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

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ABSTRACT

The nematicidal activity of acetylsalicylic acid (ASA) and oxamyl nematicide as standard nematicides werer investigated against root-knot nematode *(Meloidogyne incognita*) under laboratory and greenhouse conditions. The results of the laboratory experiment showed that ASA and oxamyl exhibited nematicdal activity against 2nd stage juveniles of nematode with LC_{50} values of 150.43 and 10.88 ppm, respectively. In the greenhouse experiment, the effect of ASA at three concentrations was tested against root-knot nematode on tomato plants in comparison with the recommended rate of 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 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 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/fed.).

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 LC_{50} 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, LC_{50} 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 acetylsalicylic 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) and Fig(1) indicated that oxamyl and ASA exhibited nematicidal activity against nematode juveniles with LC_{50} 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 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 with48 hrs-EC_{50} a value of 12.5 ppm.

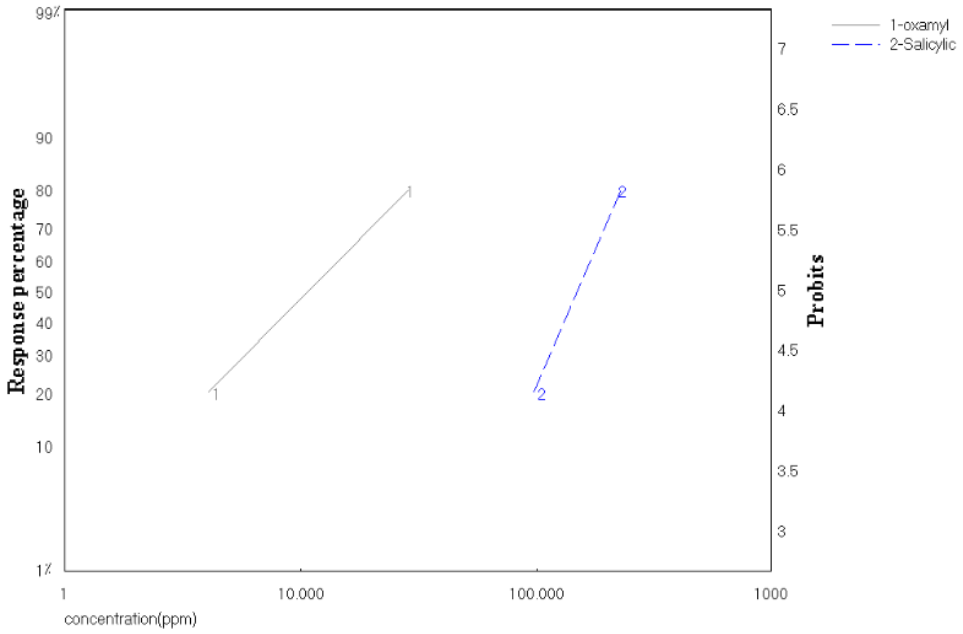

Fig (1): Toxicity regression lines of acetylsalicylic acid and oxamyl on J2 of root-knot nematode under laboratory conditions.

Table 1: Table 1: Nematicidal activity of acetylsalicylic acid and oxamyl on J2 of *Meloidogyne incognita* under laboratory conditions.

Concentrations of ASA (ppm)	% Mortality	LC_{50} value (Lower-upper limits)	Slop value	Concentrations of oxamyl (ppm)	% Mortality	LC_{50} value (Lower-upper limits)	Slop value
50	4.10	**150.43**	4.55±0.38	2	16.30	**10.88**	1.98±0.155
100	18.70			4	23.80		
150	43.50	(107.42		8	34.70	(6.17 -	
200	65.00	- 202.2)		16	51.72	19.58)	
250	94.00			20	70.36		
300	100.00			32	95.70		

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.

Treatments		No. of Galls	No. of Larvae in soil (J2)	No. of developmental	No. of eggmasses	No. of Eggs/ Egg mass	Final population (Pf)	Reproduction factor (Rf)
Acetyl salicylic acid	Concentration (ppm)							
	300	12.67^{cd}	33.33^{c}	11.00^{b}	3.00^{c}	62.67^{c}	235.33	0.24
	200	18.67^{c}	100.00^{b}	15.00^{b}	10.33^{b}	84.33^{b}	996.78	1.00
	100	28.67^{b}	141.67^{b}	15.33^{b}	14.33^{b}	96.67^{b}	1556.89	1.56
Oxamyl*		6.33^{d}	16.67^{c}	2.33^{c}	2.67^{c}	94.33^{b}	222.78	0.22
Control		113.00^{a}	258.33^{a}	32.67^{a}	53.67^{a}	158.33^{a}	8841.89	8.84
L.S.D at 5%		6.78	42.34	7.00	6.82	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.
*oxamyl was used according to it's recommended rate.

