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Evaluation of some Chemical Substances as Inducers for Tomato Resistance Against Root-Knot Nematode, *Meloidogyne incognita*

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Abstract

The potency of the some chemicals from different groups known as inducers of systemic acquired resistance (SAR) viz., acetylsalicylic acid (ASA), DL-3-aminobutyric acid (BABA), 2,6-dichloroisonicotinic acid (INA), 5-chlorosalicylic acid (CSA), nitrosalicylic acid (NSA),salicylic acid (SA),ascorbic acid (AS), and selenium (SE) in reducing reproduction *Meloidogyne incognita* in tomato plants cv. Castel rock was investigated under greenhouse conditions. Supplying chemicals three days-before nematode inoculation showed maximum efficacy in reducing nematode galls, egg-masses and eggs numbers followed by synchronized addition with inoculation, while post-inoculation treatment was less effective. Reiterative doses post-inoculation were improved the efficacy of single dose, also three doses used were more effective than one or two, while, differences between two or three doses were insignificant. On the other hand, plant fitness was slightly impaired with third dose than second one. INA and SE showed pronounced effect in inhibition nematode population after third dose compared with the rest chemicals, which showed mild increase in their efficacy from second to third doses. Unfortunately, three doses of SE were reduced plant fitness after enhanced by double doses, while INA was showed obvious phytotoxicity gradually increased by repeating doses. Gathering between the most effective application time (before inoculation) and the proper activated dose after inoculation was studies for emphasized their action and comparing with pre-inoculation only in suppressing *M. incognita* population. Chemical activators showed enhancing in peroxidase and polyphenoloxidase activities. In conclusion, CSA, NSA, BABA and SA were showed highest efficacy as resistance inducers. This collectively showed reduction of total population with pre-inoculation time application and pre plus post-inoculation application, 57.6&83.8%, 56.5&81.6%, 55.4&79.2% and 54.5&78.1%, respectively. Also the fecundity of nematode was taking similar trend as total population. The results suggest that tested chemicals especially CSA, NSA, BABA and SA have potential to suppress root-knot nematode infection in tomato plants through induced systemic resistance.

Key words: Induced resistance, chemical inducers, *Meloidogyne incognita*, tomato.

Introduction

Root-knot nematodes, *Meloidogyne* species are the most important plant parasitic nematodes and wide spread on a wide plant hosts range including agronomic and vegetable crops, ornamental, fruit trees and weeds, especially in tropical and subtropical countries causing economic losses **(Amin, 1994).** In vegetable crops production, especially tomato in greenhouses, most of the damage from continuous cropping is caused by soil-borne diseases & nematodes **(Molinari and Baser, 2010 and Amin & Mona, 2014).**

Control of nematode is complex and usually demands integrated management practices. The methods most widely used include chemical and biological control and resistant cultivars. However, the use of chemical nematicides, apart from the expenses incurred, can result in chemical residues harmful to humans and the environment as well as selecting for resistant nematodes **(Ghini and Kimati, 2000).**

Between tomato cultivars few were recorded as resistant to this pest. Genetic control to these important nematode species is limited mainly by the scarcity of high-resistance material by different meaning the lack of resistance for several crops or is present only in wild species or undeveloped genotypes represent a challenge. Resistance is typically a highly specific trait and is effective against only a single or a few nematode species. It may not be durable because of the selection of resistance-breaking populations that render the resistance effective in specific locations **(Starr and Roberts, 2004).** Other factors are also important, such as restriction to region, climate and nematode species **(Franzener *et al.,* 2007).**

Consequently, new strategies for the control of plant-parasitic nematodes have actively been sought in the last few years. Investigation has focused on biological control, organic and inorganic amendments, naturally occurring nematicides and induced resistance **(Oka *et al.,* 2000).** Induction of resistance has attracted the interest of researchers is the use of resistance inducers. Resistance inducers or elicitors can take the form of a chemicals or biotic agents whose function is to activate the plant's defense mechanisms **(Baysal *et al.,* 2003; Silva *et al.,* 2004; Bonaldo *et al.,* 2005 and Dias-Arieira *et al.,* 2012).** Systemic acquired resistance (SAR) and induced systemic resistance (ISR) are two known ways of inducing plant resistance to disease.

Resistance to pathogens can be chemically induced by applying to plants salicylic acid (SA) and compounds which can mimic the action of SA, such as acibenzolar-S-methyl(ASM) and 2,6-dicholoroisonicotinic acid (INA) **(Molinari and Baser, 2010).**

In this study, some chemical elicitors have been tested as inducers of resistance to RKNs taking into account the effect of different application times, the effect of doses number post-inoculation and the combination between the best time of application and proper number of doses.

Materials and Methods

Single egg-mass of *Meloidogyne incognita* was reared on tomato plants cv. Castel rock in 25 cm-diam. earthen pots containing more than one kg sand clay soil. Six weeks later, nematode second stage juveniles (J_2) were extracted by allowing egg-masses to hatch in Petri-dishes. Nematode inoculation was done using 1000 freshly hatched juveniles (J_2)/ pot.

Three experiments were carried out in sterilized soil (3:1 sand:clay v: v) in 25 cm-diam. earthen pots. Five-week old tomato seedlings, *Lycopersicon esculentum* Mill cv. Castel rock were grown in all experiments as susceptible host.

The first experiment (Time of application) was divided into three groups: the first group was received chemicals three day before nematode inoculation, the second group was received chemicals synchronize with inoculation time and the third group was received chemicals three days after inoculation time. One thousand freshly hatched juveniles of *M. incognita* were added per pot (each pot contains one tomato seedling).

Pots soil were drenched by 100 ml distilled sterilized water per plant with either 2.5 mM of acetylsalicylic acid (ASA), salicylic acid (SA) or 5-chlorosalicylic acid (CSA) or with either 1.25 mM nitro salicylic acid (NSA), 20 mM ascorbic acid (AS) or 20 mMDL-3-aminobutyric acid (BABA) or with either 0.62 mM selenium (SE) or 0.25mM 2.6-dichloroisonicotinic acid (INA). Four untreated inoculated pots were drenched with 100ml distilled sterilized water left as check treatment. The previous chemicals as the same concentrations were used in the present three experiments.

The second experiment (Effect of reiterative doses) was divided into three groups: the first group was received single dose of chemicals after 7 days from nematode inoculation time, the second group was received two doses of chemicals after 7 and 14 days from nematode inoculation time and third group was received three time of chemicals after 7, 14 and 21 days from nematode inoculation time.

The third experiment was divided into two groups: the first group was received chemicals at 3 days before nematode inoculation time (one dose). The second group was received chemicals at 3 days before nematode inoculation time (first dose) and 7 days after nematode inoculation time (second dose).

