Pakistan Journal of Nematology, 2014, Vol. 32(2): 195-209

Evaluation of inducers for tomato resistance against *Meloidogyne incognita*

A.A. Anter, A.W. Amin[†], A.H. Ashoub[*] and A.S. El-Nuby[*]

Nematology and Agriculture Zoology Department, Faculty of Agriculture, Cairo University, Egypt
[*]*Nematology Unit, Plant Protection Department, Desert Research Center, Cairo, Egypt*

[†]Corresponding author email: aminamin280@gmail.com

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 development and reproduction of *Meloidogyne incognita* in tomato plants cv. Castel Rock was investigated under greenhouse conditions. All inducers were applied as soil drench to tomato plants grown in 25 cm-diam. earthen pots. Three days-before nematode inoculation time treatment maximized the efficacy of tested chemicals 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 revealed that three doses remained more effective than one or two. While, differences between two or three time 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. However, three doses of SE were reduced plant fitness after enhanced by double doses. While, INA showed 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. Enzyme activity of both peroxidase and polyphenol oxidase were elevated in infected tomato roots than healthy ones. Chemical activators showed enhancing in these antioxidant activities, indicating the SAR four chemicals was occurred. In conclusion, CSA, NSA, BABA and SA have potential to suppress root-knot nematode infection in tomato plants through induced systemic resistance.

Keywords: Different inducers, tomato resistance, *Meloidogyne incognita*, drench method

Root-knot nematodes *Meloidogyne* species are one of 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 and nematodes (Molinari & Baser, 2010; 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 resulted in chemical residues harmful to humans and the environment as well as selecting for resistant nematodes (Ghini & Kimati, 2000). Between tomato cultivars few were recorded as resistant to this pest. Genetic control to these important nematode species are 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 & 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 agent to activate the plant's defense mechanisms (Baysal *et al*., 2003; Silva *et al*., 2004; Bonaldo *et al*., 2005; 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 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-dichloroisonicotinic acid (INA) (Oostendorp *et al*., 2001). In this study, some chemical substances 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 root-knot nematode *Meloidogyne incognita* was reared on tomato *Lycopersicon esculentum* Mill cv. Castel Rock in 15 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 sandy clay v:v) in 25 cm-diam. earthen pots. Five week-old tomato seedlings were grown in all experiments as susceptible host. The first experiment (time of application) was divided into three groups: the first group received chemicals three days before nematode inoculation, the second group received chemicals synchronize with inoculation time and the third group 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 2.5 mM of acetylsalicylic acid (ASA), salicylic acid (SA) or 5-chlorosalicylic acid (CSA) or with 1.25 mM nitro salicylic acid (NSA), 20 mM ascorbic acid (AS) or 20 mM DL-3-aminobutyric acid (BABA) or with 0.62 mM selenium (SE) or 0.25 mM 2.6-dichloroisonicotinic acid (INA). Four untreated inoculated pots were drenched with 100 ml 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 received single dose of chemicals after 7 days from nematode inoculation time, the second group received two times of chemicals after 7 and 14 days from nematode inoculation time and third group 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 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 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 separated by the least significant difference LSD at ($p = 0.05$) using PLABSTAT program Version 3.

Chemical analysis: Enzymes extract was prepared according to Maxwell and Batemen (1967) by grinding the root tissues which collected from healthy and chemicals treated tomato plants in 0.1 µ sodium phosphate buffer at pH 7.1 (2 ml/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 centrifuged at 1500 g for 20 min at 4 °C, the supernatant fluids used for the enzymes assay. Changes in peroxidase activity associated with the different treatments and healthy plants were determined following the procedure described by Sridhar & Ou (1974). Peroxidase activity was expressed as change in absorbance (Δ O.D 470 nm) per min/gram tomato fresh weight. Changes in polyphenol oxidase activity associated with the different treatments and healthy plants were determined following the procedure described by Maxwell and Batemen (1967). The activity of polyphenol oxidase was expressed as change in absorbance (Δ O.D 495 nm)/1.0 ml of extract per min per gram fresh weight done. Three replicates for each treatment were analyzed to determine plant enzymes. Relative activity percentage compared with healthy tomato plant was calculated.