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

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 acetylsalicylic acid acid and oxamyl on the growth of tomato plants infected with root -knot nematode (*Meloidogyne incognita*) under greenhouse coditions.

Treatments		Length (cm)		Weight (g)	
Acetyl salicylic acid	Concentration (ppm)	Shoot	Root	Shoot	Root
	300	64.33^{a}	22.00^{a}	11.00^{b}	4.50^{b}
	200	62.33^{ab}	20.00^{ab}	9.00^{c}	3.83^{b}
	100	60.00^{ab}	19.67^{b}	8.33^{c}	3.67^{bc}
Oxamyl*		57.33^{bc}	19.00^{b}	13.33^{a}	6.00^{a}
Control		53.33^{c}	19.33^{b}	6.00^{d}	2.77^{c}
LSD 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.
*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 *etal*., 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.

REFERENCES

Abbott, W.S.(1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, 18: 265-267.

Abd-alla, H.I, Hala S.Ibrahim, S.E.S. Hamouda and A. M. A. El-kady.(2013). Formulation and Nematicidal Efficiencyof Some Alternative Pesticides against *Meloidogyne spp. American Journal of Research Communication*, 1(6): 273-290.

Anter, A.A., Amin, A.W., Ashoub, A.H. & El-Nuby, A.S. (2014). Evaluation of inducers for tomato resistance against *Meloidogyne incognita*. *Pakistan Journal of Nematology*, 32: 195-209.

Dumer, J.; Shah,J. and K1essig,D.F. (1997). Salicylicacidand disease resistance in plants. *Trends in Plant Science*, 2:266-274.

Finney, D.J.(1971). Probit Analysis (3rd edition). Cambridge University Press, Cambridge, UK. ISBN 0-521-08041-X. OCLC 174198382.

Ganguly, A.K.; Sirohi, A.; and Singh, V. (1999). Salicylic acid induced resistance in tomato against *Meloidogyne incognita*. *Indian journal of Nematol*ogy, 29 (2): 182-184.

Hartman, K.M. and Sasser, J.N. (1985). Identification of *Meloidogyne* species on the basis of differential host test and perineal pattern morphology. In: An advanced treatise on *Meloidogyne*,Vol. II; Barker K.R., Carter, C.C., Sasser J.N. (eds). pp.: 69-77. Graphics, North Carolina State University, USA.

Hussey, R.S. & Barker, K.R. (1973). A comparison of methods of collecting inocula for *Meloidogyne* spp., including a new technique. Pl. Dis. Reptr., 57: 1025-1028.

Ibrahim, I.K.A. (2011). Nematode pests parasitic on agricultural field crops. Alexandria (VA): Manshaat El-Maaref. p. 250.

Lamb, C.J. (1994). Plant disease resistance genes in signal perception and transduction. *Plant Cell*, 76:419-422.

Malamy,J. &K1essig, D.F. (1992). Salicylic acid and plant disease resistance. *The Plant journal*, 2:643-654.

Meher, H.C.; Gajbhiye, V.T and Singh, G.(2011). Salicylic acid-induced glutathione status in tomato crop and resistance to root-knot nematode, *Meloidogyne incognita*. *Journal of Xenobiotics*, 1:e 5:22-28.

Metwally, W.E.; Mostafa, A.M. and Refaei, A.R.(2015). In vitro study on the antagonistic activity of different native isolates of rhizobacteria against *Meloidogyne incognita*. *Egyption Journal of Agronematology*, 14(1): 1-9.

Molinari, S. (2008). Salicylic acid as an elicitor of resistance to root -knot nematodes in tomato. *Acta Horticulture*, 789:119-125.

Molinari, S. and Loffredo, E.(2006). The role of salicylic acid in defense responseof tomato to root-knot nematodes. *Physiological and Molecular Plant Pathology*, 68 : 69–78.

Molinari, S.(2006).Salicylic acid as an elicitor of resistance to root-knot nematodes in tomato. *Acta horticulturae*, 10:119-126.

Moslemi, F.; Fatemy, S. and Bernard, F. (2016). Inhibitory effects of salicylic acid on *Meloidogyne javanica* reproduction in tomato plants. *Spanish Journal of Agricultural Research,* 14(1): 1- 7.

Mostafanezhad, H.; Sahebani, N. and Zarghani, S.N.(2014a). Control of root-knot nematode (*Meloidogyne javanica*) with combination of *Arthrobotrys oligospora* and salicylic acid and study of some plant defense responses. *Biocontrol Science and Technology*, 24(2):203–215.

Mostafanezhad, H.; Sahebani, N. and Zarghani, S.N.(2014b). Induction of resistance in tomato against root-knot nematode *Meloidogyne javanica* with salicylic acid. *Journal of Crop Protection*,3 (4):499-508.

Nandi, B.; Sukul, N.C. and Babu, S.P.S. (2000). Exogenous salicylic acid reduces Meloidogyne incognita infestation of tomato. *Allelopathy Journal*, 7: 285-288.

