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Inducing the systemic resistance of tomato plants by root-knot nematode females extract against *Meloidogyne javanica* infection in Egypt

Ahmed El-Sayed Ismail

Plant Pathology Department, Nematology Laboratory, National Research Center, Giza, Egypt

Abstract : Twenty females of *Meloidogyne javanica* were homogenized with 6 ml of distilled water. The dilute homogenate suspension was applied as foliar spray on tomato plants pre, post- and non-inoculated with *Meloidogyne javanica* juveniles. Nematode females extract (NFE) significantly (P≤ 0.05 or 0.01%) increased growth of plants, increased protein content of roots and reduced the nematode infection. The pre-inoculation treatment was more effective than post-inoculation one. Clearly, the nematode females extract is thought to induce systemic resistance in tomato plants..
Key words: systemic resistance, tomato, root-knot nematode, *Meloidogyne javanica*.

Introduction

In Egypt the fast growing population has necessitated increased food production and this in turn has put tremendous pressure on our environment. Also, nematodes comprise a large phylum of animals that includes plant and animal parasitic nematodes as well as many free living species. Plant parasitic nematodes are obligate parasites, obtaining their food as nutrition only from the cytoplasm of living plant cells (Al-Sayed[1], Datta et al. [2], Ismail and Hassabo[3]). Root-knot nematodes, *Meloidogyne* spp., are becoming a real threat to almost all vegetable crops and they have been considered to be a limiting factor in crop production (Ibrahim,[4]). There are environmental restrictions on nematicidal use for controlling plant parasitic nematodes (Lue et al.[5], Landau and Tucker[6], Loria et al.[7], Ismail and Mohamed[8]). Many of botanical nematicides though effective and easily biodegradable (Datta et al. [2]) are not easily available in large quantities from natural sources. A safe alternative to the conventional means of nematode management is to induce the natural defense system of host plants against nematode attack. Bowles[9] reported that plants unlike mammals are known to respond to pathogens by synthesizing some proteins. He also stated that the positive response of these molecular recognition events in response to a local stimulus are also transmitted to distant regions of the plant. Very few investigators in the literature studied the role of the root-knot nematode females extract as aqueous suspension on foliage as induce substance to the systemic resistance of a host plant. For example, Roy et al.[10] and Datta et al.[2] found that *Meloidogyne incognita* females extract, when applied as foliar spray in aqueous suspension on tomato plants inoculated with *M. incognita* juveniles, reduced nematode infestation and promoted plant growth. Also, they stated that the nematode females extract is thought to induce systemic resistance in tomato. Hammond-Kosack et al. [11] reported that pathogenesis-related proteins are known to accumulate in the potato leaves following root invasion by cyst nematode, *Globodera rostochiensis*. In this study, the natural defense system in the tomato plants was studied by applying root-knot nematode females extract as foliar spray. Clearly, the idea of this study was designed to determine the efficacy of *M. javanica* females extract in controlling *M. javanica* on tomato plants under greenhouse conditions in Egypt.

Materials and Methods

Preparation of nematode females extract (NFE):

Females of *Meloidogyne javanica* race 1 were collected from infected tomato roots with same size before egg-masses formation. The species of nematode was identified as *M. javanica* based on morphological and morphometrical characters (Eisenback [12]).The females were washed repeatedly in sterile distilled water, thoroughly homogenized with sterile distilled water at the rate of 20 females per 6 ml of water using a homogenizer according to Ismail and Hassabo [13] and stored at – 10 ºC as needed.