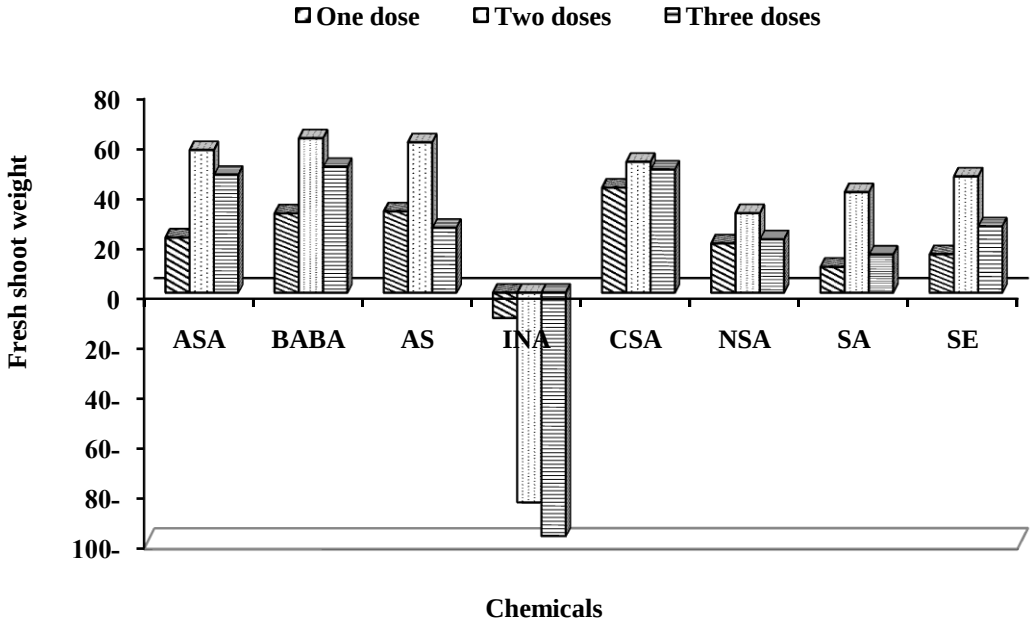
Results

The results in Table 1 and Fig. 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 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 reduction percentages recorded 91.1, 88.9, 86.7 and 82.2, respectively. 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 reduction percentages. The minimum reduction was registered by SE and AS. The most effective chemicals were the same four chemicals when applied of such chemicals before nematode inoculation time. These chemicals occupied a descending order as CSA, NSA, BABA and SA, respectively according to galls formation, egg-masses production and total population reduction percentages. The lowest efficacy was related to AS. Application of chemicals three days after nematode inoculation time was recorded the same positions in the previous treatments and with the same descending order. SE treatment considered as the less effective inducer for suppressing nematode population.

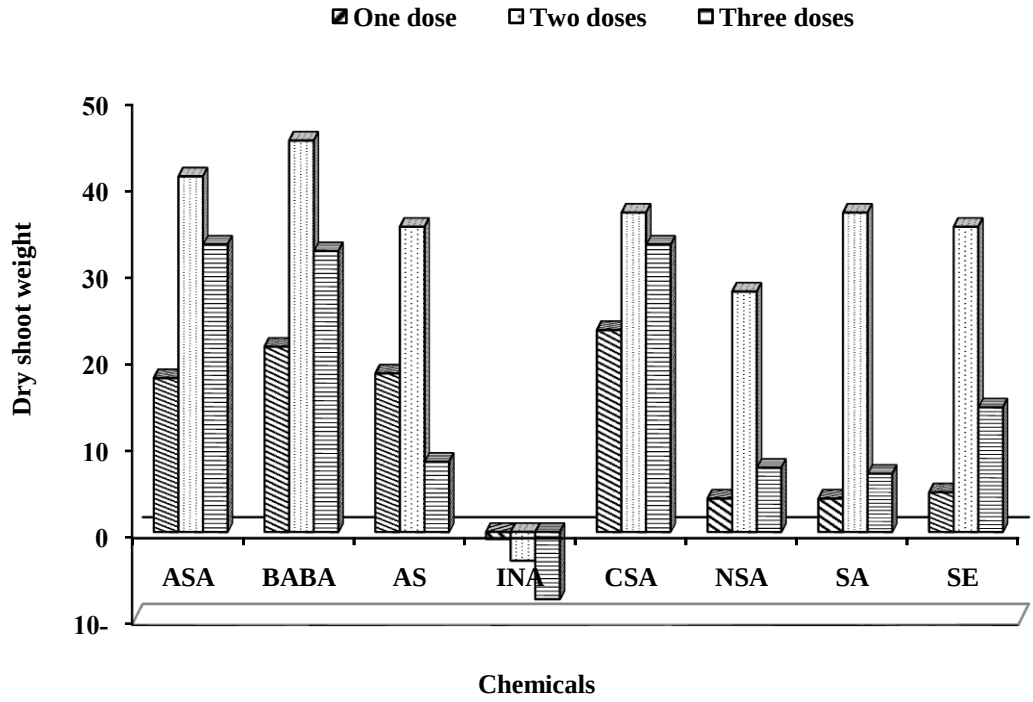
Table 1. Effect of chemical substances on development and reproduction of *Meloidogyne incognita* infected tomato roots.

