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Effect of chemical inducers on plant resistance to *Meloidogyne* spp in tomato plants under greenhouse conditions

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Abstract

The effect of certain chemical inducers such as jasmonic acid (JA), salicylic acid (SA), B-amino-n-butyric acid (BABA), acibenzolar-s-methyl (Actigard) and Potassium chloride (KCl) with concentration of 100 ppm on tomato plant resistance to *Meloidogyne* spp. was studied under greenhouse conditions. The experiment was conducted by spraying all chemical inducers on tomato plants (20 ml / plant) growing in pots 30 cm - diam. Two days after application 1000 eggs of nematode were inoculated on the root of plant roots. Nematodes and plant parameters were recorded two months post inoculation. Jasmonic acid (JA), acibenzolar-s-methyl (Actigard), salicylic acid (SA) and B-amino-n- butyric acid (BABA) reduced the numbers of eggs with 72.27, 54.9, 82.94 and 78.4%, respectively compared to inoculated untreated plants. Potassium chloride (KCl) had no significant effect (4.8%) in reduction % of total eggs numbers. Also, the compounds jasmonic acid (JA), SA, Actigard and (BABA) decreased number of root galls. The reduction values were 52.58, 41.43, 42.85 and 54.73%, respectively as compared to the untreated control inoculated. Results concluded that foliar application of Jasmonic acid (JA), (Actigard) and B- aminobutric acid (BABA) activate resistance in tomato plants to *Meloidogyne* spp. Therefore, they may be considered as good elements, especially in successful release of some schemes of integrated management of nematodes.

Keywords: Jasmonic acid, salicylic acid , acibenzolar-s-methyl, B- amino-n-butyric acid, and Potassium chloride, root-knot nematode, systemic acquired resistance, *Meloidogyne* spp., Actigard.

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Introduction

The initial infection in the plant results in the production of internally transmitted signals to parts of the plant that is far from the initial site of infection. Induced systemic acquired resistance (SAR) is activated the ability of plants to become resistant after application of chemical inducers or exposure to stress (EL Roby, 2005), An initial infestation event in plants leads to the production of chemical signals transmitted endogenously to plant parts that are far from the initial site of infestation. These signaling chemicals have been used to stimulate plant resistance against many fungal, bacterial, viral pathogens and insect infestation in more than 30 species of plants belonging to both dicotyledonous and monocotyledonous plants (EL Roby 2005; Sticher 1997). Also, induced resistance in plants was studied for plant parasitic nematodes i.e. Ibrahim and Lewis (1986) mentioned that plants which are susceptible to *Meloidogyne arenaria*, when preinoculated with *Meloidogyne incognita* showed increased resistance to this nematode. Ogallo and McClure (1995) showed that the prior inoculation into tomato plants with species of nematodes, *M. incognita* or *M. javanica*, induced resistance to *M. hapla* that infected tomato plants. Ji et al. (2015) showed that BABA induced resistance in rice plants against *M. graminicola* and has’nt toxicity to nematode. It inhibited nematode penetration and delayed giant cell development. BABA when applied as a foliar spray and root drench to cucumber and tomato induced resistance against *M. javanicum* (Oka et al., 2001; Oka et al., 1999) also in wheat against the *M. marylandi* (Oka et al., 2001). BABA protected tomato against *M. ingognita* (Anter et al., 2014). Fatemy et al. (2012) mentioned that a treatment tomato seed with BABA protected the plants from *M. javanica*. Ahmed et al. (2009) observed the efficiency of BABA against *M. javanica* in mung bean. Mongae and Moleleki (2015) showed that treatment potato plants with of BABA protected them from infestation with *M. incognita*. Fujimoto et al. (2011) showed that the foliar treatment with methyl jasmonate at 0.5 mM or higher concentrations significantly reduced the infection of *M. incognita* in tomato plants. Nahar et al. (2011) showed that application of JA onto the shoots induced systemic resistance in the rice plants against *M. graminicola*. The defense pathway is involving the up-regulation of PR genes and protecting the rice root from infection by *M. graminicola*. The importance of SA in plant defense against root knot nematodes studied by Owen et al. (2002), Nandi et al. (2003), Branch (2004) and Loake and Grant (2007), and cyst nematodes (Wubben et al., 2008). Bhattarai (2008) indicated that the minimum concentration of SA is sufficient for induced resistance in tomato (*Solanum lycopersicum*) against root knot nematodes (Degife 2017). Fatemy et al. (2012) indicated that foliar applications of JA, BABA and SA to plants induce a systemic effect that can suppress root knot nematodes infestation. They showed that these chemicals play a significant role in foliar defenses and have an important role of in the protection of root tissues against root herbivorous. The same author suggested that the role of gibberellins in induced plant resistance against *Meloidogyne incognita* caused a reduction in second-stage juveniles in soil and in egg-masses on roots. Also, salicylic acid (SA) and jasmonic acid (JA) are natural compounds, and their potency in inducing SAR was reviewed (Degife 2017; Ahmed et al. 2009; Schneider

