

Effect of Salicylic Acid on Enhancing Growth, Some Nutrients Concentration and Carbohydrates Metabolism on Pomegranate Seedlings Infected with *Meloidogyne javanica*

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Abstract

The effect of salicylic acid (SA) on pomegranate seedlings (Manfalouty cultivar) infected with root-knot nematode, *Meloidogyne javanica* was studied in this research. Salicylic acid was applied with different concentrations (0,10 and 40 mM) on foliar and soil drenching. Some traits and characteristics were determined, i.e. shoot and root lengths (cm), shoot and root fresh and dry biomass (g), nematodes reproduction, potassium and magnesium mg/g of both shoot and root, total chlorophyll content and total carbohydrates content (g/100g). Results showed that treated seedlings with SA had a healthier appearance under nematode infection, compare to untreated ones. Moreover, SA significantly enhanced most of vegetative growth parameters, and increased the potassium and magnesium uptake as well as their translocation from root to shoot, and increased the total carbohydrates content. Results also reveal that, all treatments either spraying or drenching significantly reduced all related nematode parameters i.e. number of galls/g root, number of egg masses/g root, as well as number of juveniles/100 g soil (after 45 or 90 days) compared to untreated plants.

Keywords: *Pomegranate, Root-knot nematodes, SPAD-502, Nutrients translocation.*

Introduction

In Egypt, Pomegranate is one of the most important fruit trees cultivated in warm regions such as Assiut province (375 km south of Cairo) where the climate is characterized by long hot summer and low air humidity. Such weather is ideal for the growth and fruiting of this crop. The most important cultivar is Manfalouty, which characterized by a good acidic taste and attractive color. Many investigators found that pomegranates have great beneficial effects on various human diseases (Lansky and Newman, 2007 and Hayrapetyan *et al.*, 2012).

Pomegranate is attacked by several pathogenic organisms i.e. fungi, bacteria, virus, plant-parasitic nematodes as well as some insects causing serious losses. Nematodes are arguably the most numerous animals on earth (Blaxter and Koutsovoulos, 2014). Root-knot nematodes (RKNs), *Meloidogyne* spp., are significant agricultural pests worldwide that cause acute injury to many cultivated plant species. By invading the plant and feeding on its tissues, they cause mechanical damage and physiological changes by injecting toxins (Cadet and Spaul, 2005). The symptoms of nematode attack vary according to the

species, but it usually takes a form of galls produced by *Meloidogyne* spp. However, there are other showed symptoms, such as very poor root systems, peeling or tearing of the cortex and tuber necrosis (Ferraz *et al.*, 2010). These symptoms may be confused for abiotic problems or outbreaks of other pathogens (Agrios, 2005). The main symptoms on vegetative parts are a reduction in plant growth due to lack of nutrients, wilting during the hottest times of day and a fall in crop yield, and that is mainly because of the root damage that lowers its capacity to absorb and translocate water and nutrients (Decraemer and Hunt, 2006).

Plant root is a very specialized organ, which has considerable functions; the most effective and well-studied function is to conquest water and minerals from the soil. Consequently, the root has improved specialized functions to react to numerous biotic and abiotic stresses to maintain the supply of nutrients and water under all conditions. Under root-knot nematodes infection, mineral lack is a mutual symptom in all attacked plants. Histological investigations have shown a disruption of the xylem in *Meloidogyne* galls as well as the presence of abnormally formed xylem vessels causing the interruption of water transport (Kirkpatrick *et al.*, 1991) and consequently the translocation of absorbed nutrients to the shoot.

As chemical nematicides generally cause environmental pollution (Landau and Tucker, 1984), beside it contaminate underlying soils and groundwater (Loria *et al.*, 1986), and also leave undesirable residues in the

parts of plants that is edible (Iue *et al.*, 1984). Control strategies are today directed towards the use of natural products. Salicylic acid is known as a significant effect on several biological aspects in plants, it can influence in a variable way; inhibiting some processes and promoting others (Raskin, 1992). It has been discovered that SA is essential in the signal transduction for inducing systemic acquired resistance (SAR) against some pathogenic infections (Gaffney *et al.*, 1993 and Vernooij *et al.*, 1994).

More recently, Molinari *et al.*, (2014) found that the repression of host defense SA signaling is associated with the successful development of RKNs, and that SA exogenously added as a soil drench is able to trigger a SAR-like response to RKNs in tomato.

Thus the objectives of this study were to: 1) Determine some physiological responses of pomegranate seedlings infected with root-knot nematode. 2) Investigate the effect of using SA, as an elicitor of resistance in pomegranate seedlings on some vegetative and physiological parameters attacked by RKNs.

Materials and Methods

The host plant and nematode:

This experiment was conducted at the greenhouse of the Plant Pathology Department, Faculty of Agriculture, Assiut University. One-year old seedlings of Manfalouty pomegranate cultivar (*Punica granatum* L.), were grown in 30 cm clay pots filled with sterilized sandy clay soil (1:1). After one month the seedlings were inoculated with 2000 newly hatched juveniles/plant of *Meloidogyne javanica*.

Salicylic acid treatments:

Salicylic acid (SA; 2-hydroxybenzoic acid) was initially dissolved in 10 ml ethanol alcoholic and completed with distilled water containing 0.02 % Tween 20.

After two weeks of inoculation the infected plants were treated with different concentrations of SA (0, 10 and 40 mM) by two ways: 1) Foliar application in such a way that the aerial parts of the seedlings were fully wet with different repeated times (once and twice), and 2) Drenching to soil. Each treatment was replicated three times.

Shoot and root fresh weight (FW) and length (cm) were determined directly after harvest of plants (after 45 and 90-day of SA treatment). The dry weight (DW) was obtained after drying the plant tissues for 48 h at 72 °C. Number of galls/1 g roots, number of J2/100 g soil and number of egg messes/1 g roots was determined at 45 and 90-day.

