Nematicidal Activity of Acetylsalicylic Acid Comparing With Oxamyl Against Root-Knot Nematode on Tomato Plants.

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INTRODUCTION

Root-knot nematodes, 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 Although chemical nematicides are the most rapid and effective control measure, they have withdrawn from the market due to human health and environmental hazards (Rich et al., 2004).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 acetylsalicylic acid (ASA) on the second stage juvenile of root-knot nematode motility in vitro, also on tomato root-knot disease incidence under in vivo conditions.

**MATERIALS AND METHODS**

Salicylic acid (SA) used as acetyl derivative acetylsalicylic 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 and oxamyl on newly hatched J2s after 48 hrs. Six concentrations (50, 100, 150, 200, 250, and 300 ppm) of ASA were prepared using distilled water and dimethyl sulfoxide (DMSO 1%). Serial concentrations of oxamyl (2,4,8,16,20 and 32ppm) were also prepared. 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 compounds. 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
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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 acetylsalicylic acid (ASA) against *M. incognita* was studied under laboratory and greenhouse conditions.

The laboratory experiment was conducted to evaluate LC50 value of acetyl salicylic acid on J2 of *M. incognita* after 48 hrs. The results of Table (1) and Fig(1) indicated that oxamyl and ASA exhibited nematicidal activity against nematode juveniles with LC50 values of 10.88 and 150.43 ppm, respectively. This result is in agreement with that obtained by Wuyts *et al.* (2005) who reported that the LC50 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-EC50 a value of 12.5 ppm.

![Fig (1): Toxicity regression lines of acetylsalicylic acid and oxamyl on J2 of root-knot nematode under laboratory conditions.](image-url)
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 acetylsalicylic acid and oxamyl on the development and reproduction of root-knot nematode (*Meloidogyne incognita*) infecting tomato plants under greenhouse conditions.

<table>
<thead>
<tr>
<th>Concentrations of ASA (ppm)</th>
<th>% Mortality</th>
<th>Slope value</th>
<th>Concentrations of oxamyl (ppm)</th>
<th>% Mortality</th>
<th>Slope value</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>4.10</td>
<td>4.55±0.38</td>
<td>2</td>
<td>16.30</td>
<td>1.98±0.155</td>
</tr>
<tr>
<td>100</td>
<td>18.70</td>
<td>(107.42 - 202.2)</td>
<td>4</td>
<td>23.80</td>
<td>(6.17 - 19.58)</td>
</tr>
<tr>
<td>150</td>
<td>43.50</td>
<td></td>
<td>8</td>
<td>34.70</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>65.00</td>
<td></td>
<td>16</td>
<td>51.72</td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>94.00</td>
<td></td>
<td>20</td>
<td>70.36</td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>100.00</td>
<td></td>
<td>32</td>
<td>95.70</td>
<td></td>
</tr>
</tbody>
</table>

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 2nd 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 2nd juveniles and other
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developmental stages of *M. javanica* in tomato plants. Application of 50 μg/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 acetylsalicylic acid acid and oxamyl on the growth of tomato plants infected with root-knot nematode (*Meloidogyne incognita*) under greenhouse coditions.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Length (cm)</th>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration (ppm)</td>
<td>Shoot</td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td>300</td>
<td>64.33a</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>62.33ab</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>60.00bc</td>
</tr>
<tr>
<td>Oxamyl*</td>
<td></td>
<td>57.33bc</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>53.33c</td>
</tr>
<tr>
<td>LSD at 5%</td>
<td></td>
<td>6.42</td>
</tr>
</tbody>
</table>

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.

*oxamyl was used according to it's recommended rate..

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., 1997; and 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.
REFERENCES


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**ARABIC SUMMARY**

الفاعية الإبادية لمشتق الاستال لحامض السالسليك مقارنة بمبيد اوكساميل ضد نيماeroxما تستخد ما كميينى عن الطماطم

حسنى محمد راضي. إييهاب السيد السيد كرات

قسم وقاية النباتات. كلية الزراعة بالقاهرة. جامعة الأزهر

أجريت تجارب معملية وتجارب في اصص تحت ظروف الصوبة لتقييم الفاعية الإبادية لمشتق الاستال لحامض السالسليك ومبيد اوكساميل ضد نيماeroxما تستخد ما كميينى عن الطماطم. استخدمت تجارب العملية في اصطحاب لحاسم السالسليك (Meloidogyne incognita) ان مشتق الاستال لحاسم السالسليك له فاعية إبادية على الطماطم، حيث كانت قيمة التركيز النصف ميت (LC50) هي 150٪ عند الجذر في المليون مقارنة بمبيد اوكساميل (10.33٪) حسب التجربة. الصوبة تم اختبار تأثير مركب الاستال سالسليك (300٪) على معدل الانصابيات بضعة تركيزات (100٪) و (300٪) حسب التجربة. استخدمت وأوكساميل كمبيد استخدمت مصورة لنفسي المبيدات الحيوية (لو هيتجل) و (ياهنغ الينون). كعيدي محايدة للمبيدات. استخدمت هذه المبيدات في التجارب. لاحظت هذه التجربة أن جميع المعادلات أحدثت تأثيرات عضوية في البطاطس على جذور النباتات. حيث كان متوسط عدد الكائنات 6.3، 6.7 و 18.67، 28.67 و 28.67 لمعادلات اوكساميل (300٪) و (300٪) حسب التجربة. استخدمت كديل Áمن للمبيدات الكيماء في برامج مكافحة النماذج. استخدمت كديل Áمن للمبيدات الكيماء في برامج مكافحة النماذج.