1996). Sticher (1997) observed that Polyunsaturated and oxygenated fatty acids such as arachidonic, linolenic, and oleic acids induced SAR in certain solanaceous plants. The study was carried out to study the efficiency of certain chemical compounds, in inducing resistance to tomato plants against root-knot nematode *Meloidogyne* spp. under greenhouse conditions.

Materials and methods

Eggs of *Meloidogyne* spp. was cultured on root tomato plants, *Solanum lycopersicum* cv. Trust-1 under greenhouse conditions. After 6 weeks from infection time nematode eggs were extracted using 1% sodium hypochlorite solution NaOCl (Byrd et al., 1972) and used in various experiments.

Effect of chemical inducers in inducing plant resistance against root-knot nematode *Meloidogyne* spp.: Tomato plants grown in 30 cm- diameter pots containing clay loam soil. Twenty days after transplanting, plants were sprayed with 20 ml aqueous suspension of 100 ppm from Jasmonic acid (98.7% a.i.), B-amino-n-butyric acid (BABA) (99% a.i.), Salicylic acid (SA) (99% a.i.), Potassium chloride (KCl)) (99% a.i.) and acibenzolar-s-methyl 50% a.i. (Actigard 50 WG), one thousand eggs of *Meloidogyne* spp. in 10 ml were inoculated around the roots of each plant and after two days post application, the study was conducted during two successive seasons. Experiment was designed as a randomized blocks with three replicates at each treatment. The plants were treated again 20 and 35 days later with the same concentrations of the previous compounds. All chemicals were purchased from chemical Gomhoria Company, Cairo, Egypt except (Actigard) which obtained from Syngenta Company in Egypt. Plants were irrigated and fertilized as needed. Two months after the inoculation time, number of galls / root and number of egg-masses / root were recorded total egg production / plant and the percentage of their reduction were calculated as the following equation:

$$R\% = \frac{\text{Treatment - infected control}}{\text{Infected control}} \times 100$$

Plant growth parameters i.e. shoot length (cm), plant highs, avg. numbers of leaves/plant, fresh shoot and root weights (g) were determined. Root washed smoothly with water, cut into small pieces, and shaken individually in 1% NaOCl (Byrd et al., 1972) to collect *Meloidogyne*. sp. eggs. The experiment was carried out at greenhouse conditions in plant protection Department, Faculty of Agriculture, Minia University, Mnia, Egypt throughout two successive seasons 2016 spring and summer seasons and the average of two seasons were analyzed statistically. The reduction or increasing in the growth characteristics were calculated as

$$R\% = \frac{\text{Treatment - infected control}}{\text{Infected control}} \times 100$$

Statistical Analysis: All the data were pooled together and means were analyzed statistically using the Duncan's Multible Range Test (DMRT) according to Snedecor and Cochran (1999).

Results and Discussion

In tomato plants infected with *Meloidogyne* spp, application of chemical inducers reduced nematode eggs (Table 1). The tested compounds showed significant differences at the level of P 0.01 in their effects on nematode egg production as shown in Table 1. Results showed that Jasmonic acid (JA), acibenzolar-s-methyl (Actigard), salicylic acid (SA) and B-amino-n- butyric acid (BABA) reduced the total eggs numbers with 72.27, 82.49, 54.9, and 78.4 % respectively compared to infected untreated plants. Potassium chloride (KCl)) had no significant effect (8.3%) in reduction % of total eggs numbers. Growth characters of tomato plants based on (shoot length, plant height, number of leaves and branches / plant, both fresh shoot and roots weights) were significantly decreased in *Meloidogyne* spp infected plants than in the non-infected plants. The application of chemical inducers to *Meloidogyne* spp infected tomato plants increased all the plant growth parameters except fresh root weight decreased.