Chemical Parameters:

• Total chlorophyll:

Leaves were collected from the mid-section of branches of seedlings, in order to minimize leaf age variability effects. Chlorophyll was measured with SPAD-502 meter equipment (Minolta Camera Co. Ltd., 1989). Chlorophyll is measured as SPAD unit.

• Potassium content:

The shoots and roots were oven-dried at 75°C for 22 h and 110°C for 2 h and ground. Samples of 0.2 g were digested according to **Jackson (1973)** and analyzed by flame photometry (Digital Flame Analyzer, Cole Parmer, Illinois, USA) for potassium content. The values of K were calcu-

lated as mg/g DW.

• Magnesium content:

Magnesium (Mg) content in the shoots and the roots were determined simultaneously by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) (Fassel and Kniseley, 1974; Dahlquist and Knoll, 1978). The values of Mg were calculated as mg/g DW.

• Total soluble carbohydrate content:

Carbohydrate content was determined in the aqueous solution with anthrone sulfuric acid reagent according to Fales (1951) and Schlegel (1956) using glucose as a standard. Soluble and total carbohydrate contents were finally calculated as g/100g DW.

Statistical analysis:

The layout of experimental treatment was in a randomized complete block design (RCBD) with a factorial setup containing 3 replicates. Means \pm standard errors (SE) were compared using the least significant differences (LSD) at 5% level of probability according to Gomez and Gomez (1984).

Results and Discussion

An understanding of the correlation between nematode and the host, as reflected by the host's physiological processes and the visible impact of the host-parasite system on crop yield requires concurrent measurement of several morphological and physiological parameters over a range of nematode inoculation.

Seedlings growth rate:

Data in Fig. 1 show the growth rate during 45 and 90-day of growing under nematode infection. SA application, generally increased seedlings

growth (shoots and roots length). The foliar application with 10 mM SA for once or twice showed the highest value of shoot length (111.7 and 108.6 cm, respectively) at 90-day of inoculation, compared to 0 mM SA sprayed nematode infected plants (82.3 cm). These findings are consistent with Nandi *et al.*, (2000) who found similar increasing in plant growth in terms of shoot and root length of tomato plants in response to SA, compared to untreated plants. Also, Molinari (2001a) and Molinari (2002) reported that foliar application of SA onto tomato leaves could induce some resistance to *Meloidogyne incognita* and improve plant growth. It can be easily distinguished that plants inoculated with nematode had an increase in root length at 45 and 90-day, in compare with non-inoculated ones. This increase could be attributed to the nematode promotion to root growth by raising the concentration of plant hormones Hutangura *et al.*, (1999). The treatment with 10 mM SA as spraying once showed the highest values of root length under nematode infection (56.6 cm), followed by spraying with 40 mM for twice (54.6 cm) at 90 days of the experiment, compared to 0 mM sprayed infected-plants once and

twice (47.4, 48.8 cm, respectively).

The seedlings relative growth rate, which is uttered as shoot/root ratio, was almost stable at 45 and 90-day in all treatments. The control plants at 45-day had the highest ratio; the root was smaller than shoot because the priority of growth was for shoot at the initial date of planting. Then this ratio decreased at 90-day, which the root growth increased. The nematode-inoculated seedlings had a stable ratio as a result of limitation in the vegetative growth due to the root growth under infection or the reduction of nutrient supplementary from roots. These findings are similar to Sharp and Davies (1979) who recorded that the increasing in roots to shoots ratio could be due to stress-stimulated reduction in the growth of shoots relatives to roots, or from a stress-stimulated increase in the growth of roots. According to Brouwer (1962), the balance between carbohydrates and nitrogen plays a key role in marking the volume of above and below ground growth. Also, Ericsson (1995) reported that the decrease in the shoot: root ratio as a result to a reduction in availability of nutrients is typical for all plant species and cultural methods.

