EFFECT OF RENIFORM NEMATODE, *ROTYLENCHULUS RENIFORMIS* AS BIOTIC INDUCER OF RESISTANCE AGAINST ROOT-KNOT NEMATODE, *MELOIDOGYNE INCOGNITA* IN POTATO

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Abstract: A greenhouse experiment was conducted to evaluate the preinoculation of reniform nematode, *Rotylenchulus reniformis* for inducing resistance against *Meloidogyne incognita* in potato plants. Potato plants were inoculated with reniform nematode three days before the same plants received a root knot nematode inoculation. There was a reduction in the population parameters of the root knot nematode compared to plants given only a single inoculation. The studied potato plants which had two inoculations, also had enhanced growth parameters. The activity of the enzymes; peroxidase, polyphenol oxidase, and chitinase increased in the inoculated plants compared to the non-inoculated control.

Key words: Induced resistance, *Rotylenchulus reniformis*, *Meloidogyne incognita,* resistance enzymes, potato

INTRODUCTION

Root-knot nematodes, *Meloidogyne* spp. are obligate parasites and very damaging plant pests which limit agricultural productivity. Most cultivated plant species are susceptible to root-knot nematode infection (Sasser and Carter 1985). In Egypt, 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 2011). There are environmental restrictions on nematicidal use for controlling plant parasitic nematodes. For this reason, biological control and other eco-friendly disease control measures have recently gained interest.

Infective juveniles (J_2) of root-knot nematode, *Meloidogyne* spp. generally locate and penetrate roots of susceptible and resistant plants in equal numbers (McClure *et al.* 1974; Kaplan *et al.* 1980; Huang 1985). The majority of J_2 that enter susceptible plants establish and multiply. The majority of those that enter resistant plants fail to establish and often egress from the roots 3 to 5 days later. Herman *et al.* (1991) reported that 87% of *M. incognita* egressed from resistant soybean (*Glycine max*) within 5 days, compared to 4% from susceptible soybean. The immigration of juveniles was attributed to the accumulation of defensive substances that inhibit the establishment of the nematode population. Veech and McClure (1977) observed an association between the expression of incompatibility in cotton (*Gossypium hirsutum*) to *M. incognita* and post-infection increase in phytoalexins, such as methoxy-substituted terpenoid aldehydes. Lower levels of these compounds were detected in compatible interactions. Such induced resistance to fungal, bacterial, and viral pathogens after prior inoculation of plants with weakly aggressive strains, avirulent, or incompatible forms of the disease – causing organisms has been reported (Dean and Kuc 1985; van Peer *et al.* 1991). Ibrahim and Lewis (1986) reported that prior inoculation of *M. incognita* on *M. arenaria* – susceptible soybean decreased root galls and egg mass production by *M. arenaria*. Eisenback (1983), also reported that tobacco cv. NC95 resistant to the *M. incognita* race 1 lost resistance when *M. arenaria* or *M. hapla* Chitwood was applied three weeks earlier and prior inoculation with *M. javanica* or *M. incognita* race 4 had no effect. Ogallo and McClure (1996) found that advanced inoculation of the tomato cv. Celebrity or the pyrethrum clone 223 with host-incompatible *M. incognita* or *M. javanica,* elicited induced resistance to hosts compatible to *M. hapla* in pot and field experiments. Induced resistance increased with the length of the time between inoculations and with the population density of the induction inoculum. The optimum interval before using a challenge inoculation, or population density of inocu-

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lum for inducing resistance was l0 days and 5,000 infective nematodes per 500 cm^3 pot. Hence, the aim of this research was to study the potential of the advanced infection of potato plants with incompatible or mildly virulent nematode species *R. reniformis* to the normally compatible nematode *M. incognita*, where *M. incognita* was used as a biological control.

MATERIALS AND METHODS

Sprouting eyes from tubers of potato *Solanum tuberosum* L.cv. Spunta was sown in 15 cm diameter-clay pots filled with one kg solarized sandy loam soil (1:1 w/w). One month after germination, plants were fertilized as recommended in potato culture and treated as follows:

1. The single inoculation with the root-knot nematode, *M. incognita*, was done with 500 second stage juveniles (J_2).
2. The single inoculation with the reniform nematode, *R. reniformis*, was done with 250 unswollen pre-mature females.
3. The combined inoculation with the *M. incognita* and R. *reniformis*, was done by inoculation of the root-knot nematode, three days after inoculation with the reniform nematode.
4. Non-inoculated pots served as the control.

