number/tree:

Data in Table (1) clearly show that subjecting the trees to GA_3 significantly improved yield expressed in terms of fruit weight and number per tree relative to the control. There was a gradual increase in fruit yield, fruit weight and fruit number/tree with increasing concentrations of each material. The maximum yield (58.90, 52.67 kg/tree) was recorded in trees that sprayed with GA_3 at 1000 ppm, while, the lowest yield (41.00, 36.67 kg/tree) was recorded in the untreated trees in both seasons. Similarly, GA_3 at 1000 ppm gave the highest values for fruit weight and fruit number /tree in both seasons. Control, on the other hand gave the lowest values in both seasons.

Table 1. Effect of spraying application with ascorbic acid (ASA), gibberelic acid (GA_3) and salicylic acid (SA) on yield (kg/tree), fruit number/tree and fruit weight of Manfalouty pomegranate trees during 2016/2017 season.

Treatments	Conc. (ppm)	yield (kg/tree)		fruit number/tree		fruit weight	
		2016	2017	2016	2017	2016	2017
Control	-	41.00	36.67	121.67	120.67	298.78	296.20
Ascorbic acid	250	41.33	38.00	131.33	126.67	302.88	298.78
Ascorbic acid	500	47.33	38.00	144.67	134.00	308.35	302.89
Ascorbic acid	1000	43.83	42.00	145.67	141.67	326.55	308.35
Gibberelic acid	250	52.50	46.56	157.67	149.00	366.00	342.49
Gibberelic acid	500	58.66	49.80	161.67	158.67	378.00	366.05
Gibberelic acid	1000	58.90	52.67	165.55	177.67	381.00	372.80
Salicylic acid	200	47.33	41.13	145.30	136.67	340.49	313.23
Salicylic acid	400	50.03	48.67	143.33	147.33	342.45	317.13
Salicylic acid	600	50.00	44.67	155.00	151.33	382.33	366.35
New LSD at 5%		5.12	4.93	17.72	17.17	35.11	40.2

2- Fruit cracking %:

Data illustrated in Table (2) clearly show that treating the trees with GA_3, ascorbic acid and salicylic acid significantly reduced fruit cracking percentage compared to control. There were significant differences among all the treatments. The application of GA_3 at 1000 ppm resulted in the lowest cracked fruits (1.23 and 1.27 %), meanwhile control resulted in the highest cracked fruits (3.23, 3.27). in both seasons, respectively.

Table 2. Effect of spraying application with GA_3, Ascorbic acid and Salicylic acid on grain weight %, Fruit cracking % and rind weight % of Manfalouty pomegranate during 2005 season.

Treatments	Conc. (ppm)	grain weight (%)		Fruit cracking (%)		rind weight (%)	
		2016	2017	2016	2017	2016	2017
Control	-	52.6	49.47	3.23	3.27	39.16	40.20
Ascorbic acid	250	53.33	51.73	2.43	2.43	42.10	41.33
Ascorbic acid	500	53.67	54.73	3.00	3.07	42.10	41.66
Ascorbic acid	1000	56.60	55.27	2.36	2.23	42.76	44.73
Gibberelic acid	250	56.85	56.33	1.70	1.50	43.46	45.26
Gibberelic acid	500	57.10	57.57	1.30	1.33	45.23	50.53
Gibberelic acid	1000	58.90	58.70	1.23	1.27	46.66	57.76
Salicylic acid	200	56.53	54.80	2.36	2.23	42.20	43.6
Salicylic acid	400	56.07	56.04	2.21	2.17	42.93	43.93
Salicylic acid	600	56.90	56.4	2.05	2.10	46.33	48.26
New LSD at 5%		2.91	3.34	0.84	0.71	2.83	3.21

3- Fruit quality:

Data in Table (1, 2 and 3) indicate that treating the trees with GA_3, ascorbic acid and salicylic acid significantly improved both physical and chemical characteristics of the fruit. The highest values in grain weight %, rind weight, T.S.S, and T.S.S/acid ratio, and the lowest values in acidity % were recorded in application of the highest rate of GA_3. The opposite was true for values obtained from control in both seasons.