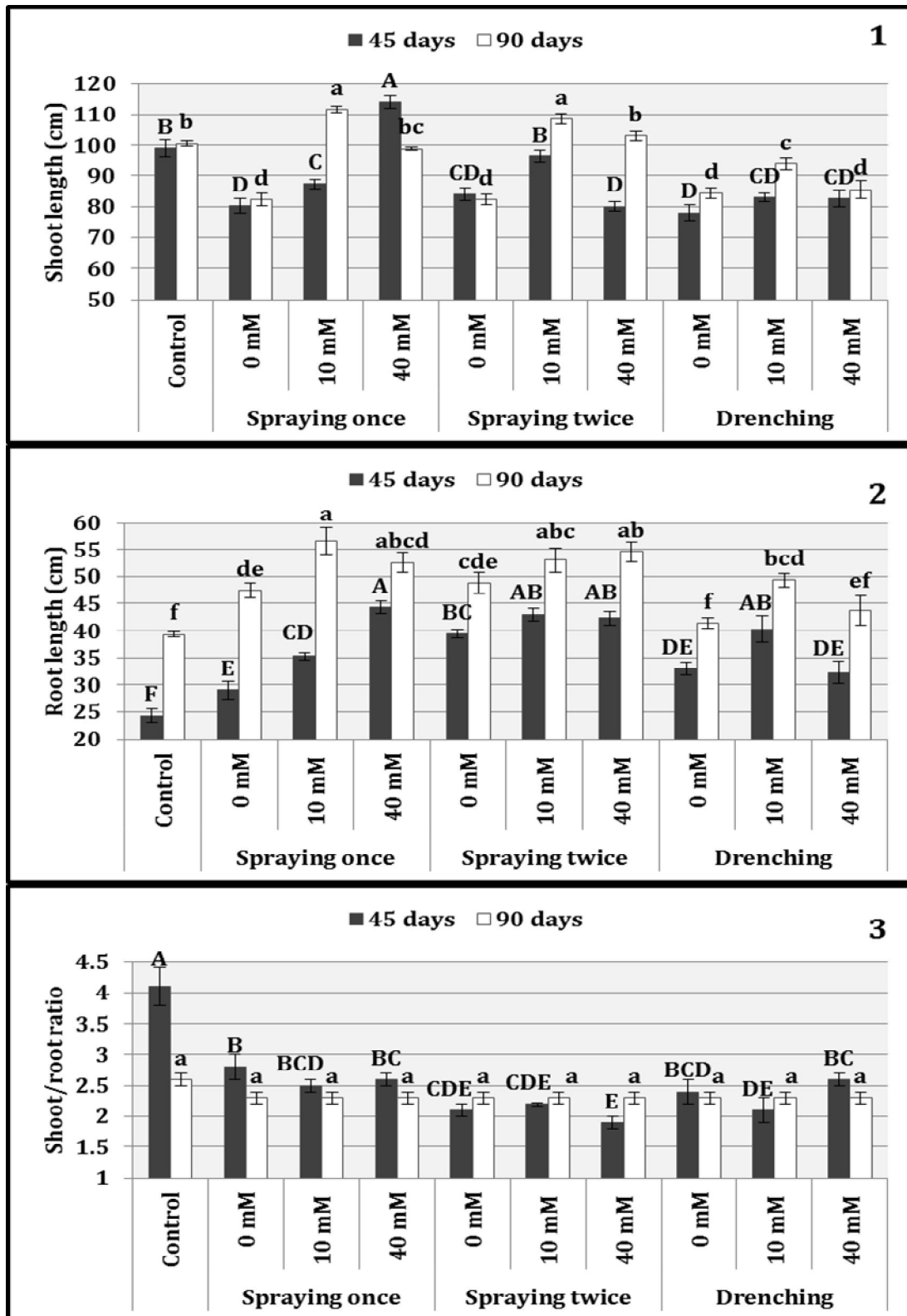


Fig. 1: Effect of SA application on 1) shoot length (cm), 2) root length (cm) and 3) shoot/root ratio of the pomegranate seedlings after 45 and 90-day of nematodes inoculation. Means \pm SE expressed with bars with lines (n=3). Bars with different letters are significantly different at $P \leq 0.05$ (Capital letters for 45-day and small letters for 90-day).

Seedlings fresh and dry bio-masses are presented in Fig. 2 and Fig. 3. The presence of nematode, in the culture medium, reduced shoot growth of seedlings than the non-inoculated ones. In contrary, the nematode-infected roots had the same weight as the non-inoculated plants at 45-day or even higher at 90-day. These results are agreement with the previous studies, which recorded that the reduction in the shoot mass seems to be very closely related to the growing of root, and might be a result of the strong carbon sink represented by gall formation and the emission of

new secondary roots (Oteifa and El-gindi, 1962; Wallace, 1971; Fatemy and Evans, 1986). Also, Carneiro *et al.*, (2002) reported that there is a trend toward a reduction of shoot weight due to an increase of root weight. It is obvious that the plants which showed the best nematode reproduction had the highest increase of the root mass. No doubt that the root galls gave a share in this. In this respect, Hutangura *et al.*, (1999) suggested that nematodes increased the plant hormones and that leads to more root formation, subsequently, higher mass.

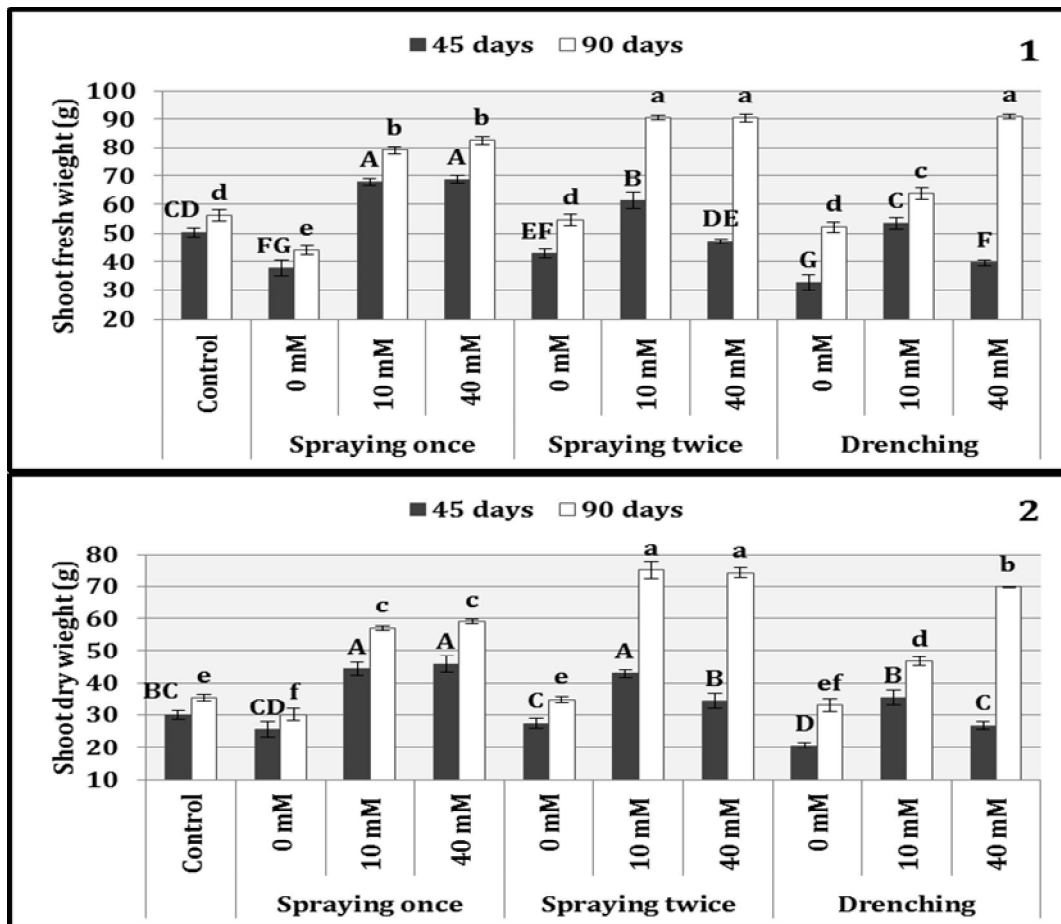


Fig. 2: Effect of SA application on 1) shoot fresh weight and 2) shoot dry weight of the pomegranate seedlings after 45 and 90 days of nematodes inoculation. Means \pm SE expressed with bars with lines. (n=3). Bars with different letters are significantly different at $P \leq 0.05$ (Capital letters for 45 days and small letters for 90 days).