Each treatment was replicated five times. For enzyme determination, infected roots from each inoculated treatment and from the control (without nematodes) were taken before inoculation, and then 5 and 10 days after inoculation. Two months after inoculation, other replicates of plants were uprooted and the nematodes in the soil and roots were counted. The plant growth parameters were recorded. Enzymes were assayed according to the methods described by Lee (1973) for peroxidase, Bashan *et al.* (1987) for polyphenol oxidase, and Reid and Ogrydziak (1981) for chitinase.

Statistical Analysis

Data were analyzed statistically with the Least Significant Difference (LSD) test, using the Master of Statics (MSTAT) statistical program version 4.

RESULTS

Table 1 indicated that with the combined inoculation, *M. incognita* had fewer galls, egg masses and females/plant and juveniles/pot than the corresponding *M. incognita* alone, as indicated by the percentage reductions, 83, 87, 87 and 88%, respectively. Also, there was a reduction in *R. reniformis* reproductivity in the combined inoculation, measured as the percentage reduction in the number of females and egg masses/plant and juveniles/pot by 33, 33 and 5%, respectively.

As for the effect of the *M. incognita* and *R. reniformis* combined inoculation on potato growth, table (2) showed that the combined inoculation improved root length, root and shoot fresh and dry weights as the percentage increases were 13, 83, 44, 163, and 22% , respectively compared to the single *M. incognita* inoculation.

Data presented in table 3 indicated that when the plants were treated with *R. reniformis* alone, all the tested enzyme activities increased, compared to the control (without nematode inoculation or with plants infected with *M. incognita* alone). As for the peroxidase enzyme, the enzyme activities increased by 333% and 231% for the control (non-inoculated plants), after 5 and 10 days of inoculation, respectively. As for polyphenol oxidase, the enzyme activity increased by 1,138% and 1,100% for the control (non-inoculated plants), after 5 and 10 days of inoculation, respectively. Chitinase activity was shown to

Table 1. Effect of prior inoculation of *R. reniformis* on the pathogenicity of *M. incognita* infection in potato plants

Treatments	Developmental Stages		Galls		Egg masses		Females		Juveniles/Pot	
	No.	[%] red.	No.	[%] red.	No.	[%] red.	No.	[%] red.	No.	[%] red.
Meloidogyne alone	05	00	24	00	23	00	30	00	217	00
*Combined treatment (*Rotylenchulus* + *Meloidogyne)*	04	20	04	83	03	87	04	87	27	88
LSD at ($p \leq 0.05$)	n.s.	–	4.984	–	2.927	–	8.121	–	38.97	–
Rotylenchulus alone	–	–	–	–	03	00	03	00	56	00
Combined treatment (*Rotylenchulus* + *Meloidogyne)*	–				02	33	02	33	53	05
LSD at ($p \leq 0.05$)	–	–		–	n.s.	–	n.s.	–	2.267	–

*combined inoculation was done by inoculating *M. incognita* three days after a *R. reniformis* inoculation

Means with different letters within each column are significantly ($p \leq 0.05$) different according to the LSD test

Each value represents the mean of five replicates

Red. – reduction

n.s. – not significant

Table 2. Effect of the *R. reniformis* inoculation on plant growth parameters of potato plants infected by *M. incognita*

Treatments	Lengths [cm]				Fresh weights [g]				Dry weights [g]			
	root	[%] inc.	shoot	[%] inc.	root	[%] inc.	shoot	[%] inc.	root	[%] inc.	shoot	[%] inc.
M. incognita alone	25.80	00	92.00	00	13.98	00	52.40	00	0.89	00	5.93	00
*Combined treatment (*Meloidogyne* + *Rotylenchulus*)	29.20	13	78.20	00	25.60	83	75.20	44	2.34	163	7.22	22
LSD at ($p \leq 0.05$)	n.s.	–	8.14	–	3.00	–	7.30	–	0.28	–	1.23	–

*combined inoculation was done by inoculating *M. incognita* three days after a *R. reniformis* inoculation

Inc. – inoculation

Means with different letters within each column are significantly ($p \leq 0.05$) different according to the LSD test

Each value represents the mean of five replicate

n.s. – not significant

Table 3. Effect of the *R. reniformis* inoculation on the enzyme activity in potato plants prior to infection with *M. incognita* (% of control values)

Treatments	Enzyme activities								
	peroxidase activity			polyphenol oxidase activity			chitinase activity		
	before inoculation	days after inoculation		before inoculation	days after inoculation		before inoculation	days after inoculation	
		5	10		5	10		5	10
R. reniformis	83	333	231	157	1138	1100	273	160	229
*combined treatment (*R. reniformis* + *M. incognita)*	83	308	225	168	688	983	273	132	182
M. incognita	100	166	69	100	138	116	100	120	167
The control non-inoculated	100	100	100	100	100	100	100	100	100

*combined treatment was done by inoculating *M. incognita* three days after a *R. reniformis* inoculation

be 160% and 229% for the control, after 5 and 10 days of inoculation, respectively.