Table 3. Effect of spraying application with GA_3, ascorbic acid and salicylic acid on chemical fruit properties of Manfalouty pomegranate during 2005 season.

Treatments	Conc. (ppm)	TSS		Acidity %		TSS/Acid ratio	
		2016	2017	2016	2017	2016	2017
Control	-	12.63	13.33	1.63	1.30	7.87	10.40
Ascorbic acid	250	13.60	13.93	1.33	1.29	10.22	10.79
Ascorbic acid	500	13.75	14.00	1.28	1.26	10.74	11.11
Ascorbic acid	1000	13.55	14.03	1.22	1.17	11.10	11.90
Gibberelic acid	250	14.33	14.17	1.27	1.13	11.28	12.53
Gibberelic acid	500	15.66	14.37	1.17	1.09	13.38	13.18
Gibberelic acid	1000	15.84	14.53	1.11	1.04	14.27	13.97
Salicylic acid	200	13.66	13.97	1.32	1.24	10.34	11.26
Salicylic acid	400	13.81	14.10	1.28	1.18	10.78	11.94
Salicylic acid	600	13.76	14.27	1.21	1. 04	11.37	13.72
New LSD at 5%		0.45	0.38	0.08	0.07	1.63	1.57

Efficiency of foliar applications of elicitors on pomegranate aphid, *A. punicae*

Foliar application of ASA, GA_3 and SA on the pomegranate aphid under field conditions resulted in significant reduction of aphid populations (Figure 1 and 2).

Reduction Percentage of aphids was increased as the concentrations of ASA, GA_3 and SA increased (Figures 1 and 2) after treatment at periods of 3, 6, 9 and 12 days, but no significant difference was recorded among them after one day of treatment in both seasons. Foliar spray of 600 ppm SA gave the higher average reduction efficiency in population of pomegranate aphid compared to other elicitors in the both seasons. On the other hand, the ASA treatments had lower effect on reduction of aphid population. Our result showed that no difference between the 200 and 400 ppm SA and 1000 ppm GA_3 treatments in both seasons. SA induced with 600 ppm a maximum reduction in aphid population, 75.21% (F= 24.87, *P*= <.0001) and 78.97 % (F= 12.76, *P*= <.0001) at 9 day after spraying in first and second season, respectively. Spray of 4 mM SA caused average reduction of aphid population of about 52.25 % (F= 21.35, *P*= <.0001) and 56.80 % (F= 24.10, *P*= <.0001) in first and second seasons, respectively. The results clearly exhibit the effectiveness of the elicitors against pomegranate aphids.

