Nematicidal Activity of Acetyl Salicylic Acid against Root-Knot Nematode on Tomato Plants.

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ABSTRACT
The nematicidal activity of acetyl salicylic acid (ASA) was investigated against root-knot nematode (*Meloidogyne incognita*) under laboratory and greenhouse conditions. The results of the laboratory experiment showed that ASA exhibited nematicidal activity against 2nd stage juveniles of nematode with a LC$_{50}$ value of 150.43 ppm. In the greenhouse experiment, the effect of ASA at three concentrations was tested against root-knot nematode on tomato plants in comparison with oxamyl nematicide as standard treatment. All plants were examined to assess disease severity based on the number of galls per root, egg masses per root, developmental stage within the root system, and average of eggs number per egg mass as well as plant growth characteristics were estimated. The results showed that all tested treatments reduced tomato root galling significantly compared with the untreated treatment. The numbers of galls/root system were (6.33, 12.67, 18.67, and 28.67) for the treatments of oxamyl and 300, 200 and 100 ppm of ASA, respectively, compared with the untreated check (113) and the other parameter were in the same trend. All treatments increased shoot and root weights and lengths compared with the untreated check. In conclusion, ASA has nematicidal activity to suppress root-knot nematode infection in tomato plants. Thus, this approach could be a safe alternative to use chemical nematicides for the management of plant-parasitic nematodes.

INTRODUCTION
Root-knot nematode, *Meloidogyne* spp., are important agricultural pests worldwide that cause severe damage to many cultivated plant species. They are responsible for destroying crops worth around 100 billion dollars every year worldwide (Ralmi *et al.*, 2016). In Egypt, Root-Knot nematode. *Meloidogyne* spp., are becoming a real threat to almost all vegetable crops (Ibrahim, 2011). Management of plant-parasitic nematodes has always been difficult and various strategies have been extensively used to manage root-knot nematodes in infested areas such as organic amendments, biological control and chemical nematicides. Although chemical nematicides are the most rapid and effective control measure, they have withdrawn from the market due to human health and environmental hazards (Rich *et al.*, 2004). Therefore, attention to other alternative methods is increasing.

The application of exogenous salicylic acid (SA) (chemical inducer of plant resistance) is probably one of the possible alternatives and environmentally safe
management practices for protecting plants from various pathogenic infections involved in the plant-parasitic nematode (Walters et al., 2013 and Radwan et al., 2017). Salicylic acid is a natural phenolic compound present in many plants and is involved in the induction of pathogenesis-related proteins in various crops.

Recently there are many approaches for controlling nematode using plant resistance inducing chemicals such as salicylic acid (SA) against nematodes (Ganguly et al., 1999; Molinari, 2006; Moslemi et al., 2016 and Radwan et al., 2017). Other reports mentioned that SA was promoted plant growth and decreased nematode infection by induction of plant resistance against root-knot nematode (Nandi et al., 2000; Molinari and Loffredo, 2006; Meher et al., 2011 and Mostafanezhad et al., 2014b). Also, SA affected chemotaxis, motility, viability and hatching of Meloidogyne incognita in vitro (Wuyts et al., 2005).

The objective of the present study is to evaluate the effects of acetyl salicylic acid (ASA) on the second stage juvenile of root-knot nematode motility in vivo, also on tomato root-knot disease incidence under in vitro conditions.

**MATERIALS AND METHODS**

Salicylic acid (SA) used as acetyl derivative acetyl salicylic acid (2-(CH3CO2)C6H4CO2H, M. w 180.16, Purity ≥ 99% w/w, Melting point: 134-136 °C) which purchased from Electro Scient Chemicals Company, Kasr El-Eieny, Cairo. Oxamyl (Vydate 24% SL) is used as a chemical nematicide at the recommended rate (3 L/fe).

**Preparation of Nematode Inoculums:**

Culture of root-knot nematode, Meloidogyne incognita was prepared from naturally infected eggplant (Solanum melongena) roots collected from the fields. Individual egg-mass with her mature female was removed from root tissues and placed in a small glass capsule containing fresh water. The female was preserved in 4% formaldehyde solution to be identified into species according to the basis cited by Hartman and Sasser (1985).

A pure stock culture of M. incognita was prepared from tomato seedling planted in a clay pot (25 cm in diameter) filled with previous steam-sterilized sandy loam soil was inoculated with egg-mass. Inoculated pots were kept in a greenhouse and irrigated regularly. The inoculum was propagated on tomato seedlings. Infected tomato plants were the source of experimental inoculum.

