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Monitoring of Systemic Resistance Induction in Tomato Against *Meloidogyne incognita*

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Abstract

The potency of three chemical compounds as resistance inducer in tomato plants against root-knot nematode (*Meloidogyne incognita*) were evaluated using split root system technique. Salicylic acid (SA), Ascorbic acid (AS) and Dipotassium hydrogen phosphate (DKP) was assessed at three concentrations (10, 20 and 50 mM) and the activity of cytoplasmic peroxidase and Phenol oxidase in tomato leaves were measured. Results indicated that application of these inducers significantly reduced all nematode related parameters in tomato plants under greenhouse conditions. Salicylic acid (SA) at 50 mM demonstrated the highest reduction in number of second stage juveniles in 250 mg soil, number of galls, egg masses and females/tomato root system compared to inoculated untreated control. These treatments significantly enhanced plant growth parameters of tomato plants i.e., plant height, root length, fresh root, shoot weight and dry shoot weight. Moreover, these resistance inducers enhanced the synthesis and activity of defence enzymes in tomato plants. Phenol oxidase and peroxidase drastically increased in the treated plants compared with un-treated control plants. Application of SA, AS and DKP may provide an environmentally friendly management strategy against root-knot nematode infection in tomato through induction of systemic resistance.

Keywords: Tomato; Resistance inducer; Defense enzymes; Nematode control

Introduction

Plant-parasitic nematodes (PPN) can act as pests on a wide range of important agricultural crops. The root-knot nematodes (*Meloidogyne* spp.) are among the most destructive agricultural pests globally. They have a wide host range of plants, causing changeable yield losses especially in tropical and sub-tropical agriculture [1]. Recently, *Meloidogyne* has a vital role as limitation factor for several crop cultivation [2]. The control of root-knot nematodes is very challenging [3]. Multiple control methods such as regulatory, cultural, physical, biological and chemical methods used for nematode control on host plants with different advantages and disadvantages [4]. Currently the use of nematicides is being limited, which are expensive, given the increasing concern for human health as well as the environment. Scientists are also looking for other nematode management strategies that aim to reduce pesticide use and to promote non-chemical management practices as much as possible. One of the proposed environmentally friendly options is Induced Systemic Resistance (ISR). It is accepted as one of the most promising methods for controlling plant diseases. Induced resistance (IR) offers a natural defense mechanism of plants as an alternative, non-traditional and eco-friendly control methods. It is also promising for control of soil-borne pathogens [5]. This will enhance the sustainable agricultural system [6]. There are a wide number of abiotic and biotic agents which can induce host resistance to the pathogen [7]. Molecules such as plant hormones or their derivatives or some nontoxic chemical substances can elicit and act as activator of natural inducible defense mechanisms [8,9]. The phytohormone salicylic acid (SA) was one of the most essential signal molecules involved in activator defense responses and/or in sensitizing plant cells for response to pathogen infection [10]. SA is not only involved in photosynthesis, bio-productivity, plant water relations, growth and various enzyme activities but also helps plants against various biotic and abiotic stresses [11]. Ascorbic acid, previously used for induction of plant resistance in plants [12] can control of different fungal diseases [13-15] and plant parasitic nematodes, such as root rot and Root-knot nematodes [16,17]. Dipotassium hydrogen phosphate (DKP) presented many advantages in agriculture used not only preferable source of potassium and phosphorus but also may induce resistance against several plant pathogens [18]. The dipotassium hydrogen phosphate (K_2HPO_4) enhancing mechanisms that similar to those by necrotizing microorganisms which induce resistance [19]. Therefore, the aim of the present research is studying the role of three chemical elicitors as inducers of tomato resistance to RKNs using split root system technique to verify induction ability. Defense enzymes phenol oxidase and peroxidase secreted by the host responsibility were evaluated for possible enhancement of the systemic acquired resistance.