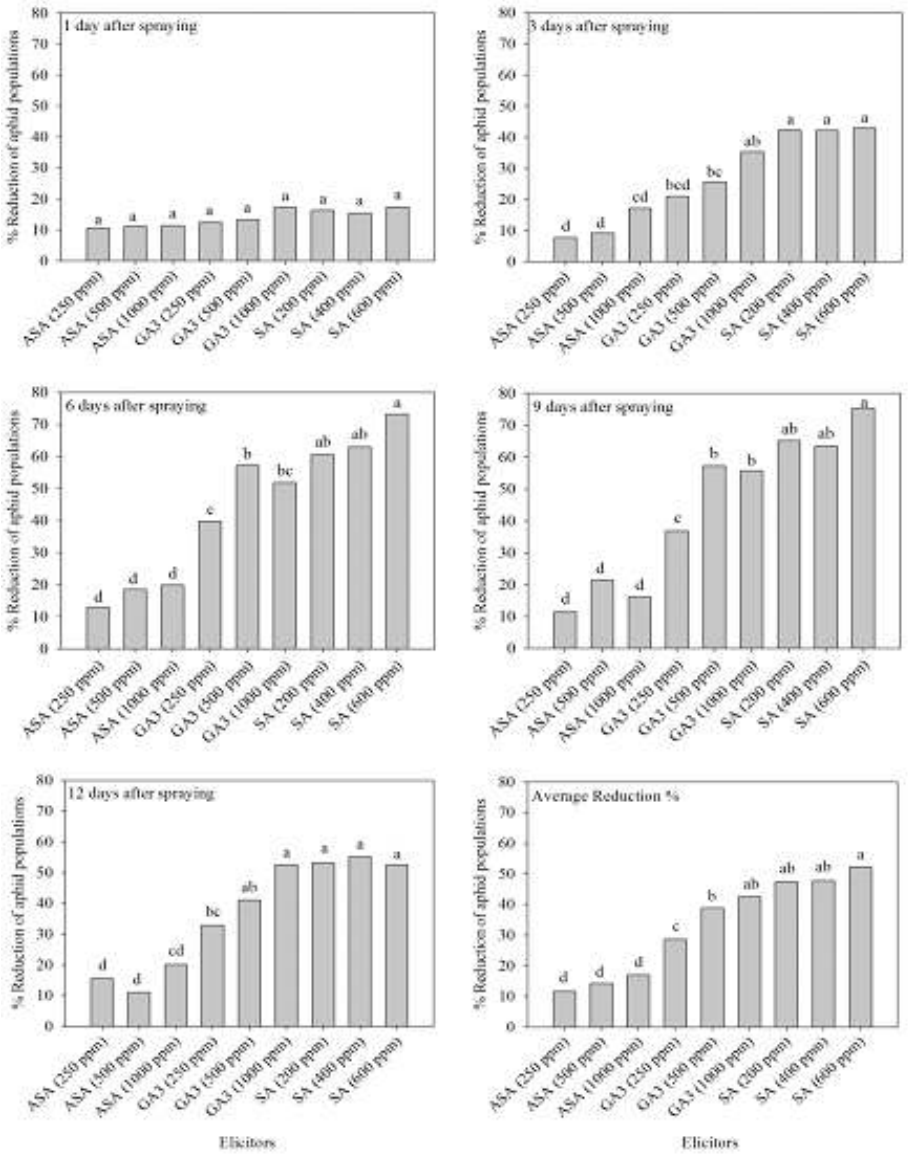

Fig. 1.Efficacy of foliar applications of elicitors on pomegranate aphid, *A. punicae* under field condition at 1, 3, 6, 9 and 12 day after treatments in 2017 season. ASA: Ascorbic acid, GA_3: Giberellic acid, SA: Salicylic acid. Columns headed by different letter(s), within the same treatment date, are significantly different ($P \leq 0.05$) according to New LSD Test.