Treatment of tomato leaves with the nematode females extract:

Tomato, *Solanum lycopersicum* L. cv. Super Strain-B was used as a host plant in this study. Aseptically germinated seeds were sown three seeds / pot (25 cm diameter) containing sterilized loamy sand soil (sand 93%, silt 4% and clay 3%). Two weeks after germination, seedlings were thinned to one plant per pot. The pots were divided into five groups of five pots each including equal numbers of non-treated pots served as the control as following: a) uninoculated untreated; b) inoculated untreated; c) NFE- pretreated inoculated; d) post-inoculated NFE- treated and e) uninoculated NFE-treated. When the seedlings were at the 6-leaf stage (14 days), they were inoculated with 3000 ± 50 freshly hatched 2nd stage juveniles of *M. javanica* / plant. The nematode inoculum was obtained from a pure stock culture on tomato plants grown in a greenhouse which prepared by collect the infected sample from a tomato roots and single egg mass was used to establish a population on tomato cv. Super Strain-B for the experiment. All the pots were arranged in a randomized complete block design under greenhouse conditions at 30± 5ºC. NFE was applied as foliar spray by using an atomizer and avoiding soil splashing according to Al-Sayed [1] at the rate of 6 ml / plant in two steps spanned with an interval of 45 minute. The pre- and post-treatment groups were treated with NFE spray 3 days before and 3 days after inoculation; respectively. The plants were uprooted 55 days after inoculation and length and fresh weight of both shoot and root were determined. Also, the number of galls per root system and gall index were counted and rated for root-knot intensity following 0-5 scale according to Taylor and Sasser[14] and the nematode populations per 250 g soil were extracted by sieving and decanting technique according to Barker[15].The number of juveniles from roots, extracted by incubation method based on Southey[16], was counted. Five samples of root pieces were taken at random from each plant group and the total protein in each sample was estimated by Folin-Phenol method (Chatterjee and Sukul[17]).

Statistical analyses:

All obtained data were statistically analyzed by using the analysis of variance and means were compared using the Least Significant Difference (LSD) at $P \leq 0.05$ and 0.01 (Steel and Torrie [18]).

Results and Discussion

The obtained data revealed that NFE significantly ($P \leq 0.05$ or 0.01) increased length and weight of both shoot and root of inoculated tomato plants as compared to the inoculated and the uninoculated control (Table 1). Plants pretreated with an extract of *M. javanica* females and then inoculated achieved the highest increase in their growth parameters (shoot length was 99.7%; shoot weight was 222.1%; root length was 76.6% and root weight was 82.4%) and increased root protein as compared with the inoculated and then treated with *M. javanica* extract treatment (Table 1). Also, it is worth to notice that NFE- treated plants had fewer root galls and number of juveniles in both roots and rhizospheric soil as compared with inoculated untreated plants (Table 2). Treatment with NFE prior to inoculation with nematodes produced better results as compared to the post-inoculation treatment with NFE in terms of improved plant growth and reduced root-knot disease. Also, NFE treatment of uninoculated plants attained significantly ($P \leq 0.05$ or 0.01) highest plant growth as compared to other test groups. Root protein - as biological signal to induce systemic resistance against root-knot nematode in tomato plants – which decreased in inoculated tomato increased significantly with NE treatment (Table 2). Root protein increased significantly in inoculated and uninoculated NE-treated tomato plants but decreased significantly in plants treated after inoculation.

Table1. Tomato growth as influenced with treatment by foliar spray at 6 ml / plant with an aqueous extract of *M. javanica* females (20 females / 3 ml distilled water)

Treatment	Shoot system				Root system			
	Length (cm)	Inc.%*	Weight (g)	Inc.%	Length (cm)	Inc.%	Weight (g)	Inc.%
Uninoculated untreated	45.8	40.1	64.6	92.8	17.4	35.9	7.9	- 33.6
Inoculated untreated	32.7	---	33.5	---	12.8	---	11.9	---
Pretreated with an extract of *M. javanica* females and then inoculated	65.3	99.7	107.9	222.1	22.6	76.6	21.7	82.4
Inoculated and then treated with *M. javanica* extract	53.9	64.8	73.4	119.1	16.9	32.0	20.8	74.8
Uninoculated and treated with *M. javanica* extract	61.7	88.7	123.6	269.0	24.8	93.8	24.3	104.2
L.S.D. 0.05 L.S.D. 0.01	14.3 20.0		20.8 29.5		5.3 7.5		4.7 6.2	

*Inc. % = Increase in growth as compared with inoculated untreated plants.