Treatments	Chemicals substances	Number of			Total eggs
		Galls	Egg-masses	Eggs/egg mass	
3 days before inoculation	Acetyl salicylic acid	12	11	206	2266
	β-aminobutyric acid	8	6	151	906
	Ascorbic acid	17	16	275	4400
	Chloroisonicotinic acid	11	10	198	1980
	Chlorosalicylic acid	5	4	131	524
	Nitro salicylic acid	7	5	141	705
	Salicylic acid	9	8	160	1280
	Selenium	16	14	232	3248
With inoculation	Acetyl salicylic acid	18	17	249	4233
	β-aminobutyric acid	13	12	180	2160
	Ascorbic acid	23	22	311	6842
	Chloroisonicotinic acid	15	15	219	3285
	Chlorosalicylic acid	11	10	149	1490
	Nitro salicylic acid	12	11	176	1936
	Salicylic acid	14	14	189	2646
	Selenium	21	20	258	5160
3 days after inoculation	Acetyl salicylic acid	32	20	339	6780
	β-aminobutyric acid	25	15	220	3300
	Ascorbic acid	38	26	374	9724
	Chloroisonicotinic acid	31	19	320	6080
	Chlorosalicylic acid	22	13	182	2366
	Nitro salicylic acid	23	14	212	2968
	Salicylic acid	26	17	227	3859
	Selenium	34	24	357	8568
Nematode infested plant		49	45	415	18675
LSD 0.05 Chemicals (C)		1.4	1.1	10.0	-
LSD 0.05 Time (T)		1.1	0.8	0.8	-
LSD 0.05 (CxT)		2.5	2.0	17.4	-

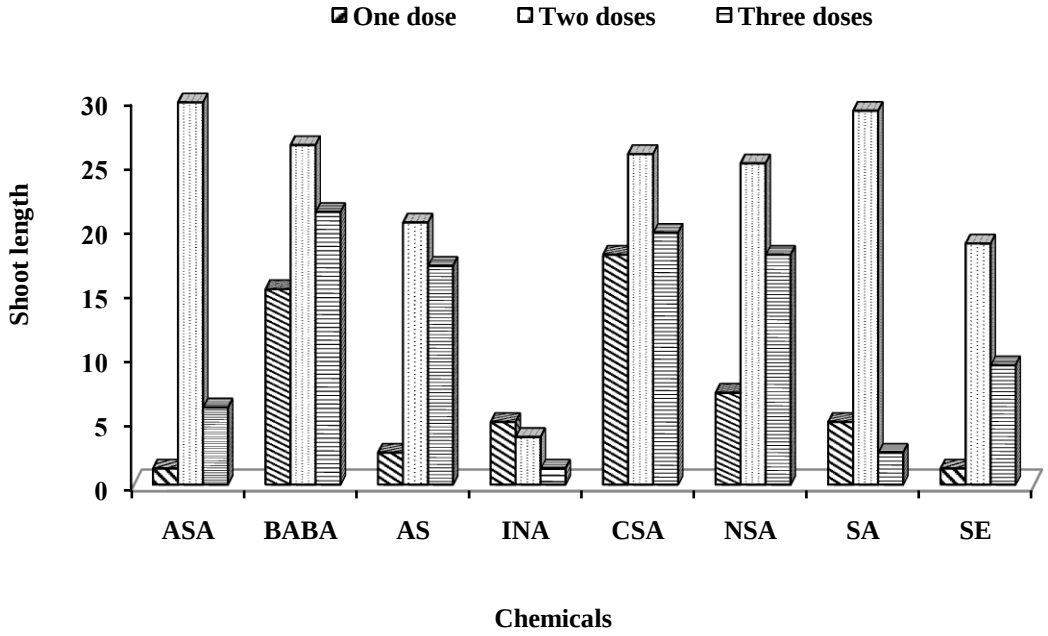
A


One dose
Two doses
Three doses
Fresh shoot weight
80
60
40
20
0
20-
40-
60-
80-
100-
ASA
BABA
AS
INA
CSA
NSA
SA
SE
Chemicals