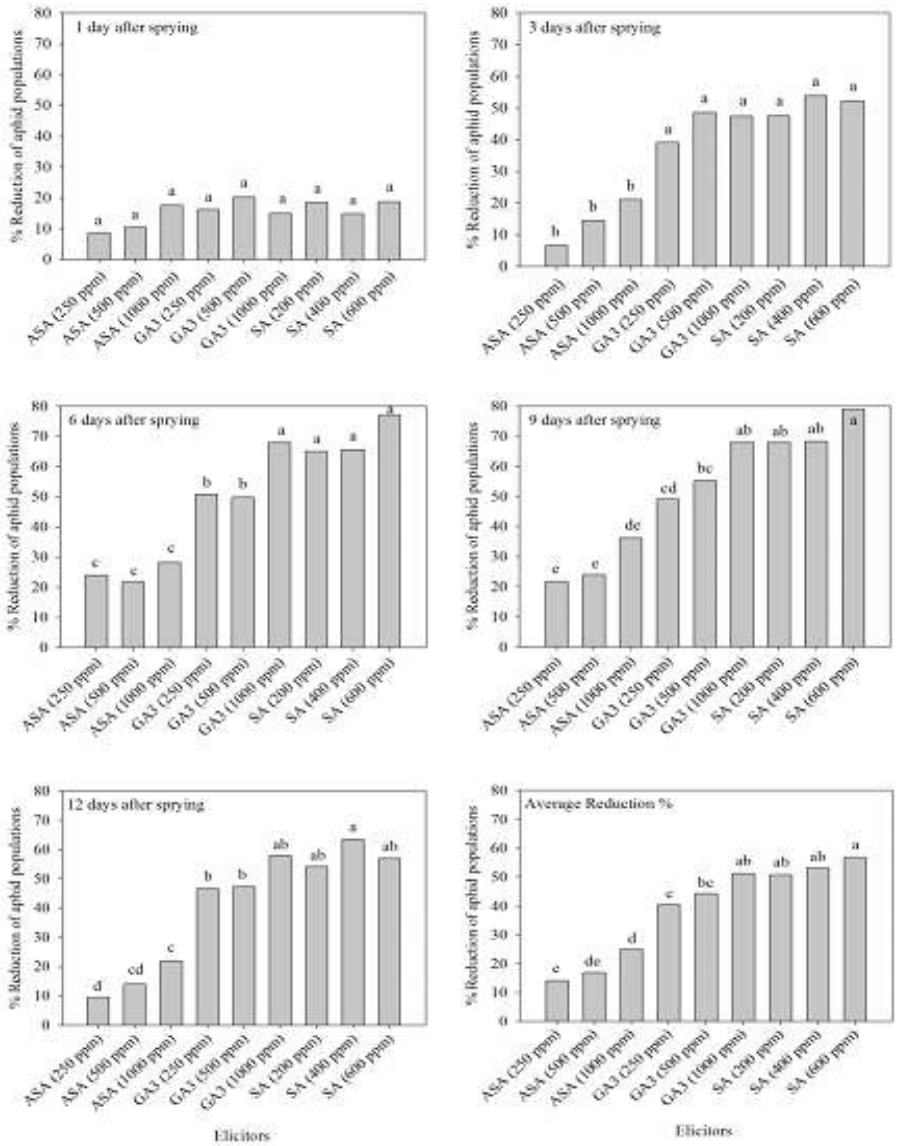

Fig. 2.Efficacy of foliar applications of elicitors on pomegranate aphid, *A. punicae* under field condition at 1, 3, 6, 9 and 12 day after treatments in 2018 season. ASA: Ascorbic acid, GA_3: Giberellic acid, SA: Salicylic acid.Columns headed by different letter(s), within the same treatment date, are significantly different ($P \leq 0.05$) according to New LSD Test.

Discussion

The results from this study clearly showed that tested elicitors affected growth of pomegranate trees, and subsequently influenced the performance of the pomegranate aphids. Our data showed that foliar application of ASA, GA_3 and SA on pomegranate trees significantly improved plant growth, quantity and quality of fruits.

When phytohormones are applied, the plant undergoes changes in carbohydrate and amino acids in leaves (Khodary 2004; Liang *et al.* 2006; Chandra *et al.* 2007; Tytgat *et al.* 2013), but less is known about phytohormone induced changes in the phloem sap composition. Spraying gebrillic acid enhanced total yield, TSS and reduced cracking fruits and acidity (Sharifi and Sepahi 1984, Ghosh *et al.* 2009, Yılmaz and Özgüven 2009, Shanmugasundaram, and Balakrishnamurthy 2017).

In pomegranates, application of SA enhanced total and marketable yield, fruit weight and diameter, TSS, and reduced acidity and fruit cracking (Abdel Aziz *et al*. 2017). In pomegranates, application of SA enhanced total and marketable yield, fruit weight and diameter, TSS, and reduced acidity and fruit cracking (Abdel Aziz *et al* 2017).

Application of salicylic acid, also, increased total yield, number of fruit/plant, fruit weight and TSS and decreased titratable acidity in Mango (Ngullie *et al*. 2014), grape (El-kenawy 2017) and strawberry (Mohamed *et al*. 2017; Qureshi *et al*. 2013). Application of ASA increased total and marketable yield, fruit weight and diameter, TSS, and reduced acidity and fruit cracking. Application of salicylic acid, also, increased total yield, number of fruit/plant, fruit weight and TSS and decreased acidity in orange (El-Badawy *et al.* 2017).

Pomegranate growth and development depends largely as any other plant on phyto-hormones, which

control metabolic processes and tolerance to biotic and abiotic stresses. These hormones play a vital role in the plant signalling in the processes of biosynthesis of metabolites that contribute to vegetative and reproduction stages and the plant response to biotic and abiotic stress.