The plants under greenhouse were irrigated and fertilized according to the recommendations of the Egyptians Ministry of Agriculture. The treatments were replicated four times (4 pots) in a completely randomized block design. After 45 days of nematode inoculation, roots of plants were carefully uprooted and nematodes in soil and roots were counted and recorded based on galls, No. of juveniles in soil, developmental stages, mature female, egg masses numbers per plant and eggs per egg-mass. Reproductive factor (RF) compared to untreated pots was calculated for root-knot nematodes. The data were subjected to analysis of variance and means were separated by the least significant difference LSD at (p=0.05) using PLABSTAT program Version 3.

Enzymes extraction: Enzyme extract were prepared according to **Maxwell and Batemen (1967)** by grinding the root tissues which were collected from healthy and chemicals treated tomato plants in 0.1 µ sodium phosphate buffer at pH 7.1(2ml/gm fresh plant) for 1 min at high speed in a small homogenizer. These triturate tissues were strained through four layers of cheese cloth and the filtrates were centrifuged at 1500 g for 20 min at 4°C, the supernatant fluids were used for the enzymes assay.

Changes in peroxidase (POX) activity associated with the different treatments and healthy plants were determined following the procedure described by **Sridhar and Ou (1974).** Peroxidase activity was expressed as change in absorbance (Δ O.D 470 nm) per min/gram fresh weight of tomato roots.

Changes in polyphenol oxidase (PPO) activity associated with the different treatments and healthy plants were determined following the procedure described by **Maxwell and Batemen (1967).** The activity of PPO oxidase was expressed as change in absorbance (Δ O.D 495 nm)/1.0 ml of extract per min per gram fresh weight. Three replicates for each treatment were analyzed to determine of plant enzymes activity. Relative activity percentage compared with healthy tomato plant was calculated, (activity of treated/healthy) x100, and recorded.

Results

The results in Table 1 indicated that application of such chemicals (Effect of application time) three days pre-inoculation time was most effective than at or post-inoculation time. Moreover, CSA, NSA, BABA and SA were found to be more efficacious chemicals in suppressing *M. incognita* reproduction and developments. According to the previous arrangement, the galls reduction percentages were 89.8, 85.7, 83.7 and 81.6, while egg-masses (EM) reduction percentages recorded 91.1, 88.9, 86.7 and 82.2. The reduction percentages of total eggs deposited by these chemicals were 97.2, 96.2, 95.1 and 93.1 for CSA, NSA, BABA and SA respectively. ASA achieved similar reduction percentage of galls and egg-masses (75.5), while INA achieved (77.6 and 77.8) and total eggs were recorded 87.9 and 89.4. The minimum reduction was registered by SE and AS. At the second treatment, synchronous addition, the most effective chemicals were the same four chemicals. These chemicals occupied a descending order as CSA, NSA, BABA and

Table (1). Effect of application time on efficacy of certain chemical substances on development and reproduction of *Meloidogyne incognita* infected tomato roots.

Treat-ments	Chemical substances	No. of Galls	*%R	No. of Egg-masses	%R	No. of Eggs / egg- mass	%R	Total eggs	%R
3 days before inoculation	**Acetyl salicylic acid**	12	75.5	11	75.6	206	50.4	2266	87.9
	***β*-aminobutyric acid**	8	83.7	6	86.7	151	63.6	906	95.1
	Ascorbic acid	17	65.3	16	64.4	275	33.7	4400	76.4
	Chloroisonicotinic acid	11	77.6	10	77.8	198	52.3	1980	89.4
	Chlorosalicylic acid	5	89.8	4	91.1	131	68.4	524	97.2
	Nitro salicylic acid	7	85.7	5	88.9	141	66.0	705	96.2
	Salicylic acid	9	81.6	8	82.2	160	61.5	1280	93.1
	Selenium	16	67.4	14	68.9	232	44.1	3248	82.6
	Control	49	-	45	-	415	-	18675	-
	Mean time	**14.89**	-	**13.67**	-	**212.1**	-	-	-
With inoculation	**Acetyl salicylic acid**	18	63.3	17	62.2	249	40.0	4233	77.3
	***β*-aminobutyric acid**	13	73.5	12	73.3	180	56.6	2160	88.4
	Ascorbic acid	23	53.1	22	51.1	311	25.1	6842	63.4
	Chloroisonicotinic acid	15	69.4	15	66.7	219	47.2	3285	82.4
	Chlorosalicylic acid	11	77.6	10	77.8	149	64.1	1490	92.0
	Nitro salicylic acid	12	75.5	11	75.6	176	57.6	1936	89.6
	Salicylic acid	14	71.4	14	68.9	189	54.5	2646	85.8
	Selenium	21	57.1	20	55.6	258	37.8	5160	72.4
	Control	49	-	45	-	415	-	18675	-
	Mean time	**19.56**	**-**	**18.89**	**-**	**238.4**	-	-	-
3 days after inoculation	**Acetyl salicylic acid**	32	34.7	20	55.6	339	18.3	6780	63.7
	***β*-aminobutyric acid**	25	49.0	15	66.7	220	47.1	3300	82.3
	Ascorbic acid	38	22.5	26	42.2	374	9.9	9724	47.9
	Chloroisonicotinic acid	31	36.7	19	57.8	320	22.9	6080	67.4
	Chlorosalicylic acid	22	55.1	13	71.1	182	56.1	2366	87.3
	Nitro salicylic acid	23	53.1	14	68.9	212	48.9	2968	84.1
	Salicylic acid	26	46.9	17	62.2	227	45.3	3859	79.3
	Selenium	34	30.6	24	46.7	357	14.0	8568	54.1
	Control	49	-	45	-	415	-	18675	-
	Mean time	**31.11**	**-**	**21.44**	**-**	**294.0**	-	-	-
Mean chemical	**Acetyl salicylic acid**	20.7	-	16.0	-	264.7	-	-	-
	***β*-aminobutyric acid**	15.3	-	11.0	-	183.7	-	-	-
	Ascorbic acid	26.0	-	21.3	-	320.0	-	-	-
	Chloroisonicotinic acid	19.0	-	14.7	-	245.7	-	-	-
	Chlorosalicylic acid	12.7	-	9.0	-	154.0	-	-	-
	Nitro salicylic acid	14.0	-	10.0	-	176.3	-	-	-
	Salicylic acid	16.3	-	13.0	-	192.0	-	-	-
	Selenium	23.7	-	19.3	-	282.3	-	-	-
LSD 0.05 Chemicals		**1.41**		**1.12**		**10.04**			
LSD 0.05 Time		**1.10**		**0.79**		**0.83**			
LSD 0.05 CxT		**2.45**		**1.95**		**17.39**			

*%R= Reduction percentage.

