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Comparative study on the effect of garlic clove and acetyl salicylic acid aqueous extracts with emphasis on inducing resistance against root knot nematode, *Meloidogyne incognita* on sugar beet

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Abstract : Garlic clove and acetylsalicylic acid (ASA) aqueous extracts at concentrations 100 and 1000ppm were treated by soil drench and foliar spraying for controlling root knot nematode, *Meloidogyne incognita* infecting sugar beet cv. Gazelle under screen house conditions. The obtained results clarified, in general, that garlic and acetyl salicylic acid extracts at concentrations of100 and 1000 ppm either as soil drench or as foliar spraying reduced nematode parameters and increased plant growth,yield and percentage total soluble solids (TSS%) criteria as influenced by the tested concentration and type of treatment compared to untreated inoculated control1. In other words, by using soil drench, the two tested materials at 1000ppm highly affected nematode criteria compared to 100ppm and control1. Contrarily, the two materials at 100ppm, when used as foliar spraying, highly affected nematode criteria than did at 1000m which may indicate that they act as resistance inducers against root knot nematode. The enzyme, chitinase was increased in the treated plants according the same trend of the tested concentrations and type of treatment compared to inoculated and uninoculated non-treated controls.
Key words: Acetyl salicylic acid, garlic clove, aqueous extracts, *Meloidogyne incognita*,sugar beet.

Introduction

The root-knot nematodes (*Meloidogyne* spp.) rank high among nematode pests in Egypt [1,2,3,4,5] including sugar beet[6,7,8]. Sugar beet yield losses caused by plant parasitic nematodes were calculated to be 12% in Egypt which means that 1, 244,462.5 metric tons (= $ 55 millions) are lost annually[7]. Salicylic acid (SA) is considered resistance inducer to plants against some pathogens[9,10]. It has been thought to play an important role in systemic acquired resistance because exogenous SA accumulates in pathogen- infected tissues[11,12] and translocated to uninfected parts of the plant[13,14]. Acetyl salicylic acid (ASA, aspirin) as pre-, post- and simultaneous treatments significantly reduced reproduction of *Heterodera glycines* on soybean by 60, 64 and 87%, respectively[15].Many researchers studied the nematicidal effect of mashed garlic, garlic oil and its components [16, 17, 18, 19, 20, 21, 22, 23,24]. However, little work was done on the effect of garlic extract when El-Nagdi and Youssef (2013)[25]and El-Nagdi *et al.* (2014)[26]found that the garlic plant extract at lower concentrations significantly ($p \leq 0.05$) lowered *M. incognita* criteria on tomato and eggplant, as an indicator of inducing resistance against this nematode. The results obtained with Mohamed and Hasabo (2005)[27] reported that at 20 days after *M. incognita* inoculation, induction of chitinase reached the maximum in resistant cotton cultivar. Also, infection with the nematode, *Meloidogyne marylandi* increased chitinase activity[28]indicating its

importance in disease defence[29] as these authors concluded that since chitinase is a PR-protein, it may result in reducing nematode population. Chitinase enzyme indirectly releases oligosaccharide signal molecules promoting plant defense against pests and diseases[30].This research aimed at evaluating garlic clove compared to acetyl salicylic acid for controlling root knot nematode, *M.incognita* on sugar beet.

Material and Methods

Fresh garlic cloves were washed under running tap water and sterile distilled water. Different concentrations of 100 and 1000 ppm of plant extract were prepared by blending 0.01 and 1g of mashed cloves each in 1000 ml-distilled water, respectively with a warring blender. Thereafter, the suspension was filtered through Whatman No.1 filter paper. The same was done by adding 0.01 and 1ml of acetyl salicylic acid to 1000 ml distilled water. Sandy-loam soil for raising sugar beet (*Beta vulgaris* L.) cv. Gazelle seedlings was used. Soil (5kg) was filled into 30cm-diameter pots, each sown with two seeds of sugar beet on November 15, 2014 which was later thinned down to one seedling per pot. One month later, soil in each pot was mixed with 200 ml of each plant extract by pouring the aqueous solution of the extract into holes made around the seedlings in each pot. The following treatments were added as soil drench or foliar spraying:

A-Soil drench:

1. Garlic clove aqueous extract at 100ppm.
2. Garlic clove aqueous extract at 1000ppm.
3. Acetyl salicylic acid (ASA) solution at 100ppm.
4. Acetyl salicylic acid solution at 1000ppm.

B-Foliar spraying:

1. Garlic clove aqueous extract at 100ppm.
2. Garlic clove aqueous extract at 1000ppm.
3. Acetyl salicylic acid solution at 100ppm.
4. Acetyl salicylic acid solution at 1000ppm.