Materials and Methods

The current study was conducted in both glasshouses and laboratories at the Agricultural Botany Department, Faculty of Agriculture, Menoufia University, Egypt. Salicylic acid (SA), Ascorbic acid (AS) and dipotassium hydrogen phosphate/K_2HPO_4 (DKP) was evaluated in this study as resistance inducing agents. Various concentrations of the three inducers (10, 20 and 50 mM) were prepared using ethanol for SA and distill water with AS and DKP. Additionally, *Meloidogyne incognita* was isolated and identified using perineal pattern technique [20] as showed in Figure 1. Nematode was reared on tomato plants (*Lycopersicum esculentum* Mill.) Cv. Beto 86 [21] in the experimental glasshouse. Tomato roots heavily infested with *M. incognita* were used for egg extraction using sodium hypochlorite (NaOCl) technique [22]. Number of harvested eggs per ml was counted under a stereomicroscope. Tomato plants (cv. Gs) were used for the greenhouse experiment to conduct the split root system technique. Tomato seedlings (25 days old) roots'

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were split for two parts using a sharp blade and transplanted in two attached pots. The inducer was to be added in one pot (inducer pot) and the other was to inoculate with nematode (responder pot). Two days after seedling transplant and upon verifying root growth, inducers were added on inducers pots. Inducer addition was repeated five times at two days intervals, while distilled water was used in nematode alone and control treatments. Three days later, responder pots were inoculated with 2000 *M. incognita* eggs/pot by pouring the collected eggs into holes around the seedlings hairy root. Non-inoculated pots served as control. Four replicates of each treatment were arranged in completely randomized block design. Plants were watered as needed and fertilized weekly using fertilizer solution [23]. Two months after nematode inoculation, tomato plants were gently uprooted and washed under running tap water. Plant growth parameters were recorded, and nematode related parameters were also recorded i.e., number of galls and egg masses and females/root system. Number of egg-masses were assessed as described by Daykin and Hussey [24] by dipping in 0.015% Phloxine-B stain solution for 20 minutes. Using a stereomicroscope number of females/root system was counted after preparation [25]. Using serial sieves and modified Baermann technique [26] number of J2S/250 g soil were determined by counting on a slide under a stereomicroscope. Reduction Percentage (R%) of Nematode parameters calculated according the following equation:

$$Reduction\ Percentage(R\%)=\frac{Control-Treatment}{Control}\times 100$$

Estimation of defense enzyme

For evaluation of treatments effect on defence enzymes peroxidase and Phenol oxidase, fresh tomato leaf samples were collected, and enzymes measured using spectrophotometer (CT-2200 Spectrophotometer – Medline, Scientific limited). Peroxidase activity was expressed as changes in absorbance per minute per gram fresh weight [27]. The increase in absorbance density at 470 nm was recorded. Activity of Phenoloxidase was expressed as the change in the absorbance of the mixture at 495 nm [28].

Statistical analysis

The obtained data were statistically analyzed using costat 6.3 version software of analysis of variance. The means were compared using Least Significant Difference (LSD) at p=0.05 as outlined before [29].

Results

Data in Table 1 illustrated the effect of Salicylic acid, Ascorbic acid (AS) and K_2HPO_4 against *M. incognita* in tomato plants. Results indicated that SA at 50 mM treatments showed significant ($p \leq 0.05$) decrease in mean number of second stage juveniles (J2s) in pots soil after two months and galls, egg masses and females/root system. It was also found that the SA at 50 mM presents a significant decrease in the number of J2s/250 mg of pots soil as it recorded 31 J2s/250 mg (93.5% reduction) followed by DKP and AS at 50 mM by 37.75 and 39.50 J2s/250 mg with no significant difference between the two treatments. The lowest number of J2s/250 mg was recoded in pots treated with AS 10 mM by 57.5 J2s/250 mg compare with 477 J2s/250 mg soil in nematode alone treatment. Observation of tomato roots showed a clarified variation among the galls number in the tested treatments. Results showed that SA at 50 mM more efficacious in suppressing the root galling by 93.92% with a significant difference. Galls formation in the treated plants with DKP and AS at 50 mM recorded 90.35 and 89.10% respectively with no significant difference observation. The least reduction percentage in root galling was recorded with AS at 10 mM by 80.35% compared with untreated infected plants as presented in Table 1. Egg-masses production was affected significantly by the used treatments. The least number of egg-masses (13/root system) was recorded in plants treated by SA at 50 mM by 91.44% reduction followed by DKP and AS at 50 mM by 17 and 18 egg mass/root system respectively. The lowest efficacy was related to AS at 10 mM by recording 24 egg mass/root system with 84.2% of reduction compared with 152 egg mass/root system in infected untreated plants. Results observed that the females number in tomato roots were significantly affected by some of the used treatments. The highest reduction was also recorded in plants treated with SA at 50 mM by 89.09% of reduction. The minimum reduction percentage (68.13%) was registered by AS at 10 mM as showed in Table 1.