SA, respectively according to galls formation, egg-masses production and total population reduction percentages. The lowest efficacy was related to AS (Table 1). Application of chemicals three days after nematode inoculation was recorded the same positions in the previous treatments and with the same descending order. SE treatment could be considered as the less effective inducer for suppressing nematode population Table 1.

Results listed in Table 2 showed an enhancement of tested chemicals efficacy at post-inoculation by repeating doses. Chlorosalicylic acid (CSA) had the highest ability to suppress the formation of galls to 95 galls and the production of egg-masses (EM) to 59 EM/plant compared to untreated control plant as one dose after inoculation, which formed 232 galls/plant and produce 143 EM/plant. On the other side, SE had the lowest capability to inhibit galls formation and egg-masses production where they recorded 165 galls and 119 EM/plant. The rested chemicals could rank in descending order according to their ability to diminish galls formation as nitrosalicylic acid (NSA)> β-aminobutyric acid (BABA)> salicylic acid (SA)> 2,6-dichloroisonicotinic acid (INA)> acetylsalicylic acid (ASA)> ascorbic acid (AS).

Concerning the second treatment, two doses, the highest effect that cease *M. incognita* galls formation was in combine with both CSA (41) and NSA (42) compared to control which formed 232 galls/plant. The feeblest effect showed by AS where it formed 150 galls/plant. The production of egg-masses took similar trend where CSA and NSA suppress EM/plant to 40 and 44 respectively. Moderate effects were related to BABA, SA and INA which produced 53, 59 and 63 respectively, while SE gave 94 EM/plant as a lesser effective chemical. The highest total population reduction was related to CSA (72.7%), NSA (70.4%), BABA (65.6%) and SA (62.5%). While the lowest reduction was 42.7 and 41.9% induced by SE and AS.

Regarding to the third treatment, three doses, there were three chemicals which could minimize gall formation; INA, SE and NSA where they recorded 34, 36 and 39 galls/plant. While the maximum gall formation was related to AS (130) compared to untreated control. INA stills the highest effective chemical on inhibiting EM/plant formation which recorded 15 EM/plant. Furthermore, AS still the lesser effective chemical where it gave 72 compared to control (143 EM/plant).

Data in presented in Table 3 showed that all chemicals with different doses encouraged plant growth criteria except INA whereas it induced decrement in weight of both shoot and root under the different doses (Fig. 1).

The efficacy of one application pre-inoculation (P) and two applications pre and post-inoculation with nematode (P.P) were tested, where this combination aims to increase the effectiveness of such chemical substance in field application (Table 4). The double application (P.P) maximized the ability of such chemical substance to

Table (2). Effect of post-inoculation reiterative doses of certain chemical substances on *Meloidogyne incognita* development and reproduction infected tomato roots.

Treatments	Chemical substances	Number of Galls	Number of Devel. stages	Number of Females	Number of Egg-masses	TP	%TPR*	Eggs / egg-mass
One dose post-inoculation	Acetyl salicylic acid	152	57	146	116	203	19.8	216
	β-aminobutyric acid	126	34	110	87	144	43.1	188
	Ascorbic acid	167	74	153	122	227	10.3	232
	Chloroisonicotinic acid	147	51	123	104	174	31.2	210
	Chlorosalicylic acid	95	30	75	59	105	58.5	138
	Nitro salicylic acid	104	36	82	63	118	53.4	173
	Salicylic acid	144	45	126	98	171	32.4	198
	Selenium	165	58	151	119	209	17.4	230
	Control	232	75	178	143	253	-	266
	Mean dose	**148.0**	**51.1**	**127.1**	**101.2**	-	-	**205.7**
Two dose post-inoculation	Acetyl salicylic acid	89	27	101	81	128	49.4	176
	β-aminobutyric acid	60	19	68	53	87	65.6	115
	Ascorbic acid	150	41	106	84	147	41.9	221
	Chloroisonicotinic acid	79	22	78	63	100	60.5	161
	Chlorosalicylic acid	41	15	54	40	69	72.7	94
	Nitro salicylic acid	42	17	58	44	75	70.4	104
	Salicylic acid	79	20	75	59	95	62.5	118
	Selenium	91	31	114	94	145	42.7	223
	Control	232	75	178	143	253	-	266
	Mean dose	**95.9**	**29.7**	**92.4**	**73.4**	-	-	**165.3**
Three doses post-inoculation	Acetyl salicylic acid	85	13	84	65	97	61.7	169
	β-aminobutyric acid	58	12	52	39	64	74.7	103
	Ascorbic acid	130	14	95	72	109	56.9	192
	Chloroisonicotinic acid	34	4	25	15	29	88.5	54
	Chlorosalicylic acid	46	8	44	33	52	79.4	62
	Nitro salicylic acid	39	9	47	35	56	77.9	84
	Salicylic acid	77	13	69	55	82	67.6	105
	Selenium	36	7	45	26	52	79.4	90
	Control	232	75	178	143	253	-	266
	Mean dose	**81.9**	**17.2**	**71.0**	**53.7**	-	-	**125.0**
Mean chemical	Acetyl salicylic acid	108.6	32.3	110.3	87.3	-	-	187.0
	-aminobutyric acidβ	81.3	21.7	76.7	59.7	-	-	135.3
	Ascorbic acid	149.0	43.0	118.0	92.7	-	-	215.0
	Chloroisonicotinic acid	86.7	25.7	75.3	60.7	-	-	141.7
	Chlorosalicylic acid	60.7	17.7	57.7	44.0	-	-	98.0
	Nitro salicylic acid	61.7	20.7	62.3	47.3	-	-	120.3
	Salicylic acid	100.0	26.0	90.0	70.7	-	-	140.3
	Selenium	97.3	32.0	103.3	79.7	-	-	184.3
LSD 0.05 Chemicals (C)		**8.45**	**2.95**	**3.40**	**5.61**			**6.94**
LSD 0.05 Doses (D)		**6.08**	**2.11**	**3.10**	**3.49**			**3.61**
LSD 0.05 CxD		**14.63**	**5.12**	**10.23**	**9.72**			**12.01**

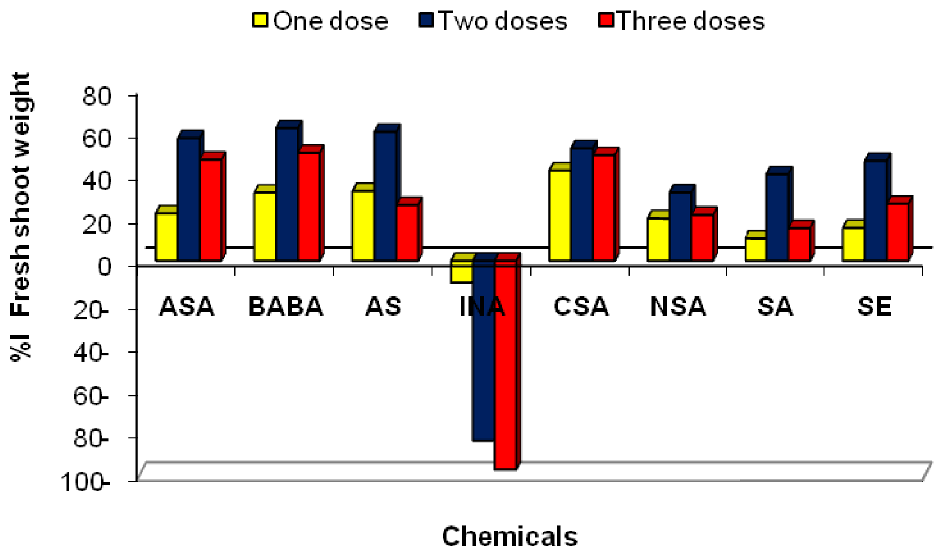
***%TPR** = Total population Reduction.