Osman, G.Y.(1993). Effect of amino acids and ascorbic acid on Meloidogynejavanica Chitw. (Tylenchide, Nematode). *Journal of Pest Science*, 66, 140-142

Radwan, M.A.; Abu-Elamayem, M.M.; Farrag, S.A. and Ahmed, N.S. (2017). Comparative suppressive effect of some organic acids against *Meloidogyne incognita* infecting tomato. *Pakistan Journal of Nematology*, 35 (2): 197-208.

Ralmi, N.H.A.A.; Khandaker, M.M. and Mat, N. (2016). Occurrence and control of root knot nematode in crops: a review. *Australian Journal of Crop Science*,1(12):16 4 9 –1654.

Rich, J.R., Dunn, R. and S. Y. & Dickson, D.W. (Eds.) Nematology: Advances and Perspectives, Vol. 2. Nematode Management and Utilization. CABI Publishing, Wallingford, UK. pp. 1041-1082.

Raskin, I. (1992). Role of salicylic acid in plants. *Annual Review of Plant Physiology and Plant Molecular BioIogy*,43:439-463.

Shirasu, K, Nakajuima, H., Rajasekhar, V.K., Dixon, R.A. and Lamb, C. (1997). Salicylic acid potentialities as against dependent grain control that amplifies pathogen signals in the activation of defence mechanism. *Plant cell*, 9:261-270.

Walters, D., Ratsep, J. & Havis, N. D. (2013). Controlling crop diseases using induced resistance: challenges for the future. *Journal of Experimental Botany*,64,1263-12 8 0.

Whitehead, A.G. and Hemming J.R., (1965). A comparision of some quantitative methods of extracting small vermiform nematodes from soil. *Annals of Applied Biology*, 55: 25-38.

Wuyts, N.; Swennen, R. and DeWaele, D. (2005). Effects of plant phenyl propanoid pathway products and selected terpenoids and alkaloids on the behavior of the plant-parasitic nematodes *Radopholus similis, Pratylenchus penetransand Meloidogyne incognita*. Nematology, 8(1):89-101.

Zinovieva, S.V; Vasyukova, N. I.; Udalova, Zh. V.; Gerasimova, N. G.(2011). Involvement of Salicylic Acid in Induction of Nematode Resistance in Plants. *Biology Bulletin*, 38, (5) 453–458.

ARABIC SUMMARY

الفاعلية الإبادية لمشتق الاستايل لحامض السالسليك مقارنة بمبيد اوكساميل ضد نيماتودا تعقد الجذور على الطماطم

.

حسنى محمد راضى- ايهاب السيد السيد كرات

قسم وقاية النبات- كلية الزراعة بالقاهرة- جامعة الازهر

اجريت تجارب معملية وتجارب فى اصص تحت ظروف الصوبة لتقيم الفاعلية الابادية لمشتق الاستايل لحامض السالسليك ومبيد اوكساميل ضد نيماتودا تعقد الجذور (*Meloidogyne incognita*), اظهرت نتائج التجارب المعملية ان مشتق الاستايل لحامض السالسليك له فاعلية إبادية على الطور اليرقى الثانى للنيماتودا حيث كانت قيمة التركيز النصف مميت (LC_{50}) هى 150.43 جزء فى المليون مقارنة بمبيد اوكساميل (10,33 جزء فى المليون) . بالنسبة لتجارب الصوبة تم اختبار تاثير مركب الاستايل سالسليك بثلاثة تركيزات (100و 200 و 300 جزء فى المليون) على معدل الاصابة بمرض التعقد الجذرى على نبات الطماطم المتسب عنه نيماتودا تعقد الجذور مع استخدام المبيد النيماتودى الكيماوى (اوكساميل) كمعاملة قياسية للمقارنة.اظهرت نتائج هذة التجارب ان جميع المعاملات احدثت خفضاً معنوياً فى معدل التعقد الجذرى على جذور نباتات الطماطم الناتج عن الاصابة بالنيماتودا مقارنة بالنباتات الغير معاملة (الكنترول). حيث كان متوسط عدد العقد لكل جذر كالاتى 6.33 و12.67 و 18.67 و 28.67 لمعاملات اوكساميل 300و 200و 100 جزء فى المليون استيل سالسليك على الترتيب وكان ذلك هو نفس الاتجاه لباقى معايير الاصابة. ايضا كل المعاملات ادت الى زيادة معنوية فى كل من وزن وطول المجموع الخضري والجذري لنباتات الطماطم مقارنة بالكنترول.

ونستخلص من النتائج, ان مشتق الاستايل لحامض السالسليك له فعالية إبادية ضد نيماتودا تعقد الجذور ويمكن استخدامه كبديل آمن للمبيدات الكيماوية فى برامج مكافحة النيماتودا.