Gibberellic acid is a plant hormone that stimulates plant growth, transitions from meristem to shoot growth and vegetative to flowering, and promotes grain development and cells elongation (Gupta and Chakrabarty 2013). GA_3 works in concert with auxins. Meanwhile, auxins increases the plasticity of cell wall, GA_3 elongates the cells. Thus, cell could be enlarged and gain weight. Salicylic acid as growth hormone influences seed germination, seedling establishment, cell growth, photosynthesis, heat tolerance, nutrient uptake and fruit yield. The effect of SA on some of these biological processes may be indirect because SA manipulates the effect of other plant hormones (Yusuf *et al* 2013). The application of SA increases fruit weight, total sugars and these observations could be attributed to the increase of photosynthesis pigments, transporting sugars and root length in plants (Hayat *et al.* 2005, Ahmed *et al.* 2013 and Larqué and Martin (2007).

Ascorbic acid is one of the major components of antioxidation system during environmental stress. ASA plays role in photo protection of photosynthesis apparatus and cell division and expansion (Smirnoff 1996).

Our results showed that the aphid populations were lower in the treated trees than in the untreated trees with SA is the most effective treatment in aphid population reduction. These observations may be attributed to elicitors' application that deterred aphids from pomegranate and increased foraging by parasitoids and predators attacking herbivorous insects of pomegranate tree. Application of SA induces systemic defense and significantly decrease aphid population in wheat (Mahmoud and Mahfouz 2015) and in tomatoes (Goggin, 2005). In pecan, the foliar application of GA_3 mitigated the injury occurred by black aphid (Hemiptera: Aphididae) (Cottrell *et al.* 2010).

Elicitors such as GA_3 and SA are important signals in the plant response against insect-herbivore, including the production of proteinase inhibitors, toxic secondary metabolites, and the production and release of volatile organic compounds (VOC). They regulate induced defense mechanisms in plants after insect attack and wounding. Most of the salicylate induced proteins (JIPs) (Vandenborre *et al.* 2009) have direct defense function against insects. SA has been reported to induce defense enzymes (War *et al.* 2011a; Noreen and Ashraf, 2009). Significant increase in polyphenol oxidase (PPO) activity in wheat was observed with application of SA (Pokhare *et al.* 2012). Exogenous application of SA at 1.5 mM induced higher (PPO) activity in chickpea (*Cicer arietinum* L.) leaves (War *et al.* 2011b). Pathogenesis-related (PR) proteins are involved in plant defense against insects and multiple signaling pathways that SA regulate their induction (War *et al*. 2012).

In conclusion, SA and GA_3 were effective in reducing of aphid population and improving plant growth and fruit quality in pomegranate. Further investigation is needed to elucidate the mechanism underlay these effects.

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استخدام الرش الورقى بأحماض الجبريلك والسالسيليك لتحسين جودة الثمار وتقليل تعداد المنّ على أشجار الرمان (Punicagranatum L.)

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[1] قسم البساتين ، كلية الزراعة ، جامعة سوهاج ، سوهاج ، مصر

[2] قسم وقاية النبات ، كلية الزراعة ، جامعة سوهاج ، سوهاج ، مصر

يعاني محصول الرمان العديد من المشاكل خلال مرحلتي نمو الأشجار وإنتاج الثمار. وتعد حشرة منّ الرمان من الآفات الخطيرة التي تهاجم الرمان في مصر. أجريت هذه التجربة لدراسة تأثير الرش بحامض الاسكوربيك (ASA) ، وحمض الجبريلك (GA3) وحمض الساليسيليك (SA) على المحصول وجودة الثمار وتعداد المنّ في الرمان. تم الرش ثلاث مرات خلال موسم النمو بمستويات (250، 500 و 1000 جزء في المليون) لـ ASA ، (250، 500 و 1000 جزء في المليون) لـ GA و (200 ، 400 و 600 جزء في المليون) لـSA على التوالي. وأوضحت نتائج الدراسة أن الرش الورقي بحمض الجبريليك (GA3) وحمض الساليسيليك (SA) أدى الي تحسن كمية المحصول (كجم / شجرة) ، وزن الثمار (جم)، عدد الثمار / شجرة ، تشقق الثمار ، نسبة المواد الصلبة الكلية، نسبة الحموضة ، نسبة المواد الصلبة الكلية/ نسبة الحموضة ، بالاضافة الي تقليل تعداد حشرة المنّ.

مفاتيح البحث :الرمان ، المحفزات ، جودة الثمار،حشرة المنّ ، المكافحة.