Table 1: Effect of chemical inducers on root galls and egg mass formation by *Meloidogyne* spp in tomato plants.

Treatments	galls/root	Reduction (%)	Egg masses / root	Eggs /mass	Total eggs / plant	Reduction in total eggs (%)
Non-infected control	0.0	0.0	0.00	0.00	0.0	0.0
Infected control	38.66^{a}	-	41.00^{a}	370.00^{a}	15170.00^{a}	-
JA	18.33^{b}	52.58^{a}	19.00^{c}	221.33^{b}	4205.30^{c}	72.27^{a}
KCl	37.66^{a}	7.95^{c}	38.66^{a}	360.00^{a}	13917.60^{a}	8.30^{c}
SA	30.00^{ab}	41.40^{ab}	26.33^{b}	260.00^{b}	6845.80^{b}	54.90^{b}
Actigard	22.60^{ab}	32.53^{b}	12.00^{c}	221.33^{b}	2655.96^{d}	82.49^{a}
BABA	17.50^{b}	54.73^{a}	13.33^{c}	245.33^{b}	3270.25^{d}	78.40a

Means followed by the same letters are not significantly different at P>0.05 using Duncan’s Multiple Range Test.

Induced systemic acquired resistance (SAR) was observed after infection by pathogens, exposure to stress, or application of chemical inducers. EL Roby (2005), Ibrahim and Lewis (1986), Makady et al. (2006) and Hussein and Abdel- Aziz (1998) indicated that resistance of plants against *Meloidogyne arenaria*, when the plant was pre-inoculated with *Meloidogyne incognita*. Also, Ogallo and McClure (1995) indicated that the prior inoculation of *M. incognita* or *M. javanica*, to tomato plants increased to *M. hapla*. After foliar treatment with methyl jasmonate the induced resistance in tomato plants (*Solanum lycopersicum*) against the root-knot nematode (*Meloidogyne* spp) was observed (Degife 2017; Nahar et al. 2011). Results in Table 2 showed that *Meloidogyne* spp caused an increasing in tomato root mass compared to the non-infected plants. The treatment tomat plants with the tested chemical inducers against *Meloidogyne* spp did not increase the root weight as compared to those infected with *Meloidogyne* spp without chemical inducers application. Among chemical inducers, root weight was lower in plants treated with

Jasmonic acid, Actigard and BABA. Foliar application of chemical inducers at concentrations 100 ppm caused a significant varied increase in tomato growth parameters compared with untreated infected control as shown in Fig (1). The results indicated that treatments with the tested chemical inducers that induced resistance in tomato plants against *Meloidogyne* spp have an indirect effect on improving the growth of tomatoes than infected plants where treatments increased the average plant height number of branches, number of leaves Table (2) and differed significantly from control infected with nematodes treatments. Also, our results were in harmony with Chinnasri et al. (2003, 2006) indicated that Actigard which haven't nematicidal effect against *M. javanica* and not prevent its penetration into the roots of cowpea.

Table 2: Effect of chemical inducers on growth parameters of tomato plants infected with *Meloidogyne* spp.

Treatments	Shoot Length (cm)	Plant height (cm)	No. of leaves /plant	No. of branches /plant	Fresh shoot weight (g)	Fresh root weight (g)
Non-infected control	48.80[a]	104.6[a]	166.0[a]	11.3[a]	4.54	0.26[c]
Infected control	27.70[e]	69.70[c]	131.2[b]	7.3[b]	3.42	0.39[a]
JA	33.10[d]	84.33[b]	158.0[a]	11.1[a]	5.52	0.29[c]
KCl	34.33[c]	70.00[c]	127.0[b]	8.5[b]	4.12	0.40[a]
SA	27.70[e]	86.90[b]	152.0[a]	11.7[a]	3.60	0.33[bc]
Actigard	33.20[d]	93.50[b]	155.0[a]	12.3[a]	4.80	0.27[c]
BABA	36.00[b]	91.60[b]	156.0[a]	11.6[a]	3.90	0.29[c]

Means followed by the same letters are not significantly different at P>0.05 using Duncan's Multiple Range Test.