To obtain second-stage juveniles for the experiments, mature egg masses obtained from the source culture were placed onto a paper tissue supported in a basket sitting in shallow water; hatched juveniles passed through the tissue and were collected daily (Whitehead and Hemming, 1965).

**Laboratory Experiment:**

The laboratory experiment was conducted to evaluate LC50 value of acetyl salicylic acid on newly hatched J2s after 48 hrs. Six concentrations (50, 100, 150, 200, 250, and 300 ppm) were prepared using distilled water and dimethyl sulfoxide (DMSO 1%). The suspension of newly hatched J2s was prepared. The mean number of 2nd stage juveniles(J2) in the suspension of 1ml (100 juvenile) was added to 1ml of each concentration of the examined compound. Three replicates for each concentration were used and the control treatment consisted of the used solvent DMSO and distilled water. The estimation of percent mortalities was calculated according to Abbott's formula (1925) after 48 hrs. The obtained data were expressed as toxicity lines, thus, LC50 value (the concentration in which 50% of the nematodes were killed) were determined by probit analysis software programme according to Finney (1971).
Greenhouse Experiment:

In a pot experiment, Three-week-old seedlings of tomato transplanted in the clay pots (15 cm in diameter) filled with 1 kg steam-sterilized soil (1:1) (sand: clay). After one week, the plants inoculated with J2 of the nematode as follows, 10 ml of nematode suspension containing about 1000 freshly hatched J2 were pipetted to each pot by pouring the J2 suspension gently in holes around the root system. The holes were plugged by pressing the pot soil and watered. Then, the plants were treated with the tested compound at 3 concentrations (100, 200, 300 ppm) of ASA and oxamyl at the recommended rate as a soil drench. After 45 days from nematode inoculation, plants were gently removed from pots and all nematode parameters (number of galls and egg masses per root system, the number of 2nd juveniles per pot and average number of eggs/egg masses) were counted according to (Hussey and Barker, 1973). Plant growth criteria were also estimated.

Nematode reproduction factor (Rf) was calculated according to the formula: \( Rf = \frac{Pf}{Pi} \)

\( Pi = \) nematode initial inoculum, \( Pf = \) nematode final population.

RESULTS AND DISCUSSION

The nematicidal effect of acetyl salicylic acid (ASA) against \( M. \) incognita was studied under laboratory and greenhouse conditions.

The laboratory experiment was conducted to evaluate LC\(_{50}\) value of acetyl salicylic acid on J2 of \( M. \) incognita after 48 hrs. The results of Table (1) indicated that ASA exhibited nematicidal activity against nematode juveniles with a LC\(_{50}\) value of 150.43 ppm. This result is in agreement with that obtained by Wuyts et al. (2005) who reported that the LC\(_{50}\) value of SA on \( M. \) incognita was 46µg/ml under in vitro conditions. Additionally, they found that SA affects the motility, viability, and hatching of \( M. \) incognita. Also, Abd-alla et al., (2013) found that a formulation of salicylic acid EC 10 % achieved a high nematicidal effect with 48 hrs-EC\(_{50}\) a value of 12.5 ppm.

Table 1: Nematicidal activity of acetyl salicylic acid on J2 of \( M. \) incognita under laboratory conditions.

| Concentrations of ASA (ppm) | % Mortality | LC\(_{50}\) value (Lower-upper limits) | Slop value |
|----------------------------|-------------|--------------------------------------|------------|
| 50                         | 4.10        |                                      |            |
| 100                        | 18.70       |                                      |            |
| 150                        | 43.50       |                                      |            |
| 200                        | 65.00       | 150.43                               | 4.55±0.38  |
| 250                        | 94.00       | (107.42 - 202.2)                     |            |
| 300                        | 100.00      |                                      |            |

A greenhouse experiment was conducted to study the effect of ASA at three concentrations in comparison with oxamyl nematicide against root-knot nematode on tomato plants.

Results listed in Table (2) showed that all treatments significantly reduced galls number, number of juveniles in soil, number of developmental stages, number of egg-masses, nematode fecundity and number of final population as compared with the untreated check. The concentrations of ASA gave markedly nematicidal activity in comparison with oxamyl nematicide. The nematicidal activity was increased with increasing the concentration of ASA. For example, the number of galls was (12.67, 18.67 and 28.67) for the treatments
300, 200 and 100 ppm of ASA, respectively and the other parameters were in the same trend (Table 2).