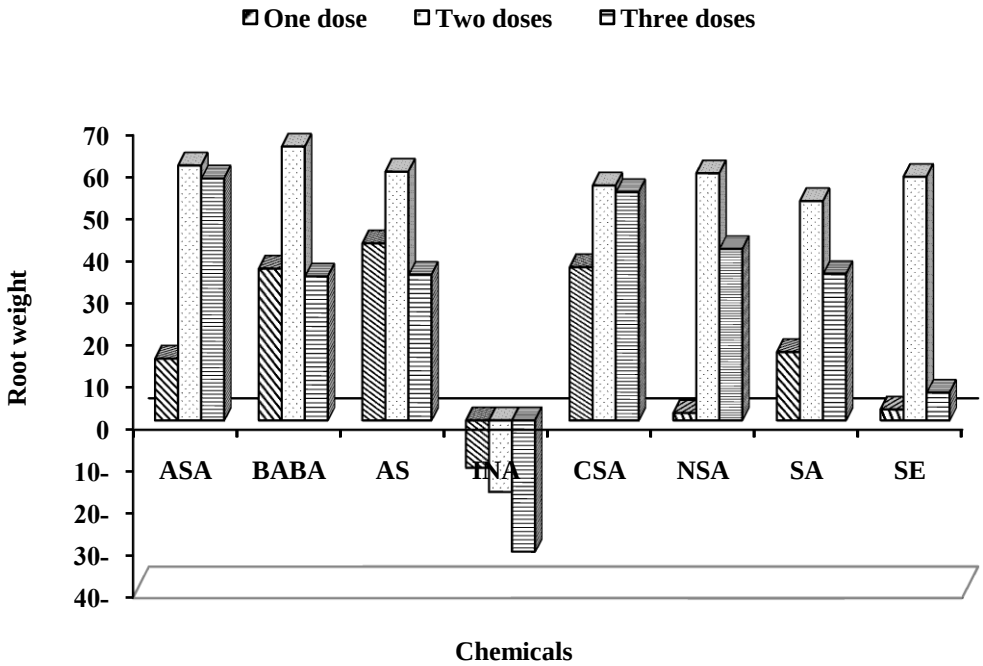
B


One dose
Two doses
Three doses
Dry shoot weight
50
40
30
20
10
0
10-
ASA
BABA
AS
INA
CSA
NSA
SA
SE
Chemicals

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

Fig. 1 (A-D). Effect of post-inoculation doses on increment (%) of tomato growth parameters infected by *M. incognita*.

Results listed in Table 2 showed that enhancement of tested chemicals efficacy at post-inoculation time. CSA has the highest ability to suppress the formation of galls to 95 galls and the production of egg-masses to 59 egg-masses/plant compared to untreated control plant as one dose after inoculation, that formed 232 galls/plant and produce 143 egg-masses/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 egg-masses/plant. The rested chemicals ranged in descending order according to their ability to diminish galls formation as NSA > BABA > SA > INA > ASA > AS.

Concerning the second treatment, two time application, the highest effect to *M. incognita* galls formation was reduced in combine with both CSA (41 galls/plant) and NSA (42 galls/plant) as compared to untreated control which formed 232 galls/plant. The feeblest effect showed by AS and formed 150 galls/plant. There were two substances INA and SA formed the same gall numbers (79 galls/plant). Other three substances were highly effective in decreasing galls formations as BABA, ASA and SE. The production of egg-masses took similar trend where CSA and NSA suppress egg-masses to 40 and 44, respectively. Moderate effects were related to BABA, SA and INA which produced 53, 59 and 63 egg-masses/plant, respectively. While, SE produced 94 egg-masses/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. Relation to the third treatment, three times doses there were three chemicals minimized gall formation INA, SE and NSA recorded 34, 36 and 39 galls/plant. While the maximum gall formation was related to AS (130) compared to untreated control 232 galls/plant. INA ranged the highest effective chemical on egg-masses which recorded 15 egg-masses/plant. Whereas, AS recoded the lesser effective chemical 72 egg-masses/plant as compared to untreated control (143 egg-masses/plant).

Data in presented in Table 3 showed that all chemicals with different doses encouraged plant growth parameters except INA induced decrement in weight of both shoot and root under the different doses.

The efficacy of one time application pre inoculation (P) and two time application pre and post nematode inoculation time application (P.P) where this combination increased the effectiveness of such chemical substance in field application (Table 4). The double application P.P maximized the ability of the chemical substance to 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 the tested chemicals exhibited effectiveness when the chemical application was encouraged when applied pre and post nematode inoculation time. The egg-masses/plant production confirmed the same previous trend. Whereas, P.P application was more successful than P application only. Besides, both of CSA and NSA were the most effective suppressive chemicals decreased 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 was 247 induced by SE with P comparing to control treatment that recorded 382, but the increment with P.P was lesser where SE registered 238 egg-masses/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.

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

Treatments	Chemicals substances	Number of					Total Population Reduction (%)	Eggs/ egg-mass
		Galls	Develo-pmental stages	Females	Egg-mass	Total population		
One dose 7days after 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
Two doses 7 and 14 days after 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
Three doses 7, 14 and 21 days after 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
	Nematode infested plant	232	75	178	143	253	-	266
LDS 0.05 Chemicals (C)		8.5	3.0	3.4	5.6	-	-	6.9
LDS 0.05 Dose (D)		6.1	2.1	3.1	3.5	-	-	3.6
LDS 0.05 (CxD)		14.6	5.1	10.2	9.7	-	-	12.0

Table 3. Effect of reiterative doses of certain chemical substances on growth responses of tomato plants infected with *Meloidogyne incognita*.