Table (3). Effect of reiterative doses of certain chemicals on growth responses of tomato plants infected by *Meloidogyne incognita*.

Doses	Chemical substances	Fresh shoot weight	Dry shoot weight	Shoot length	Root weight	Root length
One dose post-inoculation	Acetyl salicylic acid	6.6	1.5	26.3	2.7	28.6
	β-aminobutyric acid	7.5	1.6	30.7	3.6	24.6
	Ascorbic acid	7.6	1.5	26.7	3.9	24.6
	Chloroisonicotinic acid	4.7	1.2	27.3	2.0	23.0
	Chlorosalicylic acid	8.8	1.6	31.7	3.6	28.3
	Nitro salicylic acid	6.4	1.3	28.0	2.3	26.0
	Salicylic acid	5.7	1.3	27.3	2.7	23.6
	Selenium	6.1	1.3	26.3	2.3	25.6
	Control	5.1	1.3	26.0	2.3	21.6
	Mean dose	**6.50**	**1.41**	**27.81**	**2.82**	**25.10**
Two doses post-inoculation	Acetyl salicylic acid	11.9	2.1	37.0	5.7	34.0
	β-aminobutyric acid	13.4	2.3	35.3	6.5	31.0
	Ascorbic acid	12.9	1.9	32.7	5.6	29.0
	Chloroisonicotinic acid	2.8	1.2	27.0	1.9	22.6
	Chlorosalicylic acid	10.8	2.0	35.0	5.1	30.3
	Nitro salicylic acid	7.5	1.7	34.7	5.5	32.3
	Salicylic acid	8.6	2.0	36.7	4.7	31.6
	Selenium	9.6	1.9	32.0	5.4	30.3
	Control	5.1	1.3	26.0	2.3	21.6
	Mean dose	**9.17**	**1.82**	**32.92**	**4.75**	**29.19**
Three doses post-inoculation	Acetyl salicylic acid	9.7	1.9	27.7	5.3	31.6
	β-aminobutyric acid	10.3	1.9	33.0	3.5	29.0
	Ascorbic acid	6.9	1.4	31.3	3.5	29.0
	Chloroisonicotinic acid	2.6	1.2	26.3	1.7	22.0
	Chlorosalicylic acid	10.1	1.9	32.3	5.0	29.3
	Nitro salicylic acid	6.5	1.4	31.7	3.8	27.7
	Salicylic acid	6.1	1.3	26.7	3.5	27.0
	Selenium	7.0	1.5	28.7	2.4	29.3
	Control	5.1	1.3	26.0	2.3	21.6
	Mean dose	**7.15**	**1.50**	**29.29**	**3.44**	**27.39**
Mean chemical	Acetyl salicylic acid	9.41	1.84	30.33	4.59	31.40
	β-aminobutyric acid	10.41	1.91	33.00	4.50	28.22
	Ascorbic acid	9.13	1.61	30.22	4.31	27.55
	Chloroisonicotinic acid	3.35	1.20	26.89	1.90	22.53
	Chlorosalicylic acid	9.90	1.83	33.00	4.56	29.30
	Nitro salicylic acid	6.81	1.46	31.44	3.88	28.66
	Salicylic acid	6.78	1.54	30.22	3.64	27.40
	Selenium	7.54	1.57	29.00	3.38	28.41
	LSD 0.05 Chemicals(C)	**0.50**	**0.08**	**1.74**	**0.18**	**1.38**
	LSD 0.05 Doses(D)	**0.33**	**0.05**	**1.28**	**0.14**	**1.32**
	LSD 0.05 CxD	**0.87**	**0.14**	**3.01**	**0.31**	**2.39**

Fig. (1): Effect of post-inoculation reiterative doses of certain chemicals on increment percentage of tomato growth parameters infected by *M. incognita*.

(A)



(B)