Impact of the treatments on the plant growth parameters

Data presented in Table 2 showed that all used chemicals with the different doses affected the plant growth parameters. The plant height was significantly higher in plants treated with all the treatments when compared with untreated/inoculated and control plants even there is no significant difference between some treatments. Results showed that root length enhanced by the treatments but there is no significant difference ($p \leq 0.05$) between the three most effective treatments SA, DKP and AS at 50 mM respectively in comparison with untreated control (nematode alone). Additionally, data clearly recorded that fresh root weight of tomato plants was affected by the inducer compare with control. The results have significant increase in fresh root weight of tomato plants treated by SA, DKP and AS at 50 mM. The minimum level was observed in plants infected by nematode only and non-infected control. It was also noticed that fresh shoot weight obviously response to the inducer as a stimulatory effector compare with control. No significance difference appeared between the studied treatments

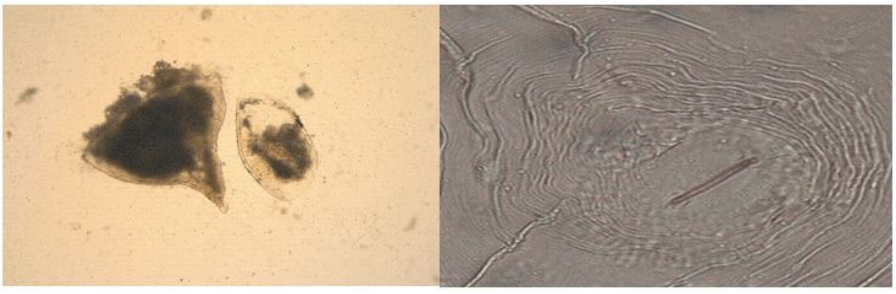

Figure 1: Female preparation and the perineal pattern of *M. incognita*.

SA, DKP and AS at 50 mM respectively. Measurement of tomato plants dry shoot weight revealed that the most effective used material was SA, DKP and AS at 50 mM compared with control.

Estimation of defence enzyme

Results showed that all treatments were significantly affect the concentration of defence enzymes peroxidase and phenol oxidase in tomato plants inoculated with *M. incognita*. Recorded data revealed that the high level of peroxidase activity was 0.882 in plants treated with SA at 50 mM followed by 0.849 and 0.825 in DKP and AS at 50 mM respectively. Lowest peroxidase activity was given by AS at 10 mM compared to the control. Activity of phenol oxidase was significantly difference and the highest level reached 0.551 Followed by 0.509 and 0.431 in plants treated by SA, DKP and AS at 50 mM respectively. Low ascorbic acid concentrations had the lowest effective compare with the inoculated untreated plants as illustrated in Figure 2.