C-Untreated inoculated (control 1).

D- Untreated uninoculated (control 2).

On January 1, 2015, each pot received 1,000 freshly hatched second stage juveniles(J_2) of root knot nematode, *M. incognita* were introduced into holes made around the roots of each plant. Five replicates were used for each treatment. Similar number of pots were not treated and inoculated with nematodes only served as control 1. Untreated uninoculated pots served as control 2.

All pots were arranged in a completely randomized block design in screen house and watered as needed. An extra sample from each replicate of the treated and untreated treatments was uprooted,one month after inoculation of *M. incognita*, for enzyme determination. Chitinase enzyme was determined by the use of the method described by Reid and Ogrydziak (1981)[31].

Six months after nematode inoculation, the experiment was terminated. Nematodes in soil were extracted by sieving and decanting methods according to Barker (1985)[32]. Roots were gently freed of adhering soil and were examined for number of galls and eggmasses. Plant growth criteria were recorded. The percentages total soluble solids (TSS%) were determined in fresh weight of roots by using hand refractometer.

Statistical Analysis:

Data were subjected to analysis by the use of the least significant differences (LSD) and Duncan's Multiple Range Test separated means among treatments by using COSTAT programme.

Results

Effect of the tested materials on nematode parameters and enzyme activity:

As shown in Table 1, data clarified that garlic and acetyl salicylic acid at concentrations 100 and 1000ppm either as soil drench or as foliar spraying significantly ($p \leq 0.05$) reduced nematode parameters and increased plant growth criteria as influenced by the tested concentration and type of treatment compared to control. On the basis of average percentages nematode reduction, garlic clove aqueous extract as soil drench at higher concentration (1000ppm) achieved the highest percentages reduction of nematode parameters including number of second stage juveniles (J_2) in soil, and number of galls and egg masses on roots of sugar beet compared to those by lower concentration (100ppm). Also, acetyl salicylic acid when treated as soil drench behaved the same trend. On the other hand, garlic clove and acetyl salicylic acid at lower concentrations, when treated by foliar spraying, affected at different way as they achieved the highest percentages reduction of the previous nematode parameters compared to untreated control. Chitinase enzyme activity increased at different treatments according the same trend of the tested concentration and type of treatment compared to controls (Table 1).

Effect of the tested materials on plant growth, yield and percentage total soluble solids:

Data in Table 2 illustrate that the tested materials significantly ($p \leq 0.05$) increased sugar beet growth parameters, yield and percentage total soluble solids (%TSS) as influenced by the tested concentration and type of treatment. On the basis of average percentages plant growth and yield increase, garlic clove and acetyl salicylic acid extracts achieved the highest percentages plant growth increase at higher concentrations compared to untreated inoculated control when treated by soil drench. By using foliar spraying, they increased the same criteria by the lower concentrations.

Table (1): Effect of garlic clove and acetyl salicylic acid (ASA) extract concentrations on root knot nematode, *Meloidogyne incognita* infecting sugar beet cv. Gazelle and enzyme activity.

Treatments	Conc. (ppm)	No.J_2in soil /pot	%Red.	No. galls	%Red.	No. egg-masses	%Red.	Average percentage nematode reduction	Chitinase activity (units/mg)
Soil drench Garlic	100	230d	69.7	17bsd	68.5	10d	73.7	70.6	1.003
	1000	150e	80.3	13d	75.9	5e	88.9	81.7	1.108
Acetyl salicylic acid	100	260cd	65.8	20bc	63.0	11cd	71.1	66.6	0.578
	1000	140e	81.6	15cd	72.2	9d	76.3	76.7	0.812
Foliar spray Garlic	100	280bc	63.2	18bcd	66.7	12bcd	68.4	66.1	1.230
	1000	300b	60.5	20bc	63.0	14bc	63.2	62.2	1.171
Acetyl salicylic acid	100	160e	78.9	14cd	74.1	9d	76.3	76.4	1.304
	1000	310b	59.2	26b	51.9	15b	60.5	57.2	1.052
Untreated inoculated control 1	-	760a	-	54a	-	38a	-	-	0.760
Untreated uninoculated control 2	-	-	-	-	-	-	-	-	0.774

-Values are averages of five replicates. Figures with different letter(s) are significantly different according to Duncan's Multiple Range Test at level 5%. Conc.= Concentration. Red.= Reduction

Table (2): Plant growth, yield and percentage total soluble solids (%TSS) of sugar beet cv. Gazelle infected by root-knot nematode, *Meloidogyne incognita* as affected by garlic clove and Acetyl salicylic acid (ASA) extract concentrations.