On the other hand, plants sprayed once with 10 and 40 mM SA gave higher shoot fresh weight at 45 days (67.9 and 68.7 g, respectively) than nematode-infected plants only (37.8 g). While at 90 days the plants sprayed twice with 10 and 40 mM gave the highest values (90.3 and 90.4 g, respectively), and these values were equal to the plants drenched

with 40 mM SA (90.8 g) at 90 days of inoculation, in compare with infected-plants sprayed or drenched with 0 mM (54.6 and 52.1 g, respectively). The same result was observed with the shoot dry weight. Increase in shoot weight and shoot length would indicate an improvement of growth of SA treated plants.

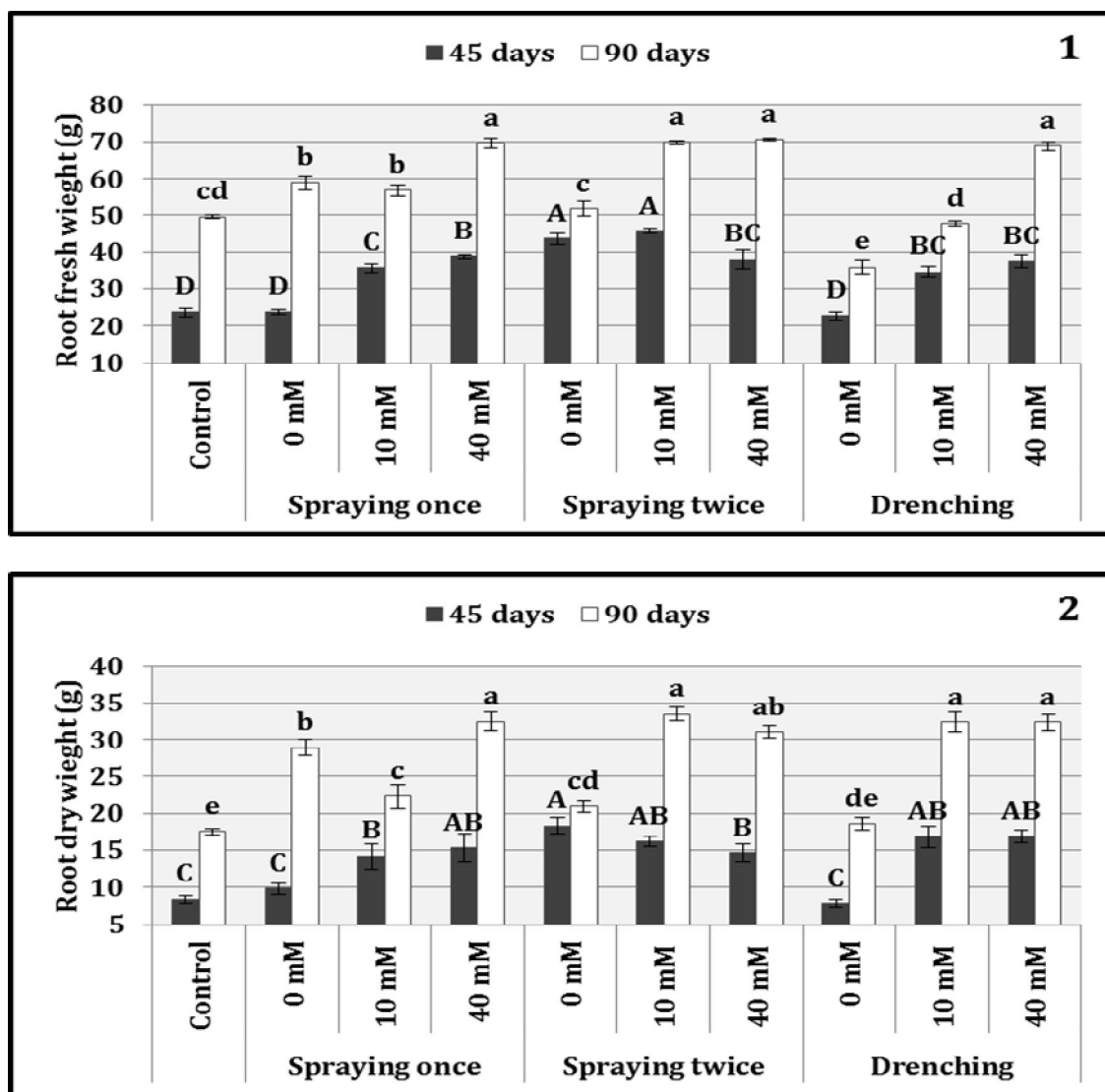


Fig. 3: Effect of SA application on 1) root fresh weight and 2) root dry weight of the pomegranate seedlings after 45 and 90 days of nematodes inoculation. Means \pm SE expressed with bars with lines. (n=3). Bars with different letters are significantly different at $P \leq 0.05$ (Capital letters for 45 days and small letters for 90 days).

Chlorophyll content:

Total chlorophyll content, which was measured with SPAD-502, is described in Fig. 4. Although, neither inoculated nor uninoculated plants showed signs of wilting, the amount of chlorophyll content was significantly decreased by nematode infection. This result is similar to Melakeberhan *et al.*, (1985) and Franco (1980), but it is in contrary to Loveys and Bird (1973) who found that the differences between infected and non-infected tomato plants were not statistically significant. The damage of root caused by the nematodes could lead to water stress in the plant and cause partial closure of the stomata, which would cause difference

in CO₂ fixation rate (Loveys and Bird, 1973).