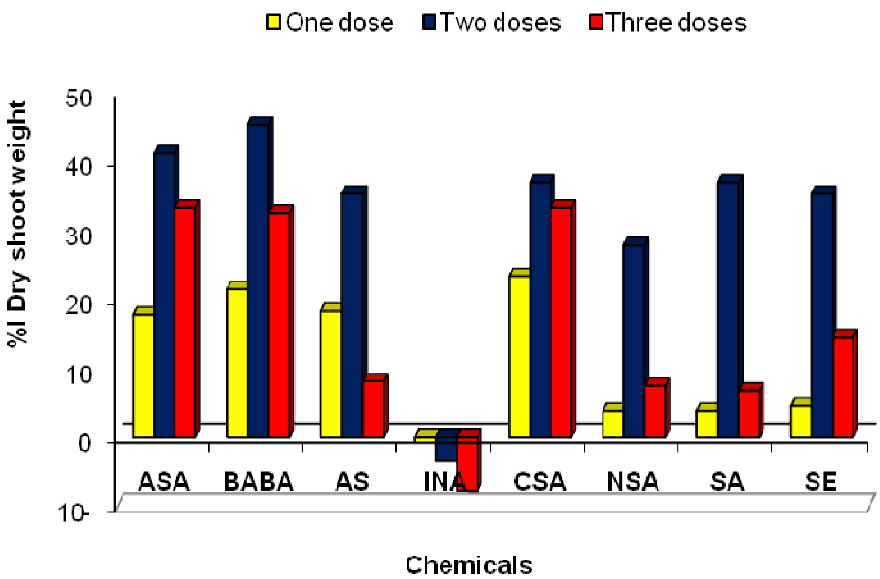


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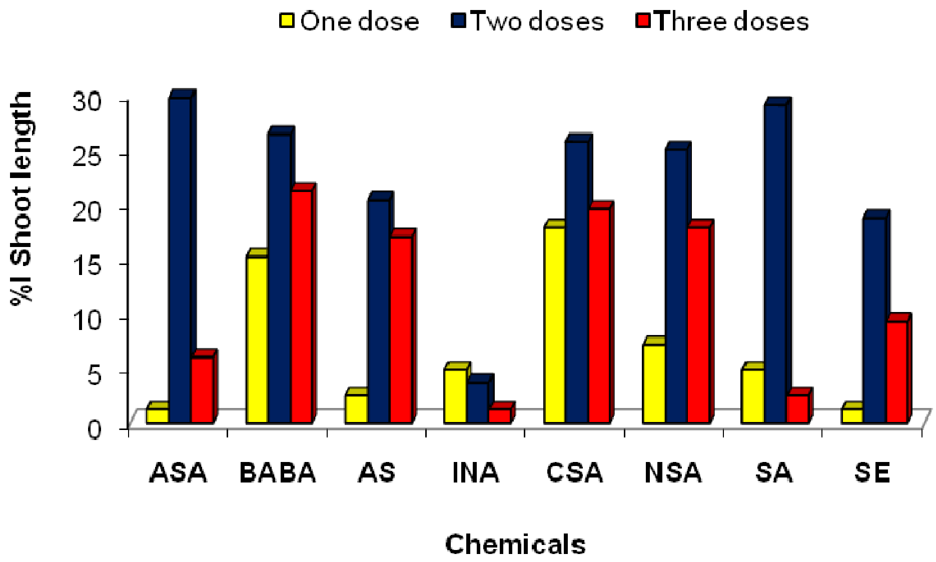
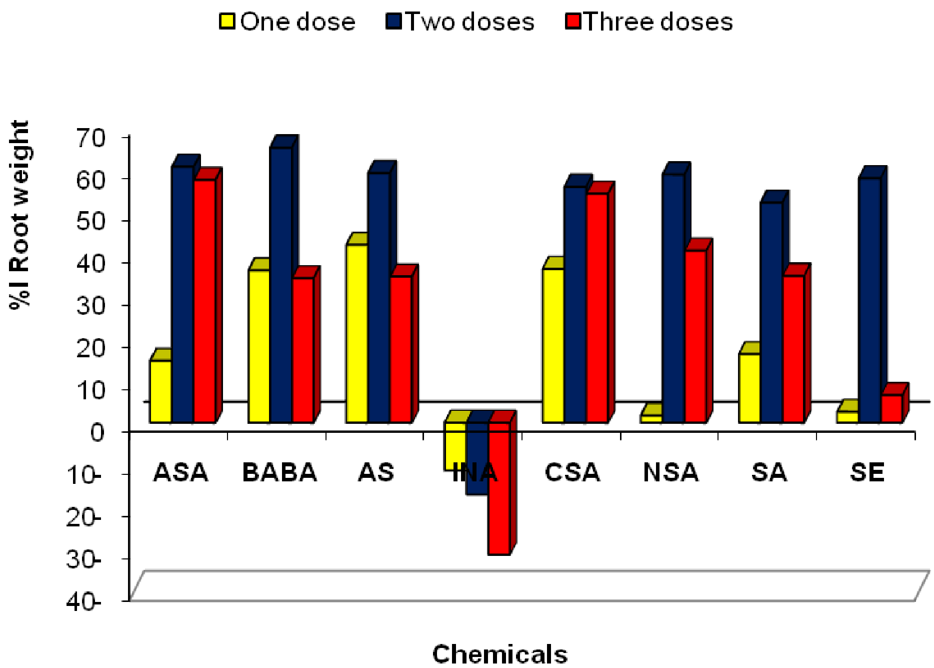
Fig. (1): Cont.

(C)



(D)



ASA=Acetylsalicylic acid, BABA= *β*-aminobutyric acid, AS= Ascorbic acid, CSA= Chlorosalicylic acid, INA= Chloroisonicotinic acid SA=Salicylic acid, NSA=Nitrosalicylic acid, SE= Selenium.

suppress nematode infection and reproduction. Thus, galls/plant formation by CSA and NSA were diminished to 112 and 116 respectively, while the same chemicals with (P) application minimized the galls to 151 and 158 consecutively. Concerning AS which gave the highest galls number (296) in (P) application comparing to untreated control, that permit to form 422, itself gave 273 with (P.P) as the highest value. However, it was noted that all tested chemicals exhibited high efficacy when the chemical applied pre and post nematode inoculation compared with pre-application only. The egg-masses/plant production confirmed the same previous trend, where as, pre and post-applications was more successful than pre-application only. Besides, both of CSA and NSA achieved the most suppressive effect, where they could decrease egg-masses production to 102 and 111 in succession with (P.P) application while these values increased to 161 and 167 order to with (P) application. The maximum egg-masses production (247) was induced by SE with (P) comparing to control treatment that recorded 382, but the increment with (P.P) was lesser where SE registered 238 EM/plant. Total population reduction was maximized by the same chemicals CSA and NSA which presented 84.0 and 81.8% under (P.P) facing to 57.6 and 56.5% with (P) application. On the other side, AS registered the lowest reduction as well as 35.1 and 25.7% respectively.

The plant growth parameters were positively affected by addition of tested chemical both pre (p) or pre and post (P.P) nematode inoculation with *M. incognita* except with INA (Table 5). Concerning plant shoots, salicylic acid (SA) maximized both of fresh and dry weight for P or P.P application, however the P.P treatment was more effective where it recorded 40.5 (P.P), 31.7 (P) and 5.5 (P.P), 4.7 (P) gm for fresh and dry weight, respectively. Also SA gave the highest length as 47.3 cm with the P.P treatment, while ASA registered the maximum shoot length (43 cm) with (P). Root weight was maximized by ASA (13.12 gm) without significant differences between it and SA (12.7gm) or CSA (12.27 gm.) with pre and post treatment.

Data in Table 6 showed that activity of two enzymes was elevated in chemically-treated tomato plants. The maximum activity of peroxidase was related to the treatment of CSA, (2.916) and the minimum activity was recorded by SE (0.898) compared to the untreated and inoculated control (0.782), while, the healthy plant registered (0.338). The rest chemicals were arranged according to their ability to enhance POX activity in descending order as follow: NSA, BABA, SA, INA, ASA, AS respectively. CSA substance maximized the activity of polyphenol oxidase enzyme (2.027), and SE is the substance that minimizes the activity of this enzyme (0.560) compared to the infected and untreated control (0.204) and the healthy plant (0.107). On the other hand, the other chemicals could rank in descending order similar to with POX.

Table (4). Effect of pre and post-inoculation application of chemical substances on *Meloidogyne incognita* development and reproduction infected with tomato plants under greenhouse conditions.