Table 2. *Meloidogyne javanica* decreasing of tomato following treatment by foliar spray at 6 ml / plant with an aqueous extract of *M. javanica* females (20 females / 6 ml distilled water).

Treatment	No. of galls/ root system	Gall index*	Number of juveniles		Root protein (mg/g)
			5 gm root	250 gm soil	
Uninoculated untreated	---	---	---	---	87.5
Inoculated untreated	158.6	5.0	675	956	94.2
Pretreated with an extract of *M. javanica* females and then inoculated	56.4 (64.4)#	4.0 (20.0)	110 (83.7)	123 (87.1)	99.7
Inoculated and then treated with *M. javanica* extract	89.7 (43.4)	4.0 (20.0)	177 (73.8)	344 (64.0)	96.2
Uninoculated and treated with *M. javanica* extract	---	---	---	---	104.3
L.S.D. 0.05 L.S.D. 0.01	40.1 55.6		64.0 83.0	88.0 102.0	3.7 5.2

The values between brackets represent reduction percent as compared with inoculated untreated plants. *Gall index: No gall = 0; 1-2 gall = 1; 3-10 gall = 2; 11-30 gall = 3; 31-100 gall = 4 and more than 100 = 5 according to Taylor and Sasser (1978).

Application of NFE, on the leaves at very low doses, might have stimulated the host plants to induce their natural defense mechanisms against the nematode attack and the resistance induced by NFE was systemic. Also, the mechanism for the control of the root-knot nematode using root-knot nematode females extract as foliar spray could induce synthesis of some antagonistic substances in the treated plants[2] resulting in poor penetration and later retardation in different activities of the 2nd stage juveniles such as feeding and/or reproduction as suggested by Bunt[19]. In addition, this mechanism might explain throughout the poor root-knot

development which attributed to formation different components in *M. javanica* females bodies such as amino acids, lipids, fatty acids proteins, hormones and enzymes that play role in plant movement as resistance agent in tomato plants. Also, lectins accumulated in galled regions of root of *Hibiscus esculentus* infected with *M. incognita* Das et al. [20]. There is evidence that defense gene expression and changes in the levels of defense – related product such as phytoalexins, callus and lignin are modulated by local and systemic signaling events arising out of parasite invasion or some foreign substances (Bunt [9]). Fassuliotis[21] reported that there are two types of resistance. The first is the pre-infectional resistance, which operates before the nematode penetrates the surface of the roots. The second is the post-infection resistance which is manifested after the nematode penetrates the plant tissues. The present data showed that NFE treatment have two types of resistance, in regard to, most of *M. javanica* larvae – to some extend- failed to penetrate roots of tomato plants when planted with both NFE treatment. Our results were harmony with Datta et al [2] and Roy et al. [10] who found that *M. incognita* females extract reduced nematode infestation and promoted plant growth and also, they stated that this treatment is thought to induce systemic resistance in tomato against *M. incognita*. Root invasion by the cyst nematode produced systemic accumulation of new proteins in the leaves of potato plants as reported by Hammond-Kosack[11]. In addition, proteinase inhibitor proteins (PIP), a defense product, accumulate in potato leaves in response to physical injury of a distant leaf[22] or of the root [9] or spraying of a distant leaf with absicisic acid (Pera-Cortez[23]). It appears that NFE might have induced PIP and other defence-related products in host plants which in turn reduced nematode infection. The root- knot nematodes are known to share common antigens with its host plants (McClure et al.[24], Elshahawy et al.[25], Mawardika et al.[26], Ghoname et al.[27], Abdalla et al.[28], Vimala Kumari et al. [29], El-Sayed and Mahdy [30]. Moreover, Osman et al.[31] found that in Egypt under greenhouse conditions, the pre-inoculation of reniform nematode, *Rotylenchulus reniformis* succeeded as inducing resistance against *M. incognita* in potato plants. Therefore, application of NFE might serve as a prophylactic, a safe environment-friendly nematode-controlling agent as well as an agent that adds to the nutritional value of the crops.

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