In this study, treatments with SA enhanced the synthesis of total chlorophyll even at 90-day. Foliar application of nematode-infected plants with 10 mM SA was the highest value when it used as twice application at 90 days (71.6), followed by 10 mM for once (69.1), in compare with 0 mM sprayed plants twice or once (52.8 and 52.1, respectively). Our findings are in agreement with El-Sherif *et al.*, (2015) who used SA and some other organic acids with different concentrations on tomato leaves and found that SA increased total chlorophyll, compared to nematode alone.

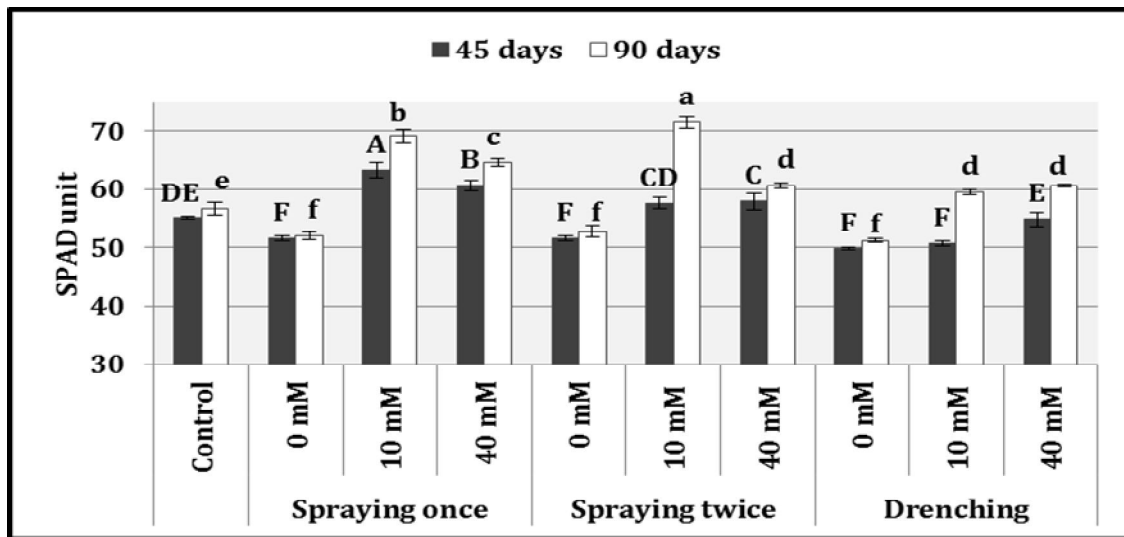


Fig. 4: Effect of SA application on total chlorophyll content (expressed as SPAD unit) of the pomegranate seedlings after 45 and 90 days of nematodes inoculation. Means \pm SE expressed with bars with lines (n=3). Bars with different letters are significantly different at $P \leq 0.05$ (Capital letters for 45 days and small letters for 90 days).

Potassium content:

Figure 5 shows the shoots and roots content of potassium and the translocation from the site of uptake (roots) to the shoots in pomegranate plants. Potassium was determined as

an indicator of root nutrients translocation efficiency. It can be figured out that, in the most of inoculated plants, the content of potassium (mg/g) in the shoots was more than in the roots. This may be implied that

translocation of this element had not affected by nematode infection. These findings agree with Melakeberhan *et al.*, (1985) who stated that the infected plants with nematode had more potassium in the shoots, compared to control. However, it was in contrary with Nasr *et al.*, (1980) who found that the potassium in roots was higher than in control plants.

SA treatments increased the content of K in both shoot and root when it applied to the infected seedlings, compared to untreated ones (0

mM). Spraying with 40 mM SA for once increased K content in shoots at 45 and 90-day by 1.8 and 1.6 mg/g DW, respectively, in compare with 0 mM treated plants (0.9 and 1.3 mg/g DW, respectively). Similarly, this concentration, when was sprayed once or twice, gave the highest values of K content in roots at 45 and 90 days of inoculation. Our results agree with El-Sherif *et al.*, (2015) who recorded that SA increased the content of K in the leaves of the plants when infected with nematode.