**Table 2:** Effect of acetyl salicylic acid on the development and reproduction of root-knot nematode (*Meloidogyne incognita*) infecting tomato plants under greenhouse conditions.

| Acetyl salicylic acid | Concentration (ppm) | No. of Galls | No. of Larvae in soil (U2) | No. of developmental | No. of egg masses | No. of Eggs/ Egg mass | Final population (Pt) | Reproduction factor (Rf) |
|-----------------------|---------------------|--------------|---------------------------|----------------------|-------------------|----------------------|-----------------------|------------------------|
| Acetyl salicylic acid | 300                 | 12.67<sup>a</sup> | 33.33<sup>b</sup> | 11.00<sup>b</sup> | 3.00<sup>c</sup> | 62.67<sup>c</sup> | 235.33 | 0.24 |
|                       | 200                 | 18.67<sup>c</sup> | 100.00<sup>c</sup> | 15.00<sup>b</sup> | 10.33<sup>b</sup> | 84.33<sup>c</sup> | 996.78 | 1.00 |
|                       | 100                 | 28.67<sup>c</sup> | 141.67<sup>b</sup> | 15.33<sup>b</sup> | 14.33<sup>b</sup> | 96.67<sup>b</sup> | 1556.89 | 1.56 |
| Oxamyl                |                     | 6.33<sup>d</sup> | 16.67<sup>c</sup> | 2.33<sup>c</sup> | 2.67<sup>c</sup> | 94.33<sup>b</sup> | 222.78 | 0.22 |
| Control               |                     | 113.00<sup>c</sup> | 258.33<sup>c</sup> | 32.67<sup>b</sup> | 53.67<sup>a</sup> | 158.33<sup>a</sup> | 8841.89 | 8.84 |
| L.S.D at 5%           |                     | 6.78          | 42.34                    | 7.00                 | 8.25              | 17.98                |                    |                        |

* Differences between means in each column followed by the same small letter (s) are not significant at P<0.05 according to Duncan's multiple range test.

Results in Table (3) indicated that the application of ASA at tested concentrations significantly enhanced the shoot and root weight of tomato plants as well as shoot and root length in comparison with untreated control. In other words, improved plant growth. From the obtained results, it was noticed that application of ASA as soil drench against *M. incognita* on tomato plants reduced tomato root galls and nematode population in soil and roots and hence enhance the plant growth are well agreement with those obtained by several authors, such as Radwan *et al.*, (2017) who found that application of ASA as soil drenching significantly reduced tomato root galls and 2<sup>nd</sup> juvenile numbers in soil compared with control through inducing tomato plant resistance to root-knot nematode infection. Also, Osman (1993) found that SA was found to reduce the number of 2<sup>nd</sup> juveniles and other developmental stages of *M. javanica* in tomato plants. Application of 50 ug/ml of SA reduced root galling (50% or lower) of tomato plants and induced resistance in tomato against *M. incognita* (Ganguly *et al.*, 1999). In another study, Molinari (2008) found 45 mM SA solution as soil drench or 0.5-1 mM root dip significantly reduced *Meloidogyne* reproduction (by 20 to 25%).

**Table 3:** Effect of acetyl salicylic acid on the growth of tomato plants infected with root-knot nematode (*Meloidogyne incognita*) under greenhouse conditions.

| Treatments | Concentration (ppm) | Shoot | Root | Shoot | Root |
|------------|---------------------|-------|------|-------|------|
| Acetyl salicylic acid | 300 | 64.33<sup>a</sup> | 22.00<sup>a</sup> | 11.00<sup>b</sup> | 4.50<sup>b</sup> |
|                       | 200 | 62.33<sup>b</sup> | 20.00<sup>ab</sup> | 9.00<sup>c</sup> | 3.83<sup>b</sup> |
|                       | 100 | 60.00<sup>b</sup> | 19.67<sup>b</sup> | 8.33<sup>c</sup> | 3.67<sup>bc</sup> |
| Oxamyl                | 57.33<sup>bc</sup> | 19.00<sup>b</sup> | 13.33<sup>a</sup> | 6.00<sup>a</sup> |
| Control               | 53.33<sup>c</sup> | 19.33<sup>b</sup> | 6.00<sup>d</sup> | 2.77<sup>c</sup> |
| L.S.D at 5%           | 6.42 | 2.25 | 1.94 | 0.99 |