Treatments	Conc. (ppm)	Height of shoots (cm)	%Inc.	Weight of shoots (g)	%Inc.	Weight of roots (tubers) (g)	%Inc.	%TSS	%Inc.	Average percentages plant growth and yield increase
Soil drench Garlic	100	50.4bc	18.6	200.6c	91.6	146.4e	45.7	16.8a	1.4	79.4
	1000	52.6ab	23.8	260.3a	148.6	249.5a	148.3	16.0a	0.6	89.3
Acetyl salicylic acid	100	40.0f	-5.9	157.8d	50.7	138.7f	38.0	15.8a	0.4	20.8
	1000	56.0a	31.8	219.4b	109.6	145.2e	44.5	15.6a	0.2	46.5
Foliar spray Garlic	100	46.8cd	10.1	215.4b	105.7	146.6e	45.9	15.8a	0.4	43.1
	1000	42.0ef	-1.1	193.5c	84.8	177.4d	76.4	15.8a	0.4	40.1
Acetyl salicylic acid	100	47.0c	10.6	196.6c	87.8	196.3b	95.3	15.0a	-	48.4
	1000	47.3c	11.3	198.1c	89.1	187.9c	87.0	16.0a	0.4	47.0
Untreated inoculated control 1	-	42.5def	-	104.7e	-	100.5h	-	15.4a	-	-
Untreated uninoculated control 2	-	45.9cde	-	150.6d	-	130.5g	-	15.6	-	-

-Values are averages of five replicates. Figures with different letter(s) are significantly different according to Duncan's Multiple Range Test at level 5%. %TSS= percentage total soluble solids. Inc. Increase.

Discussion

Different concentrations of garlic cloves and ASA were tested in this study on cowpea plants as soil drench or foliar spraying. Results proved that these materials were effective in inducing resistance against *M. incognita* depending upon the tested concentrations and method of application. This is as indicated by the number of galls, egg masses on roots and juveniles in soil compared to untreated inoculated control. Also, these treatments improved plant growth criteria, yield and percentage total soluble solids. In the present results, lower concentration of garlic clove as foliar spray only caused higher percentages nematode reduction compared to its higher concentration which emphasized that aqueous garlic extract may serve as an inducer for resistance against root-knot nematode. Abd- Elgawad *et al*. (2009)[33] reported that soil treatment with a commercial product containing aqueous garlic extract reduced the nematode root gall index and increased the activity of catalase, B-1, 3glucanase enzyme in tomato leaves comparable to untreated nematode- infected plants which was considered as an indicator of inducing resistance against root-knot nematode. At the same trend, Korayem and Hasabo (1994)[34]reported that the nematode juveniles of *M. incognita* were immobilized after 24 hrs of exposure to the extract of garlic bulb. The effect of garlic was attributed to the presence of pyruvic acid and ammonia together with diallyldisulphide[16,19]. Also, Nigh (1985)[35]reported that garlic has biochemical substances toxic against nematodes of which allelopathic substances. The volatile substance, allicin in garlic was found to be active against several plant bacteria and fungi and inhibited different plant parasitic nematodes[20,36,37,38,39].

Also, ASA belonging to AS when used as foliar spraying behaved the same trend because it reduced nematode parameters at lower concentration. The effect of ASA as AS may be explained on the basis that it

was considered an endogenous signal for the activation of certain plant defense response by expression of genes for pathogenesis-related protein (PR-1) and enhanced resistance to pathogens [40,41,42,43]. In this study, determination of certain enzymatic activity in sugar beet infected by *M. incognita* treated by garlic clove and ASA revealed an increase in the activity of the enzyme, chitinase compared to controls.

Vice versa, higher concentration from each material caused higher percentage nematode reduction when used as soil drench. This may be due to that garlic extract directly affected on nematode juveniles in soil resulting in reducing or preventing nematode juveniles to penetrate the roots[20] and acetyl salicylic acid behaved the same trend. Subsequently, plant growth criteria increased at different treatments in accordance to the percentages nematode reduction. I.e. As higher percentage nematode reduction of each treatment occurred; there was a corresponding increase in the percentage plant growth criteria. No phytotoxicity was observed by using garlic extract in the present study which contrasts the study by Sukul *et al.* (1974)[44] as they used 250 ml of 50% garlic extract per plant which caused phytotoxicity to plants. This was attributed to osmotic loss of water from plant tissues causing plant wilting.

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