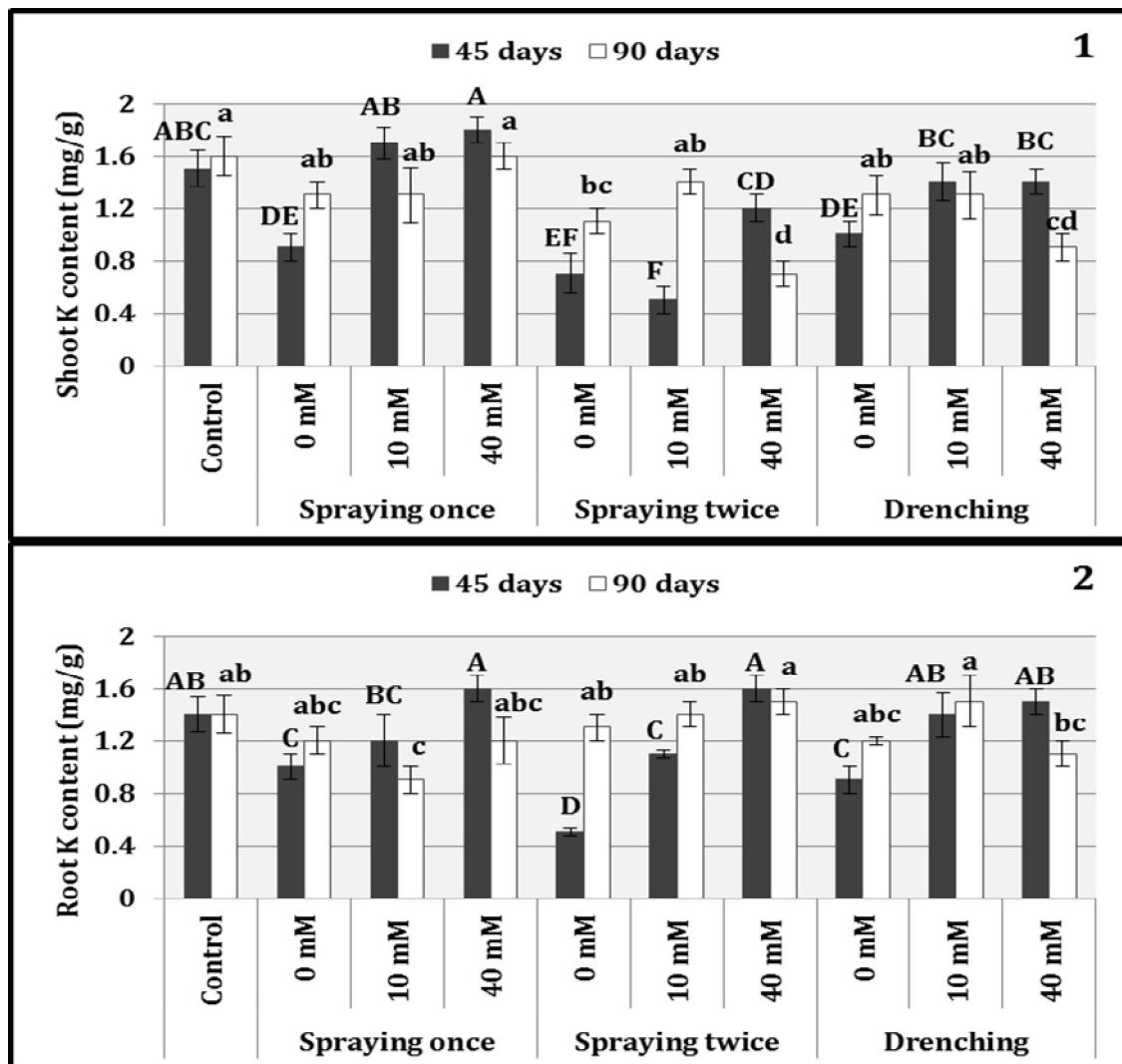


Fig. 5: Effect of SA application on K content of the pomegranate seedlings 1) in shoots and 2) in roots after 45 and 90 days of inoculation. Means \pm SE expressed with bars with lines (n=3). Bars with different letters are significantly different at $P \leq 0.05$ (Capital letters for 45 days and small letters for 90 days).

Magnesium content:

The Mg concentrations (mg/g) in shoots and roots showed in Fig. 6. Mg content in shoots was decreased in nematode-infected seedlings, compared to control ones. This finding is similar to Fatemy and Evans, 1986 and Price *et al.*, 1982. Nematode infection significantly or non-significantly inhibited the Mg accumulation in test plants in comparison with non-infected control ones at 45 and 90-day, except in case of roots at 90-day and twice spraying with 0 mM SA, where a significant stimulation was detected. The reduction in nutrient content in inoculated plants shoots could be mainly due to a reduction in dry weight in seedlings shoots. In this paper, the content of Mg in the root was increased compared to control at 45 and 90 days of inoculation and that maybe due to the

increasing in root weight in nematode-infected seedlings compared to uninoculated ones. These result agreed with the report of Carneiro *et al.*, 2002, who observed during the 50 days after inoculation, that with the increasing in the inoculum levels with nematodes, there was an increasing in nutrient concentrations, that may be due to that the inoculated plants have heavier roots than the uninoculated ones, and that led to increasing in the absorbing surface of root mass.

Foliar application with 40 mM SA increased Mg content in the shoots by 40% at 45-day of inoculation comparing to control plants. While in roots, drenching 10 mM SA gave the highest value of Mg content in compare with nematode-infected plants at 90 days. These results are consistent with those of El-Tayeb (2005) and Gunes *et al.*, (2007).

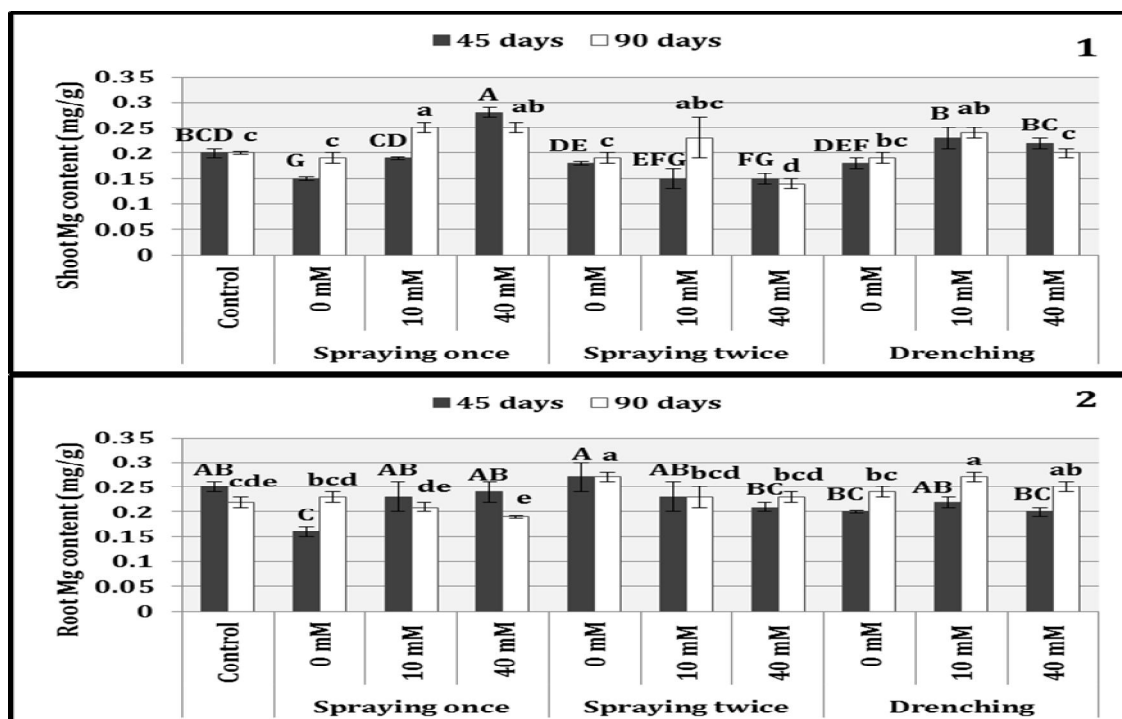


Fig. 6: Effect of SA application on Mg content of the pomegranate seedlings 1) in shoots and 2) in roots after 45 and 90 days of nematodes inoculation. Means \pm SE expressed with bars with lines (n=3). Bars with different letters are significantly different at $P \leq 0.05$ (Capital letters for 45 days and small letters for 90 days).