* Differences between means in each column followed by the same small letter (s) are not significant at P<0.05 according to Duncan's multiple range test.
Soil drench of SA and ASA reduced M. incognita reproduction on tomato roots (Anter et al., 2014). Also, Mostafanezhad et al. (2014a), found that soil drench and spraying tomatoes with SA significantly reduced the diameter of M. javanica galls, numbers of galls and egg masses. Treatments also, increased the activity of enzymes and phenolic compounds. On other hand, Moslemi et al. (2016) concluded that exogenous application of SA reduced root-knot nematode reproduction and final population on tomato.

Salicylic acid was known as an inducer of plant resistance to some pathogens. Also, it has been thought to play an important role in systemic acquired resistance because exogenous salicylic acid induces this resistance and accumulated in pathogen-infected tissue (Malamy et al., 1990). Several evidences indicate that SA binds with specific receptor molecules and activates a messenger to transduce signal for the production of defence molecules (Raskin, 1992; Lamb, 1994; Dumer et al., 1997and Shirasu et al., 1997). These defence molecules are responsible for systemically induced resistance against a wide array of pathogens including nematodes. Meher et al. (2011) concluded that SA activated glutathione metabolism, augmented glutathione status of the crop, imparted resistance against Meloidogyne incognita and improved yield and fruit quality. The study provided additional insight into the SA-induced resistance mechanism against obligate phytopathogen and indirectly assigned a number of enzyme functions. Zinovieva et al. (2011) reported that the increase in the resistance of tomato plants is related to the increased activity of phenylalanine ammonia-lyase and an increased SA content in plant tissues infected with nematodes; both these factors significantly influence nematode development.

Finally, the results of study demonstrate that ASA reduced tomato gall numbers, eggmasses, eggs, and the present 2nd stage juveniles of root-knot nematode in the soil. These results support the theory that SA involvement in the resistance inducing mechanisms in the case of plant infection with root-knot nematode. Further field experiments should be carried out to support this speculation.

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**ARABIC SUMMARY**

الفاعلية الإبادية لمشتق الاستايل لحامض السالسليك ضد نيماتودا تعقد الجذور على الطماطم.

حسني محمد راضى - عماد الدين مرزوق محمد - إيهاب السيد كرات - سامح حماده السيد حماده
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جرت تجارب معملية وتجارب في اصص تحت ظروف الصوبة لتقييم الفاعلية الإبادية لمشتق الاستايل لحامض السالسليك ضد نيماتودا تعقد الجذور (*Meloidogyne incognita*). أظهرت نتائج التجارب المعملية ان مشتق الاستايل لحامض السالسليك له فاعلية إبادية على الطور اليرقى الثاني للنميادودا حيث كانت قيمة التركيز النصف مميت (*LC*50) هي 150.43 جزء في المليون. بالنسبة لتجارب الصوبة تم اختبار تأثير مركب الاستايل سالسليك بثلاثة تركيزات (100 و 200 و 300 جزء في المليون) على معدل الإصابة بمرض التعقد الجذري على نباتات الطماطم المثبطة عن نيماتودا تعقد الجذور مع استخدام المبيد النيماتودي الكيماوي (أوكساميل) كمعايير قياسية للمقارنة. أظهرت نتائج هذه التجارب أن جميع المعاملات أحدثت نسبة معنوية في معدل التعقد الجذري على جذور نباتات الطماطم الناتج عن الإصابة بالنيماتودا مقاومة بالنباتات الغير معالمة (الكنترول). حيث كان متوسط عدد العقد لكل جذر كالياني (6.33 و 12.67 و 18.67) لمعاملات أوكساميل 300 و 200 و 100 جزء في المليون استيل سالسليك على الترتيب وكان ذلك هو نفس الاتجاه لباقي معايير الإصابة. أيضا كل المعاملات أدت إلى زيادة معنوية في كل من وزن وطول المجموع الخضري والجذري لنباتات الطماطم مقاومة بالكنترول. 

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استنتاجات النتائج: إن مشتق الاستايل لحامض السالسليك له فعالية إبادية ضد نيماتودا تعقد الجذور ويمكن استخدامه كديل آمن للمبيدات الكيماوية في برامج مكافحة النيماتودا.