Chemical substances	Juveniles in soil		Mean C	Developmental stages		Mean C	Mature females		Mean C	Egg masses		Mean C
	Pre-*	P** Post-		Pre-	P Post-		Pre-	P Post-		Pre-	P Post-	
Acetyl salicylic acid	13942 (30.5)	11995 (40.2)	**12968.5**	128 (32.5)	74 (60.7)	**101**	246 (41.7)	220 (51.7)	**233**	234 (38.7)	210 (45.0)	**222**
β-aminobutyric acid	8928 (55.5)	4073 (79.7)	**6500.5**	80 (57.5)	50 (73.4)	**65**	189 (53.3)	130 (63.3)	**159.5**	180 (52.9)	123 (67.8)	**151.5**
Ascorbic acid	14893 (25.7)	13062 (34.9)	**13977.5**	144 (23.8)	102 (45.9)	**123**	298 (35.0)	231 (40.0)	**264.5**	285 (25.3)	219 (42.7)	**252**
Chloroisonicotinic acid	9742 (51.4)	4891 (75.6)	**7316.5**	117 (37.9)	62 (67.0)	**89.5**	228 (45.0)	167 (55.0)	**197.5**	217 (43.3)	158 (58.6)	**187.5**
Chlorosalicylic acid	8517 (57.5)	3152 (84.3)	**5834.5**	69 (63.7)	41 (78.5)	**55**	168 (66.7)	108 (70.0)	**138**	161 (57.8)	102 (73.2)	**131.5**
Nitro salicylic acid	8733 (56.5)	3591 (82.1)	**6162**	72 (61.7)	47 (75.3)	**59.5**	176 (56.7)	118 (65.0)	**147**	167 (56.3)	111 (71.0)	**139**
Salicylic acid	9103 (54.6)	4268 (78.7)	**6685.5**	87 (54.0)	52 (72.3)	**69.5**	197 (50.0)	148 (60.0)	**172.5**	187 (51.1)	140 (63.4)	**163.5**
Selenium	13427 (33.1)	12469 (37.8)	**12948**	130 (31.0)	79 (58.4)	**104.5**	259 (38.3)	249 (48.3)	**254**	247 (35.3)	238 (37.6)	**242.5**
Control	20055		-	189		-	402		-	382		-
Mean Time	**11926.7**	**8617.3**	-	**112.9**	**77.3**	-	**240. 3**	**197**	-	**228.9**	**187**	-
LDS 0.05 Chemicals (C)	**729.57**			**4.83**			**9.94**			**9.63**		
LDS 0.05 Time (T)	**858.78**			**3.52**			**6.66**			**6.39**		
LDS 0.05 CxT	**1031.7**			**6.83**			**14.08**			**13.62**		

Table (4). Cont.

Chemical substances	Galls		Mean C	Eggs/eggmass		Mean C	Total population		%R Pre-	%R P Post-	Reproduction factor	
	Pre-	P Post-		Pre-	P Post-		Pre-	P.P			Pre-	P Post-
Acetyl salicylic acid	259 (38.6)	230 (45.6)	**244.5**	365 (36.2)	329 (42.5)	**347**	14316	12289	30.66	40.48	14.31	12.28
β-aminobutyric acid	169 (59.9)	142 (66.3)	**155.5**	287 (49.9)	244 (57.3)	**265.5**	9197	4253	55.45	79.40	9.19	4.25
Ascorbic acid	296 (29.9)	273 (35.2)	**284.5**	415 (27.5)	379 (33.7)	**397**	15335	13395	25.72	35.12	15.33	13.39
Chloroisonicotinic acid	220 (48.0)	194 (54.1)	**207**	346 (39.5)	314 (45.1)	**330**	10087	5120	51.14	75.20	10.08	5.12
Chlorosalicylic acid	151 (64.3)	112 (73.5)	**131.5**	277 (51.6)	212 (63.3)	**244.5**	8754	3301	57.60	84.01	8.75	3.30
Nitro salicylic acid	158 (62.6)	116 (72.5)	**137**	283 (50.5)	220 (61.6)	**251.5**	8981	3756	56.50	81.81	8.98	3.75
Salicylic acid	189 (55.1)	159 (62.2)	**174**	309 (46.0)	251 (56.1)	**280**	9387	4468	54.53	78.36	9.38	4.46
Selenium	267 (36.7)	239 (43.4)	**253**	377 (34.1)	354 (38.1)	**365.5**	13816	12797	33.08	38.02	13.81	12.79
Control	422		-	572		-	20646		-	-	20.64	
Mean Time	**236.8**	**209.7**	-	**359**	**319.4**	-	-	-	-	-	-	-
LDS 0.05 Chemicals (C)	**8.43**			**9.50**								
LDS 0.05 Time (T)	**6.84**			**11.23**								
LDS 0.05 CxT	**11.92**			**13.44**								

Pre-= pre-inoculation application only, "PPost-"= pre plus post- inoculation applications, %R=Reduction percent, Values between parentheses (%R) represented reduction%.

Table (5). Effect of pre and post-inoculation application of chemical substances efficacy versus pre-inoculation application only on growth parameters of tomato plants infected with *M. incognita* under greenhouse conditions.

Chemical substances	Shoot									Root					
	Fresh weight		Mean C	Dry weight		Mean C	length		Mean C	weight		Mean C	length		Mean C
	Pre-*	P** Post-		Pre-	P Post-		Pre-	P Post-		Pre-	P Post-		Pre-	P Post-	
Acetyl salicylic acid	30.29	37.26	**33.78**	4.41	5.29	**4.85**	43.00	46.33	**44.67**	10.78	13.12	**11.95**	33.00	35.00	**34.00**
β-amino butyric acid	29.22	35.04	**32.13**	4.12	4.66	**4.39**	39.67	44.00	**41.84**	10.43	11.52	**10.98**	32.00	33.33	**32.67**
Ascorbic acid	30.34	31.91	**31.13**	3.38	4.05	**3.72**	39.00	40.33	**39.67**	9.13	10.37	**9.75**	30.33	31.00	**30.67**
Chloroisonicotinic acid	9.94	2.04	**5.99**	1.34	0.58	**0.96**	25.67	18.00	**21.84**	3.79	1.78	**2.79**	24.00	18.33	**21.17**
Chlorosalicylic acid	25.22	30.02	**27.62**	3.54	4.47	**4.01**	41.00	44.67	**42.84**	10.60	12.27	**11.44**	31.67	34.00	**32.84**
Nitro salicylic acid	28.98	31.46	**30.22**	3.87	4.56	**4.22**	40.33	43.00	**41.67**	9.36	10.44	**9.90**	32.67	33.00	**32.84**
Salicylic acid	31.72	40.54	**36.13**	4.74	5.49	**5.12**	42.00	47.33	**44.67**	9.92	12.70	**11.31**	34.00	37.00	**35.50**
Selenium	33.41	32.26	**32.84**	3.69	3.24	**3.47**	41.00	38.67	**39.84**	9.88	9.10	**9.49**	31.33	30.67	**31.00**
Nematode infected plant	13.49		-	2.08		-	32.00		-	5.72		-	28.00		-
Mean Time	**25.85**	**28.22**	-	**3.46**	**3.82**	-	**38.19**	**39.37**	-	**8.85**	**9.67**	-	**30.78**	**31.15**	-
LSD 0.05 Chemicals	**1.58**			**0.49**			**2.42**			**0.83**			**2.49**		
LSD 0.05 Time	**2.11**			**0.41**			**2.25**			**0.31**			**1.94**		
LSD 0.05 CxT	**2.24**			**0.69**			**3.42**			**1.17**			**3.53**		

Pre-*= Pre-inoculation application only, P** Post- = Pre and post- inoculation application, C= chemicals.