Concerning the assessment of enzyme activity following the use of the inoculation of *M. incognita* alone, the results revealed a different pattern as indicated by a reduction of peroxidase down to 69% for the control (non-inoculated plants) after 10 days of nematode inoculation. However, slight increases were found in the activity of polyphenol oxidase as well as chitinase as compared with the remarkable increase found after inoculation of the plants with *R. reniformis* alone. Also, the activities of the three enzymes studied in this work, showed a pattern of increase after the *R. reniformis* inoculation took place 3 days prior to the inoculation with *M. incognita* (combined treatment). These patterns were almost the same as those found following the inoculation with *R. reniformis* alone.

DISCUSSION

Our results indicated that prior inoculation of potato plants with the host-incompatible or less virulent nematode, *R. reniformis,* induced resistance. As a result, the reproduction of the host-compatible nematode, *M. incognita,* was highly suppressed, as measured by the reduction in the number of females, galls, and egg masses. In addition, the plant growth parameters were improved. Similar results have been experimentally observed with several fungal and bacterial pathogens as well (Sequeira 1983). There were fewer *R. reniformis* than *M. incognita* in the roots of the potato cv. Spunta, which may indicate the unsuitability of this potato cultivar to the reniform nematode. There were fewer scores of gall and egg mass numbers of *M. incognita* when used as a combined inoculation, compared with the same nematode used as a single inoculation. This observation indicates that *R. reniformis* may have caused some effects on the root tissues and subsequently on *M. incognita* penetration and reproduction.

When the potato plant was triggered by the incompatible pathogen (*R. reniformis*), the defense mechanism in the plant was stimulated. Therefore, *M. incognita* population density was decreased. Our results, also agree with Ogallo and McClure (1995 and 1996), who studied the changes in host suitability of tomato and pyrethrum plants to host-incompatible *M. incognita* and host-compatible *M. hapla* determined by using split-root assays. Prior inoculation with M. *incognita* significantly suppressed reproduction of *M. hapla* applied 5 days after or even later. Aboul-Eid and Youssef (1998) found that, *M. incognita* reproduced on the roots of four tested potato cultivars, no matter whether the single or combined

inoculation with *R. reniformis* was used. However, no final population of *R. reniformis* was detected on the tested cultivars at the end of the experiment. In the combined treatment, it was noticed that *M. incognita* had fewer galls and egg masses than when the corresponding single inoculation had been used. Similarly, McKenry and Anwar (2007) reported that reproduction of the virulent *M. arenaria* population was determined 63 days after being challenged by an avirulent *M. incognita* population when a range of inoculum densities and timeframes had been used. Induction of resistance became apparent when the virulent nematode population was inoculated 7 days after the avirulent nematode population, and resistance increased thereafter. The level of induced resistance increased with increased inoculum levels of the avirulent nematode population. Also, Anwar and McKenry (2008) suggested that a resistant or susceptible response can result from prior plant inoculations involving either avirulent or virulent populations of the same nematode species

Indeed, the concept of primary plant defense as an equivalent to adaptive immunity has been recently introduced. Plants can develop an enhanced defensive capacity that is effective against a remarkably wide range of different stresses. In many cases, this induced resistance is not based on direct defense activation by the inducing agent, but on a faster and stronger activation of inducible defense mechanisms once the plant is exposed to stress. This defense sensitization is commonly referred to as "priming" and allows the plant to adjust its inducible defense system to the environmental conditions. Therefore, priming can be regarded as a form of adaptive immunity that increases the plant's ability to survive in hostile environments. Induction of priming yields broad-spectrum resistance with minimal reductions in plant growth and seed set. Although priming has been known to occur for decades, most progress in the understanding of the phenomenon has been made over the past few years.

Recent insights in the mechanisms behind systemic acquired resistance (SAR) suggest prior inoculation of the plant with incompatible pathogen and then challenging the plant with host compatible pathogen. The incompatible pathogen activates the inducible defense mechanisms "priming" which allows the plant to adjust its inducible defense system to the new conditions.

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