Treatments	Chemicals	Fresh shoot weight	Dry shoot weight	Shoot length	Root weight	Root length
One dose 7days after 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
Two doses 7 and 14 days after inoculation	Acetyl salicylic acid	11.9	2.1	37.0	5.8	34.0
	β-aminobutyric acid	13.4	2.3	35.3	6.5	31.0
	Ascorbic acid	12.9	1.9	32.7	5.5	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	8.58	2.0	36.7	4.7	31.6
	Selenium	9.6	1.9	32.0	5.4	30.3
Three doses 7, 14 and 21 days after 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	1.4	26.7	3.5	27.0
	Selenium	7.0	1.5	28.7	2.4	29.3
Nematode infested plants		5.1	1.3	26.0	21.6	
LSD 0.05 Chemicals (C)		0.5	0.08	1.7	0.18	1.4
LSD 0.05 dose (D)		0.3	0.05	1.3	0.14	1.3
LSD 0.05 (CxD)		0.9	0.14	3.0	0.31	2.4

Table 4. Effect of pre and post-inoculation time application on some chemicals substances activation on development and reproduction of *Meloidogyne incognita* on tomato plant under greenhouse conditions.

Chemical substances	J_2 in soil		Galls		Developmental stages		Mature females		Egg-masses		Total population		Eggs/ egg-mass		Reproduction factor	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Acetylsalicylic acid	13942 (30.5)	11995 (40.2)	259 (38.6)	230 (45.6)	128 (32.5)	74 (60.7)	246 (41.7)	220 (51.7)	234 (38.7)	210 (45.0)	14316 (30.7)	12289 (40.5)	365 (36.2)	329 (42.5)	14.31	12.28
β-aminobutyric acid	8928 (55.5)	4073 (79.7)	169 (59.9)	142 (66.3)	80 (57.5)	50 (73.4)	189 (53.3)	130 (63.3)	180 (52.9)	123 (67.8)	9197 (55.5)	4253 (79.4)	287 (49.9)	244 (57.3)	9.19	4.25
Ascorbic acid	14893 (25.7)	13062 (34.9)	296 (29.9)	273 (35.2)	144 (23.8)	102 (45.9)	298 (35.0)	231 (40.0)	285 (25.3)	219 (42.7)	15335 (25.3)	13395 (35.1)	415 (27.5)	379 (33.7)	15.33	13.39
Dichloroisonicotinic acid	9742 (51.4)	4891 (75.6)	220 (48.0)	194 (54.1)	117 (37.9)	62 (67.0)	228 (45.0)	167 (55.0)	217 (43.3)	158 (58.6)	10087 (51.1)	5120 (75.2)	346 (39.5)	314 (45.1)	10.08	5.12
Chlorosalicylic acid	8517 (57.5)	3152 (84.3)	151 (64.3)	112 (73.5)	69 (63.7)	41 (78.5)	168 (66.7)	108 (70.0)	161 (57.8)	102 (73.2)	8754 (57.6)	3301 (84.0)	277 (51.6)	212 (63.3)	8.75	3.30
Nitrosalicylic acid	8733 (56.5)	3591 (82.1)	158 (62.6)	116 (72.5)	72 (61.7)	47 (75.3)	176 (56.7)	118 (65.0)	167 (56.3)	111 (71.0)	8981 (56.5)	3756 (81.8)	283 (50.5)	220 (61.6)	8.98	3.75
Salicylic acid	9103 (54.6)	4268 (78.7)	189 (55.1)	159 (62.2)	87 (54.0)	52 (72.3)	197 (50.0)	148 (60.0)	187 (51.1)	140 (63.4)	9387 (54.5)	4468 (78.4)	309 (46.0)	251 (56.1)	9.38	4.46
Selenium	13427 (33.1)	12469 (37.8)	267 (36.7)	239 (43.4)	130 (31.0)	79 (58.4)	259 (38.3)	249 (48.3)	247 (35.3)	238 (37.6)	13816 (33.1)	12797 (38.0)	377 (34.1)	354 (38.1)	13.81	12.79
Nematode inoculated plant	20055 -	20055 -	422 -	422 -	189 -	189 -	402 -	402 -	382 -	382 -	20646 -	20646 -	572 -	572 -	20.64	20.64
LSD 0.05 Chemical (C)	729.6		8.4		4.8		9.9		9.6		-		9.5		-	
LSD 0.05 Time (T)	858.8		6.8		3.5		6.7		6.4		-		11.2		-	
LSD 0.05 (CxT)	1031.7		11.9		6.8		14.1		13.6		-		13.4		-	

Data in Table 6 showed that the relative activity of two enzymes which were affected by the chemical substances used in this study. 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 and AS respectively. CSA substance maximized the activity of this PPO enzyme (2.027) and SE is the substance that minimizes the activity of this enzyme (0.56) compared to the infected and untreated control (0.204) and the healthy plant (0.107). Other chemicals showed descending order similar to POX. The plant growth parameters were positively affected by addition of tested chemical both pre or pre and post nematode inoculation time with *M. incognita* except with INA (Table 5).

Table 5. Effect of pre and post-inoculation application doses on activation of SAR inducers on growth parameters of tomato plants infected with *Meloidogyne incognita* under greenhouse conditions.