Total Carbohydrates:

Carbohydrates are the important components of storage and structural materials in the plants. Data expressing total carbohydrates content in shoots of pomegranate seedlings under various treatments was shown in Fig. 7. Nematode infection after 45-day of inoculation induced an inhibitory effect on the accumulation of total carbohydrate in shoots of pomegranate plants. On the other hand, there were no significant differences after 90-day of nematode inoculation, as compared with the non-infected control plants. The fluctuation in carbohydrates accumulation may be due to tendency of fruit trees to store carbohydrates before dormancy and remobilize them for spring bud break and root growth (Gordon and Dejong, 2007). Also, high soil temperature would promote starch synthesis, and roots would act as a sugar sink and that lead to carbohydrates reduction in shoots Zwieniecki (2015). It can be figured out that the reduction in carbohydrates lead to decrease in shoots fresh and dry weight. These findings agree with Olién *et al.*, (1995) who

found that nematodes (*Criconemella xenoplax*: Cx) infection reduced dry weight and concentrations of soluble carbohydrates of all organs in Nema-guard peach rootstock.

The results obtained here reveal that different applications of SA did not induce appreciable variations in total carbohydrates accumulation in shoots of nematode-infected plants at 45-day of inoculation. While, SA at high concentration (40 mM) or low concentration repeated treatment (10 mM spraying twice) and drenching soil with both concentrations were significantly increased the carbohydrates content in shoots of infected-plants, and the values were closed to the non-infected control ones. These findings are parallel with Radwan *et al.*, (2007).

It is obvious that salicylic acid is very important to seedlings as it can regulate some process as photosynthesis, and subsequently chlorophyll and carbohydrates content and defense against abiotic and biotic stress. That was mentioned also by Herrera-Vásquez *et al.*, (2015).

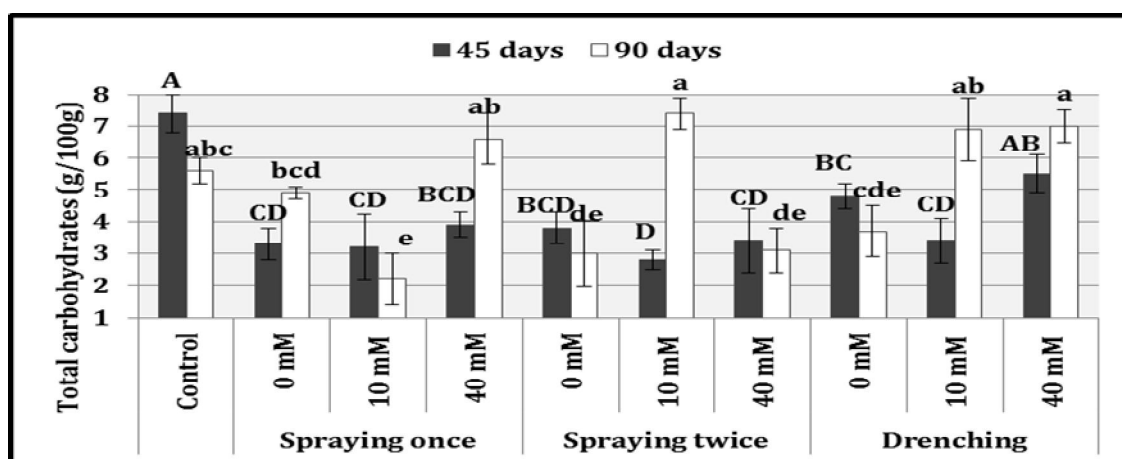


Fig. 7: Effect of SA application on total carbohydrates content (g/100g) of the pomegranate seedlings after 45 and 90 days of nematodes inoculation. Means \pm SE expressed with bars with lines (n=3). Bars with different letters are significantly different at $P < 0.05$ (Capital letters for 45 days and small letters for 90 days).

Nematode Parameters:

Results in Fig. 8 reveal that, all SA treatments either spraying or drenching significantly reduced all nematode parameters i.e. number of galls/g roots, number of egg masses/g roots, as well as number of juveniles/100 g soil either after 45 or 90 days in compare with 0 mM SA treated plants. Concerning the root galls/g roots, data indicate that, treatments with 10 and 40 mM SA as spraying twice and 10 and 40 mM SA as drenching after 90-day induced the

highest reduction of galls (39, 37, 38.33 and 35, respectively) followed by after 45-day (13, 12, 12.33 and 11, respectively). When treating once of 10 mM SA as spraying or drenching either after 45 or 90-day was the lowest effective in reducing galls (25 and 57). These results are in agreement with results of Mostafanezhad *et al.*, (2014) who found that soil drenching with SA significantly reduced the diameter of *M. javanica* galls and numbers of galls and egg masses.