Treatments	Effect on nematode parameters							
	J2/250 mg soil		Galls		Egg masses		Females	
	No.	R%	No.	R%	No.	R%	No.	R%
Ascorbic Acid 10 mM	57.50^{b}	87.95	27.25^{b}	80.54	24.00^{b}	84.21	38.00^{b}	68.13
Ascorbic Acid 20 mM	48.25^{c}	89.88	22.50^{c}	84.11	22.00$^{b, c, d}$	85.53	32.00^{c}	73.17
Ascorbic Acid 50 mM	39.50^{d}	91.72	15.25^{e}	89.11	18.50$^{d, e}$	87.83	21.00d^{e}	82.39
Salicylic Acid 10 mM	47.50^{c}	90.04	20.75$^{c, d}$	85.18	21.25$^{b, c, d}$	86.02	23.25^{d}	80.50
Salicylic Acid 20 mM	46.75^{c}	90.20	17.25$^{d, e}$	87.68	20.25$^{c, d, e}$	86.68	22.00d^{e}	81.55
Salicylic Acid 50 mM	31.00^{e}	93.50	8.50^{f}	93.93	13.00^{f}	91.45	13.00^{f}	89.10
K2Hpo4 10 mM	52.00$^{b, c}$	89.10	23.50$^{b, c}$	83.21	23.00$^{b, c}$	84.87	35.00b^{c}	70.65
K2Hpo4 20 mM	47.75^{c}	89.99	18.00$^{d, e}$	87.14	20.75$^{b, c, d}$	86.35	22.25^{d}	81.34
K2Hpo4 50 mM	37.75^{d}	92.09	13.50^{e}	90.36	17.00^{e}	88.82	17.50^{e}	85.32
Nematode Only	477.00^{a}	0.00	140.00^{a}	0.00	152.00^{a}	0.00	119.25^{a}	0.00

Columns followed by different letters are significantly different according to Duncan's Multiple Test ($p \leq 0.05$)

Table 1: Effect of Salicylic Acid, Ascorbic Acid (As) and K_2HPO_4 on mean number and reduction percentage of galls, egg masses and females/ root system of tomato plants.

Treatments	Effect on plant growth parameters						
	Plant height (cm)	Root length (cm)		Fresh root weight (g)		Fresh shoot weight (g)	Dry shoot weight (g)
		Inducer	Responder	Inducer	Responder		
Ascorbic Acid 10 mM	51.00^{d}	18.25^{e}	17.00^{f}	3.89^{c}	3.28^{d}	17.03^{c}	2.74^{d}
Ascorbic Acid 20 mM	53.00$^{b, c, d}$	18.75$^{d, e}$	18.50$^{d, e}$	4.21$^{b, c}$	3.64^{cd}	17.33^{c}	2.92$^{c, d}$
Ascorbic Acid 50 mM	56.25$^{a, b}$	21.75$^{b, c}$	20.75$^{b, c}$	5.09^{b}	4.96^{b}	20.84$^{a, b, c}$	3.50$^{b, c}$
Salicylic Acid 10 mM	53.5$^{b, c, d}$	19.00$^{d, e}$	19.00^{d}	4.65$^{b, c}$	3.83^{c}	18.21$^{b, c}$	3.10$^{b, c, d}$
Salicylic Acid 20 mM	55.5$^{a, b, c}$	20.25$^{c, d}$	20.50$^{b, c}$	4.98$^{b, c}$	4.60^{b}	19.78$^{b, c}$	3.39$^{b, c, d}$
Salicylic Acid 50 mM	58.50^{a}	24.25^{a}	22.25^{a}	6.91^{a}	6.00^{a}	24.26^{a}	4.47^{a}
K2Hpo4 10 mM	52.50$^{c, d}$	18.25^{e}	17.25$^{e, f}$	4.38$^{b, c}$	3.59$^{c, d}$	17.24^{c}	2.81^{d}
K2Hpo4 20 mM	55.00$^{a, b, c}$	19.50$^{d, e}$	19.50$^{c, d}$	4.83$^{b, c}$	4.06^{c}	18.92$^{b, c}$	3.24$^{b, c, d}$
K2Hpo4 50 mM	57.25^{a}	23.25$^{a, b}$	21.00$^{a, b}$	6.54^{a}	5.63^{a}	21.38$^{a, b}$	3.74^{b}
Nematode Alone	37.00^{f}	13.25^{g}	13.25^{g}	1.76^{e}	1.79^{f}	9.64^{e}	1.47^{f}
Non–infected Control	46.25^{e}	16.25^{f}	16.00^{f}	2.85^{d}	2.74^{e}	13.29^{d}	2.07^{e}

Columns followed by different letters are significantly different according to Duncan's Multiple Test ($p \leq 0.05$)

Table 2: *In vivo* effect of Salicylic acid (SA), Ascorbic acid (AS) and K_2HPO_4 on plant growth parameters of tomato plants.