Table (6). Effect of some chemical substances on peroxidase and polyphenol oxidase activities in tomato roots infected with *M. incognita*.

Chemical substances	Enzymes			
	Peroxidase		Polyphenol oxidase	
	Activity	Relative activity	Activity	Relative activity
Acetyl salicylic acid	1.155	342.08	1.102	1033.01
***β*-aminobutyric acid**	2.248	665.75	1.529	1432.89
Ascorbic acid	1.057	313.14	0.658	616.47
Chloroisonicotinic acid	1.262	373.66	1.138	1066.33
Chlorosalicylic acid	2.916	863.10	2.027	1899.41
Nitro salicylic acid	2.533	749.95	1.804	1691.14
Salicylic acid	1.662	492.07	1.209	1132.98
Selenium	0.898	265.77	0.560	524.84
Healthy (Uninfected untreated)	0.338	100.00	0.107	100.00
Check (Infected untreated)	0.782	231.56	0.204	191.61
LDS 0.05	**0.21**		**0.39**	

Discussion

The previous results demonstrate that the pre-inoculation addition of chemicals is more effective than the post-inoculation. These results are in accordance with **Arrigoni *et al.*, (1979); Al-Sayed, (1992) and Nandi *et al.*, (2000), (2002& 2003).** In 2005 **Pandey and Kalra** showed that ASA, INA, NSA, CSA, SA and isonicotinamide applied as pre-infection could suppress nematode reproduction. Also, **Sanz *et al.,* (2008)** found a reduction in galls in relation to the application of INA and SA to tomato two days before infection with *M. incognita*. **Molinari and Baser (2010)** confirmed these results and mentioned that the effect of the pre-inoculation indicates the persistence of defense elicitation by a determined systemic resistance acquired (SRA) effect for a long time. Possible mechanism explaining the efficacy of pre-inoculation treatment of chemical inducers was supposed by **Cohen and Gisi (1994)** they mentioned that BABA is not metabolized in tomato plants; it is thought to bind to cell-wall proteins, resulting in cell walls that are resistant to infection. They added another possible mechanism of resistance may result from synthesis in tomato roots of compounds with deleterious effects on nematode and giant sell development. Nematodes may ingest BABA directly through the giant cells, which would then interfere with normal amino acid and protein synthesis by the nematodes. It is evident that BABA has been found in tomato root exudates, **Gamliel and Katan (1993).**

Dichloroisonicotinic acid (INA) has been shown to induce disease resistance in a number of plants including green bean **(Dann and Deverall, 1995)** against a broad range of pathogens. In addition to, **Dann *et al.,* (1998)** suggested that INA treatment may stimulate inherent defense mechanisms so plant can respond more quickly against infection. INA provided as soil-drench at concentrations lower than that used here did reduce egg-masses and nematode reproduction, although with negative effects on plant fitness these finding are in agreement with **Chinnasri *et al.,* (2006).** Salicylic acid (SA) is an endogenous signal for the activation of certain plant defense responses by expression of genes for pathogenesis-related protein (PR-1) and enhanced resistance to pathogens. SA, in particular, has a biotic role in nematode susceptible plants and it has been regarded as resistance inducer **(Nandi *et al.,* 2003, Osman *et al.,* 2012 and Zinovieva *et al.,* 2013).**

The effect of repeating dose after nematode inoculation on activation of chemical inducers efficacy was obvious in our results and are in agreement with **Oka *et al.,* (1999).** They demonstrated that addition of BABA reduced the number of *M. javanica* eggs and galls on infected tomato roots. They also found that two doses after inoculation with nematode was better than one dose and near to three doses and the differences between two or three doses were not significantly different. Also in 2010 **Molinari and Baser** indicated that the efficacy of activators in eliciting resistance to root-knot nematode is strictly dependent on the amount applied which in turn determines the amount of chemicals adsorbed by the plants. Although depending on the amount of chemical provided, root adsorption may be influenced by an array of factors, such as the method of application, the age and health of the adsorbing plants and the environmental conditions.

Plant growth was positively reacted in general due to addition chemicals when used in proper dose; these data are compatible with those of **Molinari (2008).** He has shown that appropriate doses of SA provided to well-developed tomato plants may markedly reduce root-knot nematodes infestation and reproduction with no negative effects on plant fitness. It is likely that SA inhibit the penetration and/or the establishment of the feeding sites by the invading juveniles, thus encouragement in plant growth criteria occur. Repeating application of certain chemical was not always benefit for plant growth, these may due to their effect on plant physiological processes and metabolism, which became pronounced as concentrations elevated inside plant cell sap. On the other hand, some chemical can accumulated in plant tissues caused phytotoxic effect or rendering growth. Unfortunately, INA was phytotoxic to tomato and the toxicity increased by increasing the amounts added to roots. These findings are similar to recorded by **(Molinari and Baser, 2010).** Selenium may be accumulated in plants resulting toxicity when reached to such level which interfered with plant metabolic activity.

Phytotoxicity caused by the application of some SAR inducers has been increasingly documented. The mechanism responsible for the reduced plant fitness associated with SAR induction is not known **(Cipollini *et al.,* 2003),** although resource allocation tradeoff has been widely supported as a key mechanism. **Baldwin *et al.*, (1998)** found that induced responses caused an increase in nicotine content, which is a putative defense compound. In addition, **Baldwin and Callahan (1993)** found that high levels of nicotine lead to autotoxicity to plants. Recent experiments utilizing differential display or microarrays to analyze gene expression have shown that induced plant responses are associated with the coordinate up-regulation of many defense-related transcripts and the down regulation of transcripts involved in primary metabolism **(Reymond *et al.,* 2000 and Hermsmeier *et al.,* 2001).** These findings support the assumption that upon induction, resources are allocated toward defense and away from primary metabolism, leading to fitness costs in the plant.