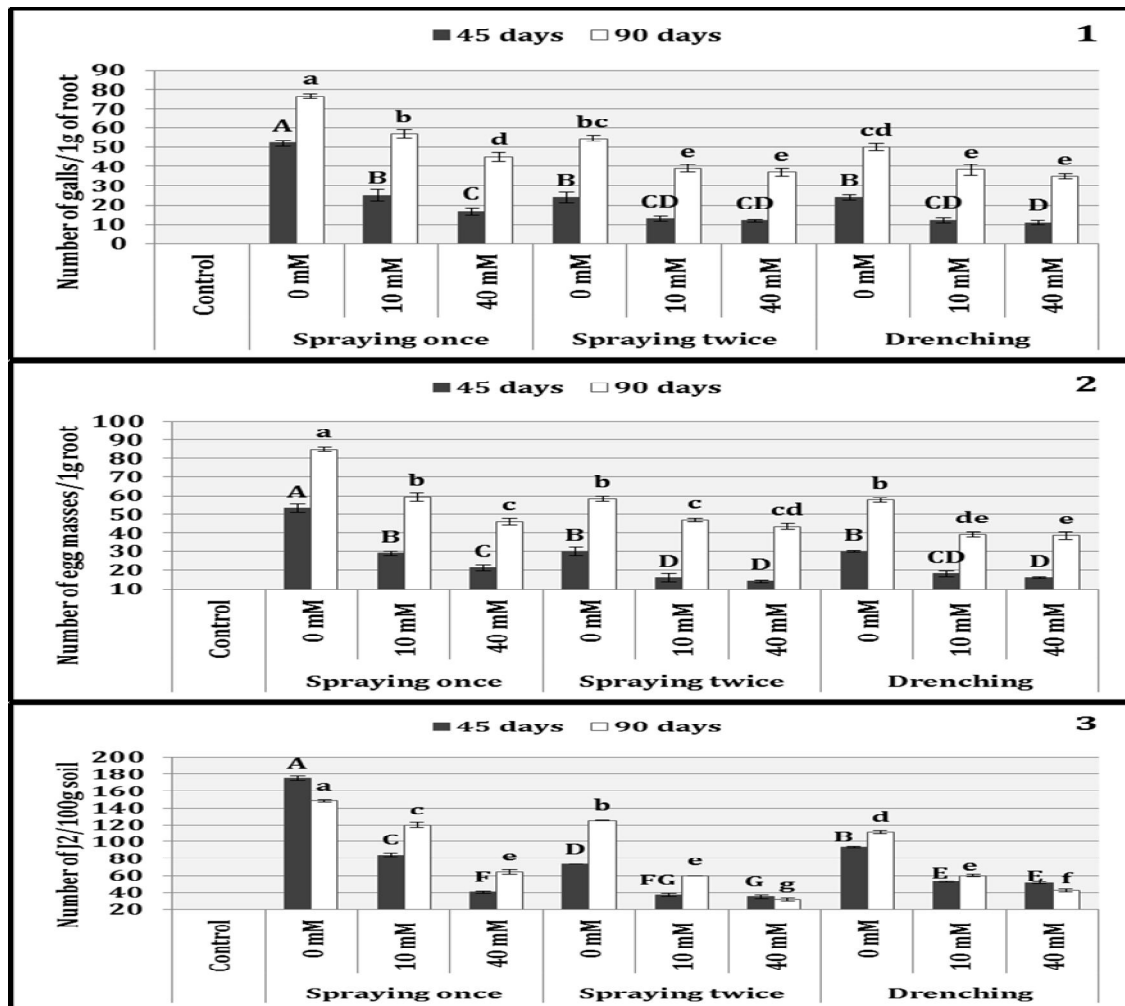


Fig. 8: Effect of SA application on 1) number of galls/1g of root, 2) number of egg masses/1g of root and 3) number of J2/100g soil after 45 and 90 days of nematodes inoculation. Means \pm SE expressed with bars with lines (n=3). Bars with different letters are significantly different at $P < 0.05$ (Capital letters for 45 days and small letters for 90 days).

Moreover, the reduction in egg masses/g roots was noticed after 45 and 90-day on inoculation. However, the highest significant reduction was obtained with 40 mM SA (38.67) followed by 10 mM SA (39.33) as drenching and then 40 mM SA twice as spraying (43.33) after 90-day. After 45 days, the highest effective treatments in reducing egg masses were 10 and 40 mM SA as spraying twice and 40 mM SA as drenching (16, 14 and 16, respectively). These results agree with Moslemi *et al.*, (2016) who stated that when using SA as a soil drench, number of egg masses that formed on tomato plants reduced by 78%. In this study, the significant reduction in J₂ were obtained after 45 and 90-day by spraying with 40 mM SA twice (35.33 and 32, respectively) followed by spraying 40 mM SA once after 45-day (40.3) and drenching 40 mM SA after 90-day (42.33). Whereas the lowest decrease in J₂ was achieved by treatment of 10 mM SA as spraying after 45 and 90 days (84 and 120, respectively). Our results are similar to the results recorded by Ganguly *et al.*, (1999).

The reduction in the numbers of galls and egg masses are probably due to the fact that SA could possibly cause the formation of defective eggs, and the new juveniles formed by the nematodes are not healthy enough to create a new population (Pankaj *et al.*, 2005).

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تأثير حمض السالسيك على النمو والعناصر الغذائية والكربوهيدرات لشتلات الرمان تحت ظروف الإصابة بالنيماطودا

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الملخص

الهدف من هذا البحث هو دراسة تأثير إضافة حمض السالسيك بتركيزات مختلفة (١٠، ٤٠ و ١٠٠ ملليمول) على شتلات الرمان (صنف المنفلوطى) تحت ظروف الإصابة بالنيماطودا. حيث تم إضافة حمض السالسيك بطرق مختلفة (رش على المجموع الخضرى (مرة واحدة أو مرتين) و إضافته للتربة). و قد تم أخذ القياسات على النمو الخضرى والمجموع الجذرى و تقدير محتوى الجذر والساق من البوتاسيوم والماغنسيوم و الكلوروفيل الكلى والكربوهيدرات الكلية. حيث أظهرت النتائج أن حمض السالسيك قد ساعد معنويا على تحسين معظم الصفات الخضرية للشتلات تحت الإصابة بالنيماطودا، كما زاد من قدرة الجذر على إمتصاص العناصر التى تم قياسها و إنتقالها الى الساق، كما زاد محتوى الشتلات من الكلوروفيل الكلى والكربوهيدرات الكلية. أيضا أظهرت النتائج أن المعاملة بحمض السالسيك قد أدت إلى انخفاض معنوى فى كل القياسات الخاصة بالنيماطودا.