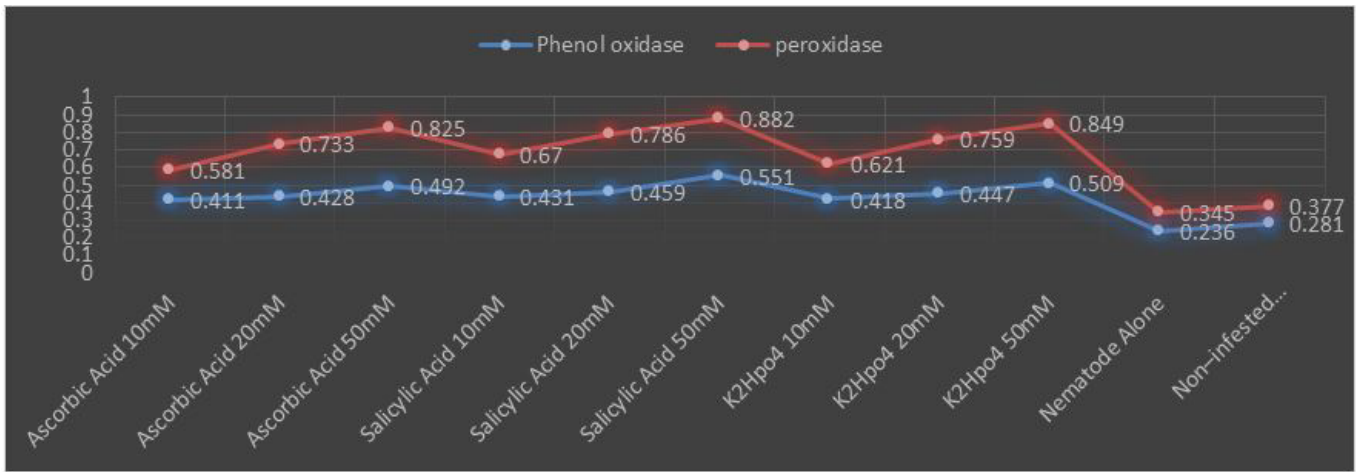

Figure 2: Effect of Salicylic acid, Ascorbic acid (AS) and K_2HPO_4 on peroxidase and phenol oxidase in tomato plants infected by *M. incognita*.