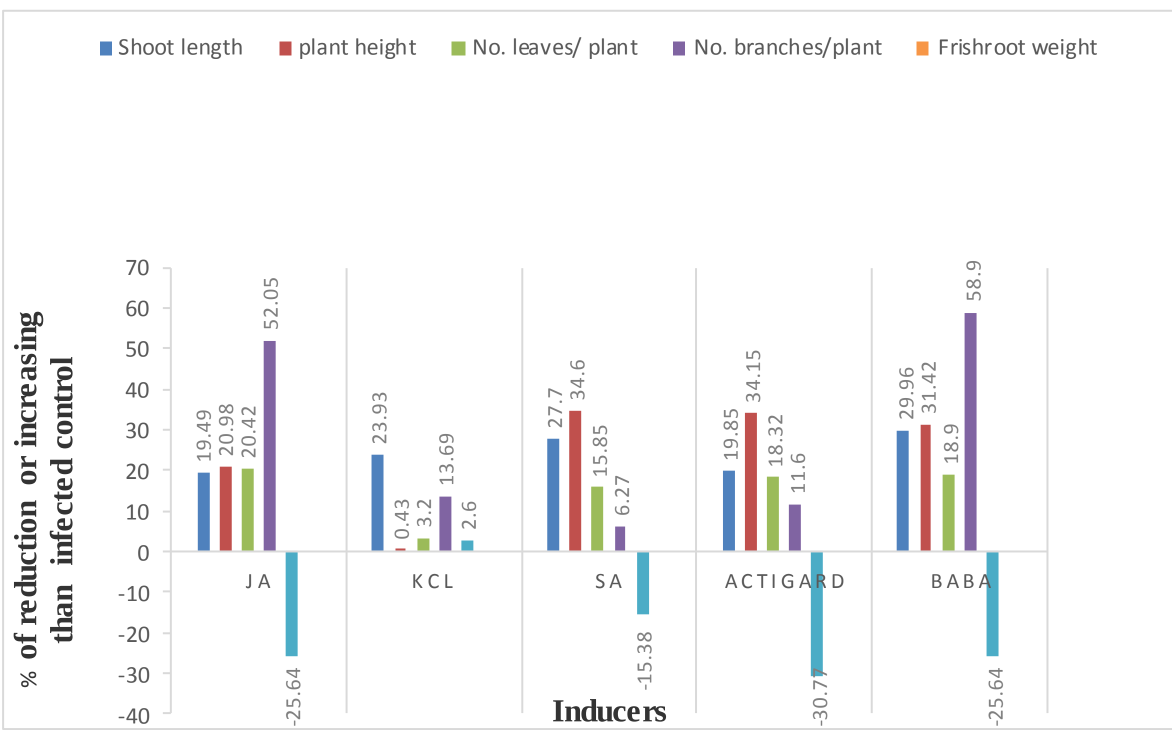

Figure 1: Percentages of reduction or increasing in tomato plant growth characters.

So, the mechanisms induced by these chemicals were a delay in nematode development and its reproduction. Similar results on the mechanisms of resistance against *Meloidogyne* spp. in grapevine and *Heterodera trifolii* in white clover crops was observed (Owen et al. 2002; Kempster et al. 2001). Oka et al. (1999) indicated that the inhibition of infection with the root-knot nematode in tomato due to changes in plant metabolism by using DL-β-amino-n-butyric acid and the cell wall of the plant has become difficult to penetrate with nematode. Also many researchers have shown that BABA is not metabolized in the plant and it is thought that it is linked to the cell wall protein making it difficult to penetrate withe nematodes (Ji et al. 2015; Cohen & Gisi, 1994). Oka et al. (2001) showed that higher activity of peroxidase enzymes accelerate lignin synthesis in tomato roots treated with BABA compared with untreated plants. Increase in plant growth parameters than infected plants showed improvement of growth of treated plants than infected un-treated plants. Therefore, effective control measures that are friendly to environment are needed. Based on our results, BABA, Actigard, and JA at 100 ppm appear to have potential to be used as an element for inducing systemic resistance against *Meloidogyne spp* in tomato plants. They showed low levels of nematode population without any phytotoxicity in the plants and without any significant reduction in tomato plant growth. So these compounds may have potential as an alternative method to nematicides or could be used in combination with nematicides or other nonchemical control measures in an integrated management program to achieve more effective nematode control in tomato in Egypt.

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