Chemical substances	Shoot						Root			
	Fresh weight		Dry weight		Length		Weight		Length	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	P	PP
Acetyl salicylic acid	30.3	37.3	4.41	5.2	43.0	46.3	10.78	13.12	33.00	35.00
β-aminobutyric	29.2	35.0	4.12	4.6	39.6	44.0	10.43	11.52	32.00	33.33
Ascorbic acid	30.3	31.9	3.38	4.0	39.0	40.3	9.13	10.37	30.33	31.00
Chloroisonicotinic	9.9	2.0	1.34	0.5	25.6	18.0	3.79	1.78	24.00	18.33
Chlorosalicylic acid	25.2	30.0	3.54	4.4	41.0	44.6	10.60	12.27	31.67	34.00
Nitro salicylic acid	29.0	31.5	3.87	4.5	40.3	43.0	9.36	10.44	32.67	33.00
Salicylic acid	31.7	40.5	4.74	5.4	42.0	47.3	9.92	12.70	34.00	37.00
Selenium	33.4	32.3	3.69	3.2	41.0	38.6	9.88	9.10	31.33	30.67
Nematode infested	13.49		2.08		32.0		5.72		28.0	
LSD 0.05 Chemicals	0.8		2.5		2.4		0.5		1.6	
LSD 0.05 time (T)	0.3		1.9		2.3		0.4		2.1	
LSD 0.05 (CxT)	1.2		3.5		3.4		0.7		2.2	

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
Uninfected untreated (Healthy)	0.338	100.00	0.107	100.00
Nematode infested plant	0.782	231.56	0.204	191.61
LDS 0.05	0.21	-	0.39	-

Concerning plant shoots SA maximized PP and P fresh and dry weight was recorded more effective 40.5, 31.7 and 5.5, 4.7 gm, respectively. On the other hand, all treatments showed insignificant differences in dry weight. Also SA provided the highest length as 47.3 cm with the P.P treatment, while ASA maximum shoot length (43 cm).

Discussion

The previous results demonstrated that the pre-inoculation addition of chemicals is more effective than the post-inoculation. These results were in accordance with Arrigoni *et al*., (1979) and Al-Sayed (1992). Pandey & Kalra (2005) showed that ASA, INA, NSA, CSA, SA and isonicotinamide applied as pre-infection suppressed nematode reproduction. Also, Sanz-Alférez *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 & Baser (2010) 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 explained the efficacy of pre-inoculation treatment of chemical inducers. BABA was not metabolized in tomato plants to bind cell-wall proteins, resulting in cell walls 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 cell development. Nematodes ingested BABA directly through the giant cells, which interfere with normal amino acid and protein synthesis by the nematodes (Cohen & Gisi, 1994). It was evident that BABA found in tomato root exudates (Gamliel & Katan, 1993).

INA showed induce disease resistance in a number of plants including green bean (Dann & Deverall, 1995) against a broad range of pathogens. In addition, Dann *et al*., (1998) reported that INA treatment stimulated inherent defense mechanisms. INA provided as soil drench at lower concentrations reduced egg-masses and nematode reproduction, although with negative effects on plant fitness finding agreed with Chinnasri *et al*., (2006). SA was 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 has particular a biotic role in nematode susceptible plants and regarded as resistance inducer (Nandi *et al*., 2003).

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. Molinari & Baser (2010) indicated that efficacy of activators in eliciting resistance to root-knot nematode was 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 influenced by an array of factors, such as the method of application, age, health of the adsorbing plants and the environmental conditions.

Plant growth was positively reacted due to addition chemicals used in proper dose (Molinari, 2008). 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. SA inhibited the penetration and/or the establishment of the feeding sites by the invading juveniles, thus encouragement in plant growth parameters occurred. Repeating application of certain chemical was not always benefit for plant growth due to their effect on plant physiological processes and metabolism, which became pronounced as concentrations elevated inside plat cell sap. On the other hand, some chemical 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 were similar to Molinari & Baser (2010). Selenium accumulation in plants resulted toxicity at high level which interfered with plant metabolic activity.

Phytotoxicity caused by the application of SAR inducers has been increasingly documented. The mechanism responsible for the reduced plant fitness associated with SAR induction was 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 was a putative defense compound. In addition, Baldwin & Callahan (1993) found that high levels of nicotine lead to auto toxicity to plants. Recent experiments utilizing differential display or microarrays to analyze gene expression showed that induced plant responses were 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; Hermsmeier *et al*., 2001). These findings support the assumption that upon induction, resources were allocated toward defense and away from primary metabolism, leading to fitness costs in the plant.