Discussion

Plants have evolved complex mechanisms to defend themselves against pathogens, and thus a great deal of attention has been directed towards elucidating the molecular nature of resistance. Salicylic acid has been shown to be a signalling molecule involved in both local defence reactions at infection sites and the induction of systemic resistance. Although it is still unclear whether this compound can serve as a long-distance messenger signalling the presence of a pathogen, its synthesis and accumulation are important requirements for defence responses. Recent advances in plant pathology field have further established the key role of the signal transduction pathways dependent on salicylic acid. Obtained results from the current study revealed that used inducer varied in their effectiveness in induction the tomato plants resistance against root-knot nematode *M. incognita*. Application via root system in split root system appears an evidence for induction of systemic resistance and the pre-inoculation treatment confirmed that. The frequency of application might be enhanced the resistance induction might provide which give a long-term Protection, this in agreement with [7]. Our study showed that the efficacy of the treatment in inducing resistance to RKNs correlated with concentration of the treatment, which refers to the adsorbed amount of the chemical by the tomato plant roots. Even if dependent on the adsorbed amount of chemical, there are many factors affecting the adsorption processing by the root such as, health and age of the roots, method of application, soil structure and environmental conditions [30]. Salicylic acid has the heist reduction in nematode parameters compared with un-treated control. The reduction was recorded in number of galls, egg masses and females per root system and number of second stage juveniles in 250 mg soil sample. Our finding similar to those obtained by Molinari and Baser [30] and Selim, Mahdy, Sorial, Dababat and Sikora [31] who found that application of SA at 200 µM induced resistance of tomato against the root-knot nematode *M. javanica*. Soil drench with SA significantly reduce number of galls, egg masses in tomato plants compared to inoculated control plants [8] reported that. In a previous study spry of salicylic acid achieved the highest reduction percentage of final number of root galls, egg- masses and nematode population of *Meloidogyne incognita* infecting tomato plants under greenhouse conditions [32]. The pioneer effect of SA in providing a good growth of tomato plants may be revealed to the reduction in root-knot nematodes infestation and reproduction and inhibition of the penetration rate and/or the establishment of the feeding sites by the invading juveniles [16]. Induction of resistance in various crops by SA recorded; the mode of action may be depend on the induction of pathogenesis-related protein (PR protein) [33]. Moreover, SA play a role as endogenous signal for the activation of certain plant defence responses by expression of genes for pathogenesis-related protein (PR-1) and enhanced resistance to pathogens [34]. Moreover, the soil-borne pathogens is correlated with fast increase in activate and induction of some PR proteins [35]. The PR proteins involved in induction defence against diseases and present a pioneer role in reducing the pathogens development and expansion in plants [36]. Ascorbic acid treatments at the three used concentrations were not effective as the other used treatments. The efficacy of AS is correlated with concentration as the high concentration give the high reduction of nematode parameters. This is agreement with finding that the plant content of ascorbic acid related with resistance to some diseases [37]. Ascorbic acid followed the SA and DKP in reduction of number of galls and egg masses and females in tomato root system. Similar results [16], mentioned that as almost inter in the synthesis of mitochondrial hydroxyproline proteins which regulate the cyanide-resistant respiration and this will be in a large amount in resistant plants [17]. Current work showed that application of DKP to the root system inducer side reduced the nematode parameters in the receptor root parts. Our fining is in accordance with [38] who revealed that application by potassium phosphate was effective in controlling *Meloidogyne marylandi* and *Heterodera avenae* in wheat and oats. Population of *Pratylenchus brachyurus* decreasing in maize by using potassium phosphate [39]. The mechanism of phosphate-mediated resistance induction associated with localized cell death, preceded by a rapid generation of superoxide and hydrogen peroxide and local and systemic increases in levels of free and conjugated SA following phosphate application [19]. The ability of DKP to induce plant defence mechanisms may be due to phytoalexins production in the plant [40]. Additionally, Phosphate-induced local and systemic accumulation of salicylic acid in cucumber plants [19]. The fresh and dry weights of both shoots and roots significantly increased by the different treatments. These results confirmed by finding of many researches [32]. The activation of plant growth parameters may be due to the ability of treatments in reducing the nematode infection on the root. Healthy or low infected roots can translocate the water and nutrients from the soil via phloem and xylem in the tomato root system, which affect the growth of tomato plants. Analysis of tomato leave samples revealed to activation of peroxidase and phenol oxidase markedly by using the selected treatments. Activation of plant defences related enzymes against pathogens and accumulation of plant defence metabolites is a vital mechanism of chemical inducers. Previous researcher reported that application of plant with abiotic or biotic stimulators or hormones can increase activity of defending enzymes, such as polyphenol oxidase and peroxidase steering to induce systemic resistance [41-43] recorded different enzymes involved in defence reactions against plant pathogens. Peroxidase is an important member of different biochemical and physiological function in the plants that affect the resistance to the pathogens such as, biosynthesis of lignin, phenol oxidation, phenolic compounds deposition into plant cell walls and defence against pathogens [44]. Further studies must be in plant-pathogen interactions, which will be providing solutions for a long-term, wide broad-spectrum protection and reduced chemical uses in tomorrow agriculture.

Conclusion

We provide an overview of the different mechanisms that have been proposed for management the effects of nematode. Consequently, our present search strongly suggests that application of tomato plants with plant resistance inducers can be used as a part of integrated nematode management programs under greenhouse and field conditions.

Future Prospective

Plants are essential resources for human beings and other living organisms. Environment harmful chemical pesticides are used to and crop production. Environmentally friendly strategies such as organic cultivation is necessary for improve crop production and control stress factors in the future. Methodologies for crop protection in organic productions are scarce throughout the world. Bio-control is a tool with a potentially broad range of stress control and potential to improve crop production without the negative environmental impact associated with chemical pesticides.

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