Many authors stated that pre and post infection application of chemical inducer were more effective than pre infection application only **(Oka *et al.,* 1999 and Mutar & Fattah, 2013).** They added that plants treated with BABA render roots less attractive to *Meloidogyne* juveniles through altered plant nutrient assimilation or render plant cell walls harder to penetrate by J_2 which caused the formation of smaller giant cells which are not able to provide enough nutrients for the developing nematodes. In addition to, **Dann, *et al.,* (1998)** suggested that INA treatment may stimulate inherent defense mechanisms so plant can respond more quickly against infection. The promised results which gained by the combination of pre and post inoculation application may be due to increasing the amount of chemical provided, so when the chemical inducer is abundance within plant the induction resistance is extended to adversely affected nematode development, as well as enhance plant growth.

Activation POX and PPO is a general response of infected plants tissue and its leaves have been correlated with resistance **(Sridhar and Ou, 1974).** In another study, **Kataria *et al.,* (1997)** found that pretreated of bean seedling with NSA, ASA and INA acquired a high level of POX activity. **Mostafa and Youssef, (2007)** stated that ethyl salicylic and jasmonic acid increased the POX activity. In particular POX activity has been reported to be biochemical marker for resistance and to be associated with systemic resistance **(Mosa, 2002 and Nawar & Kuti, 2003).** Using some chemical compounds like SA and AS showed increasing in POX and PPO activity **(Saeed, 2005).**

Thus, the measurement of POX and PPO activities may provide a convenient method for screening and quantification of inducers activity. Moreover, it is evident that enzymes in host plants play an important role in the mechanisms of resistance to nematodes, in other words nematode infection enhanced enzyme activity. Induction of *Mi*-mediated nematode resistance is correlated with increased activity of several enzymes implicated in defense; POX and PPO **(Zacheo *et al.*, 1993).** So, the increasing of these enzymes are an active response in systemic induce resistance **(Irving and Kuc, 1990).**

In conclusion: SAR inducers differed in their ability to reduce nematode reproduction on tomato, while CSA, NSA, BABA, and SA are among the most potent SAR inducers. Differential potency among SAR inducers and between nematode species may be due to different activation points along the signal transduction pathway of SAR. Also, chemical activators which correctly applied at the most effective dosages can be used for nematode management in conventional and organic tomato protected cultivation, better if included in integrated management programs. Further investigations are needed to verify whether such SAR elicitors may be effective in limiting nematode infestation to other crops, or whether their application may be feasible also in field conditions. On the other hand, INA was found to have phytotoxic effects than the other SAR elicitors used and, therefore, a lower dosage was applied to plants for induction of resistance.

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الملخص العربي

تقييم بعض المواد الكيماوية كمستحثات لمقاومة نباتات الطماطم ضد نيماتودا تعقد الجذور (ميلودوجيني انكوجنيتا)

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** قسم وقاية النبات– شعبة البيئة– مركز بحوث الصحراء – القاهرة

تعتبر نيماتودا تعقد الجذور من أخطر الآفات علي نباتات الطماطم والتي تسبب خسائر اقتصادية كبيرة ولما كان الاتجاه حديثًا لتقليل استخدام المبيدات الكيماوية لما لها من آثار خطيرة علي البيئة عامة وصحة الإنسان وحيواناته بصورة خاصة فقد أصبحت الحاجة ملحة لوسائل أكثر أمانا لمكافحة النيماتودا. من الوسائل الحديثة هو تحفيز مقاومة النبات السليقية ضد الاجهادات الحيوية (الممرضات) أو غير الحيوية. ويهدف هذا البحث إلى تقييم استخدام بعض المواد الكيماوية مثل: حمض الساليسيلك، الاستيل ساليسيلك، الكلوروساليسيلك، النيتروساليسيلك، الكلوروايزونيكوتينك، الاسكوربيك، البيتا امينوبيوتيرك ومادة السلينيوم على خفض معدل تكاثر النيماتودا تحت ظروف الصوبة وذلك من خلال تأثير ميعاد الإضافة و تكرار الجرعات بعد ظروف العدوى فقط و الجمع بين أفضل ميعاد للتطبيق و أفضل تكرار من الجرعات بعد العدوى والذي لا يؤثر سلبًا على صحة النباتات.

و قد أظهرت النتائج ما يلي:

– كان لميعاد الإضافة تأثيرًا إيجابيًّا عندما تم إضافة الكيماويات قبل العدوى بالنيماتودا بثلاث أيام تلتها الإضافة المتزامنة مع العدوى وكان أقلها تأثيرًا الإضافة بعد العدوى بثلاث أيام.

– زاد تأثير المواد على خفض تعداد النيماتودا بزيادة عدد الجرعات وقد اختلف تأثر المواد بتكرار الجرعات، حيث وجد أن مادة السلينيوم زاد تأثيرها بعد الجرعة الثالثة بفارق كبير عن الثانية ومادة الكلورايزونيكوتينك أظهرت سمية زاد تأثيرها تدريجيًّا بزيادة عدد الجرعات. وعمومًا فقد كان تأثير الجرعتان أفضل من تأثير الثلاثة عند الأخذ في الاعتبار التأثير على نمو وصحة نباتات الطماطم و كذلك التكلفة الاقتصادية.

– عند الجمع بين تأثير أفضل ميعاد للإضافة (قبل العدوى) وأفضل تكرار للجرعات بعد العدوى (جرعتان) مع مقارنة ذلك في نفس الوقت بمجموعة نباتات عوملت قبل العدوى فقط، فقد لوحظ أن تأثير الإضافة قبل وبعد العدوى أفضل من تأثيرها قبل العدوى فقط مما يدل على أن الجرعات بعد العدوى تحدث تنشيط لما قبلها وتتداخل سلبيًّا مع نمو و تطور النيماتودا.

– بالنسبة لتقدير النشاط الإنزيمي للبيروكسيديز والبولي فينول أوكسيديز فقد أظهرت النتائج حدوث زيادة في النشاط الإنزيمي لكلاهما بعد المعاملة بالمواد الكيماوية مما يدل علي حدوث نوع من استحثاث المقاومة في نبات الطماطم وقد ارتبط هذا بانخفاض في تعداد النيماتودا.

يستنتج من هذا أن تلك المواد لها قدرة علي حفز مقاومة نباتات الطماطم ضد الإصابة بنيماتودا تعقد الجذور وبالتالي يمكن استخدامها في برامج الإدارة المتكاملة لتلك الآفة، مع الأخذ في الاعتبار عمل دراسات أكثر توسعا على مستوى الحقل والتنويع في طرق التطبيق والفترات بين الإضافة و تكرارها مع تقييمها مع أنواع نباتية أخرى كل هذا مع مراقبة للحالة الصحية للنباتات لتعظيم الاستفادة من استخدام تلك المواد.