Activation POX and PPO was responded to infected plants tissue and leaves correlated with resistance (Sridhar & 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 *et al*., (2007) stated that ethyl salicylic and jasmonic acid increased the POX activity. In particular POX activity reported as biochemical marker for resistance and to be associated with systemic resistance (Mosa, 2002; Nawar & Kuti, 2003). Using chemical compounds like SA and AS showed increasing in POX and PPO activity (Saeed, 2005). POX and PPO activities provided a convenient method for screening and quantification of inducers. Moreover, enzymes in host plants played an important role in the mechanisms of resistance to nematodes, in other words nematode infection enhanced enzyme activity. Induction of Mi-mediated nematode resistance was correlated with increased activity of several enzymes implicated in defense POX and PPO (Zacheo *et al*., 1993) and increasing of these enzymes were active responses in systemic induce resistance (Irving & Kuc, 1990).

Conclusion

SAR inducers differed in their ability to reduce nematode reproduction on tomato, while CSA, NSA, BABA and SA were 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 used for nematode management in conventional and organic tomato protected cultivation. On the other hand INA found phytotoxic to other SAR elicitors therefore, a lower dosage was applied to plants for induction of resistance.

References

Al-Sayed, A.A. 1992. Effect of ascorbic acid and L-arginine on controlling *Meloidogyne incognita* as influenced by soil temperature. *Annals of Agricultural Science* 30, 1549-1558.

Amin, A.W. 1994. The root-knot nematodes (*Meloidogyne* species) in Hungary. *Bulletin EPPO/OEPP* 24, 417-422.

Amin, A.W. & Mona, A.W. 2014. Protecting from cucumber *Meloidogyne incognita* using graft onto resistant cucurbit rootstocks and antagonistic marigold as an alternative to nematicide. *Pakistan Journal of Nematology* 32, 51-58.

Arrigoni, O., Zacheo, G., Arrigoni-Liso, R., Bleve-Zacheo, T. & Lamberti, F. 1979. Relationship between ascorbic acid and resistance in tomato plants to *Meloidogyne incognita*. *Phytopathology* 69, 579-581.

Baldwin, I.T. & Callahan, P. 1993. Autotoxicity and chemical defense: Nicotine accumulation and carbon gain in solanaceous plants. *Oecologia* 94, 534-541.

Baldwin, I.T., Gorham, D., Schmelz, E.A., Lewandowski, C.A. & Lynds, G.Y. 1998. Allocation of nitrogen to an inducible defense and seed production in *Nicotiana attenuata*. *Oecologia* 115, 541-552.

Baysal, O., Soylu, E.M. & Soylu, S. 2003. Induction of defense-related enzymes and resistance by the plant activator acibenzolar S-methyl in tomato seedlings against bacterial canker caused by *Clavibacter michiganensis* ssp. *michiganensis*. *Plant Pathology* 52, 747-753.

Bonaldo, S.M., Pascholati, S.F. & Romeiro, R.S. 2005. Indução de resistência: noções básicas e perspectivas. In: Cavalcanti, L.S., Di Piero, R.M., Cia, P., Pascholati, S.F., Resende, M.L.V. & Romeiro, R.S. (Eds.). *Indução de resistência em plantas a patógenos e insetos*. *Piracicaba: FEALQ*, 11-28 pp.

Chinnasri, B., Sipes, B.S. & Schmitt, D.P. 2006. Effects of inducers of systemic acquired resistance on reproduction of *Meloidogyne*

javanica and *Rotylenchulus reniformis* in pineapple. *Journal of Nematology* 38, 319-325.

Cipollini, D., Purrington, C.B. & Bergelson, J. 2003. Costs of induced responses in plants. *Basic and Applied Ecology* 4, 79-85.

Cohen, Y. & Gisi, U. 1994. Systemic translocation of 14C-DL-β-aminobutyric acid in tomato plants in relation to induced resistance against *Phytophthora infestans*. *Physiological and Molecular Plant Pathology* 45, 441-546.

Dann, E.K. & Deverall, B.J. 1995. Effectiveness of systemic resistance in bean against foliar and soil borne pathogens as induced by biological and chemical means. *Plant Pathology* 44, 458-466.

Dann, E., Diers, B., Byrum, J. & Hammerschmidt, R. 1998. Effect of treating soybean with 2,6-dichloroisonicotinic acid (INA) and benzothiadiazole (BTH) on seed yields and the level of disease caused by *Sclerotinia sclerotiorum* in field and greenhouse studies. *European Journal of Plant Pathology* 104, 271-278.

Dias-Arieira, C.R., Marini, P.M., Fontana, L.F., Roldi, M. & Silva, T.R.B. 2012. Effect of *Azospirillum brasilense*, Stimulate® and potassium phosphate to control *Pratylenchus brachyurus* in soybean and maize. *Nematropica* 42, 170-175.

Franzener, G., Martinez-Franzener, A.S., Stangarlin, J.R., Furlanetto, C. & Schwan-Estrada, K.R.F. 2007. Proteção de tomateiro a *Meloidogyne incognita* pelo extrato aquoso de *Tagetes patula*. *Nematologia Brasileira, Brasília* 31, 27-36.

Gamliel, A. & Katan, J. 1993. Suppression of major and minor pathogens by fluorescent pseudomonads in solarized and nonsolarized soils. *Phytopathology* 83, 68-75.

Ghini, R. & Kimati, H. 2000. Resistência de fungos a fungicidas. *Jaguariúna, São Paulo: Embrapa Meio Ambiente*, 78 pp.

Hermsmeier, D., Schittko, U. & Baldwin, I.T. 2001. Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera: Sphingidae) and its natural host *Nicotiana attenuata*. I. Largescale changes in the accumulation of growth and defense-related plant mRNAs. *Plant Physiology* 125, 683-700.

Irving, H.R. & Kuć, J. 1990. Local and systemic induction of peroxidase, chitinase and resistance in cucumber plants by K_2HPO_4. *Physiology of Plant Pathology* 37, 355-366.

Kataria, H.R., Wilmsmeier, B. & Buchenaure, H. 1997. Efficacy of resistance inducers, free radical scavengers and an antagonistic strain of *Pseudomonas fluorescens* for control of *Rhizoctonia solani* AG-4 in bean and cucumber. *Plant Pathology* 46, 897-909.

Maxwell, D.P. & Bateman, F.D. 1967. Changes in the activities of some oxidases in extract of Rhizoctonia-infected bean hypocotyls in relation to lesion maturation. *Phytopathology* 57, 132-136.

Molinari, S. 2008. Salicylic acid acts as a resistance elicitor on tomato seedlings attacked by root-knot nematodes: effect of plant age. *Redia* XCI, 73-75.

Molinari, M. & Baser, N. 2010. Induction of resistance to root-knot nematodes by SAR elicitors in tomato. *Crop Protection* 29, 1354-1362.

Mosa, A.A. 2002. Induced resistance in rice against blast disease using abiotic and biotic agents. *Annals of Agricultural Science, Ain Shams University* 47, 933-1008.

Mostafa, M.H., Gado, E.A.M. & Youssef, M.M. 2007. Induction of resistance in tomato plants against root-knot nematode by some chemical and plants extracts. *Arab Universities Journal of Agricultural Sciences* 15, 177-184.

Nandi, B., Kundu, K., Banerjee, N. & Babu, S.P.S. 2003. Salicylic acid-induced suppression of *Meloidogyne incognita* of okra and cowpea. *Nematology* 5, 747-752.

Nawar, H.F. & Kuti, J.O. 2003. Wyerone acid phytoalexin synthesis and peroxidase activity as markers for resistance of broad beans to chocolate spot disease. *Phytopathology* 151, 564-570.

Oka, Y., Cohen, Y. & Spiegel, Y. 1999. Local and systemic induced resistance to the root-knot nematode in tomato by DL-b-amino-nbutyric acid. *Phytopathology* 89, 1138-1143.

Oka, Y., Koltai, H., Bar-Eyal, M., Mor, M., Sharon, E., Chet, I. & Spiegel, Y. 2000. New strategies for the control of plant-parasitic nematodes. *Pest Management Science* 56, 983-988.

Oostendorp, M., Kunz, W., Dietrich, B. & Staub, T. 2001. Induced disease resistance in plants by chemicals. *European Journal of Plant Pathology* 107, 19-28.

Reymond, P., Weber, H., Damond, M. & Farmer, E.E. 2000. Differential gene expression in response to mechanical wounding and insect feeding in *Arabidopsis*. *Plant Cell* 12, 707-719.

Saeed, M.R.M. 2005. *Utilization of some specific materials to stimulated resistance in some host plants against the root-knot nematodes*. Ph. D. Thesis. Faculty of Agriculture, Cairo University, Giza, Egypt.

Sanz-Alférez, S., Mateos, B., Alvarado, R. & Sánchez, M. 2008. SAR induction in tomato plants is not effective against root-knot nematode infection. *European Journal of Plant Pathology* 120, 417-425.

Silva, L.H.C.P., Campos, J.R., Dutra, M.R. & Campos, V.P. 2004. Aumento da resistência de cultivares de tomate a *Meloidogyne incognita* com aplicação de acibenzolar-S-meltil. *Nematologia Brasileira*, *Brasília* 28, 199-206.

Sridhar, R. & Ou, S.H. 1974. Biochemical changes associated with development of resistant and susceptible types of rice blast lesion. *Journal of Phytopathology* 79, 222-230.

Starr, J.L. & Roberts, P.A. 2004. Resistance to plant-parasitic nematodes. In: Chen, Z.X., Chen, S.Y. & Dickson, D.W. (Eds.). *Nematology: Advances and Perspectives*. CAB International, Wallingford, UK, 879-907 pp.

Zacheo, G., Orlando, C. & Bleve-Zacheo, T. 1993. Characterization of anionic peroxidases in tomato isolines infected by *Meloidogyne incognita*. *Journal of Nematology* 25, 249-256.

(Received: 2 April, 2014)