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Comparative Efficacy of Oil Seed Radish and Tomato Root Exudates on Hatching of *Meloidogyne hapla*

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Abstract: Oil seed radish (*Raphanus sativus* cv. common L.) has been used for plant-parasitic nematodes as a green manure and trap crops. This study was conducted to determine and compare the effects of root exudates of oilseed radish and susceptible tomato (*Lycopersicon esculentum* L. variety Rutgers) on *Meloidogyne hapla* greenhouse populations from Manhattan (MH), New York (NY) and Michigan (MI) in order to understand the way of effect of OSRC (Oil seed radish). In a laboratory, 3 mL exudates of OSRC, tomato and same amount of water introduced with 1000 eggs of each nematode population that come from greenhouse mass culture separately. Hatched second stage juveniles were counted for 8 days and exudates were renewed with stock solution every day. Although tomato exudates increased hatched juvenile number but there is no statistically difference between OSRC and tomato exudates. Also all different *M. hapla* populations showed same responses to every exudate. As a result it may be said that, OSRC does not effect *M. hapla* population by influencing egg hatching but, it may effect infection and/or reproduction level of nematodes.

Key words: Egg hatching, *Meloidogyne hapla*, root exudates, *Raphanus sativus*

INTRODUCTION

Management of plant-parasitic nematodes has been searched for a long time all over the world. The objective of nematode control is to prevent important yield loss and quality in crops and in longer term, to keep nematode populations small. Crop rotation and growing resistant plant varieties have been advised after having side effects of nematicides on nature, human and animal health and ozone layer (Whitehead, 1998). Plant-parasitic nematodes have 5 different biological stages as an egg, second, third, fourth stage juvenile and mostly mature female and male. Egg is the most resistant stage of nematodes due to having a strong egg shell and lipid membrane (Wharton, 2002) and egg hatching is one of the most important parts in the life cycle of nematodes. Hatching, effects the survival of nematodes, occurrence of generation thus penetration of host plants and level of damage caused by plant-parasitic nematodes. Exudates derivatives from plant roots and seasonal changes in agro ecosystem, physical and chemical factors of environment especially soil have a great importance on hatching. These factors may cause egg hatching or diapause of

infective juvenile (Perry, 1987; Shepherd and Clarke, 1971). It is too difficult to define the effects of root exudates on hatching in agroecosystems that are using for agricultural purposes. If root exudates cause the increase of hatching, penetration of plant-parasitic nematodes may be increased as well as lots of host plant would be damaged economically. Most studies had been done to search the reality of this idea by using different plant-parasitic nematodes and plants (Idowu and Fawole, 1990; Hamlen and Bloom, 1968; Inserra *et al.*, 1983).

There are a lot of records about OSRC related with the using of green manure (Al-Rhiayani *et al.*, 1999) and trap crops (Smith *et al.*, 2004). Some trap crops such as *Beta vulgaris* or *R. sativus* var. *oleifera* have been shown to stimulate hatch of plant-parasitic nematodes (Winslow and Ludwig, 1957). But, there is no information the effects of *R. sativus* root exudates on hatching of *M. hapla*. So, the objective of this study was to determine effects of *R. sativus* and compare with tomato root exudates on egg hatching of different populations of *M. hapla*. The aim of the research was also to understand the way of *R. sativus* in a using of trap crops or green manure for managing *M. hapla* in vegetable production.

MATERIALS AND METHODS

Experiments were conducted in both greenhouses and laboratories of Michigan State University, Natural Science Building, East Lansing in 2005. Experimental steps summarized as follows:

Obtaining eggs of *M. hapla* to be used in experiments:

Eggs of *M. hapla* populations from Manhattan (MH), New York (NY) and Michigan (MI) were obtained from mass culture by using tomato (*Lycopersicon esculentum* L., variety Rutgers) in greenhouses. Greenhouse growth conditions were set at $25\pm 2^{\circ}\text{C}$ with diurnal cycles of 8 h dark and 16 h light with photosynthetically active radiation of 300 to 350 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at canopy level. Eggs from each nematode population were obtained by using Hussey and Barker's (1973) bleach method. Inoculum population densities, from ten 1 mL suspensions were determined under stereobinocular microscope. One thousand eggs for each population was separated and saved in refrigerator till using.

Preparation of root exudates: Tomato and oilseed radish (*Raphanus sativus* cv. common) plants were used for research the effects of hatching. Seedlings of these plants were selected from mass-germinated seeds and transplanted into culture or experimental pots containing steam sterilized soil and watered daily as needed. Greenhouse growth conditions were same as explained above. Almost 5-6 weeks old plants were selected and roots were washed then root exudates were obtained in deionised distilled water (Castillo and Vovlas, 2002). Tomato root exudates were used as 2 different rates; one of them is non-diluted, the other is diluted half time.

Applying of root exudates to eggs: Petri dishes (35×10 mm) and egg hatching vessels were used in the experiments. One thousand eggs of each population in 1 mL water were put into hatching vessel and this vessel were settle in petri dishes adding 3 mL root exudates. Petri dishes were covered and sealed with parafilm and put into big glass tray. The bottom of tray was moistened with a little amount of water regularly and top part of tray was enclosed with an aluminum paper and put in a room temperature ($25\pm 2^{\circ}\text{C}$). Petri dishes were examined under stereobinocular microscope every day, taking the vessel out and exudates were renewed by adding 3 mL and old solutions were removed after counting second stage juveniles.

Data analysis: Data were utilized after tukey's test in SAS Statistical program (SAS Institute, 1985; Steele and Torrie, 1980) to determine the effects of root exudates

and population on hatching of *M. hapla*. The experiment was repeated and they are designated as Test 1 and Test 2. In each test, 48 experimental units (3 *M. hapla* population×3 plant exudates + water = 4×4 replicates) were set. The petri dishes were randomly arranged and the experiment was terminated when no newly second stage juveniles were observed. Results from repeated assays (Test 1 and 2) were similar so combined together for analysis.

RESULTS AND DISCUSSION

The current study reports the toxicity of aqueous root extracts *in vitro* against eggs of *M. hapla* is less and there were no statistically significant differences between the tomato and OSRC root extracts against the egg hatching. Root extracts of OSRC reduced *M. hapla* egg hatch but there is no statistically differences between tomato and OSRC root extracts on egg hatching of different populations of *M. hapla*. Egg counting were terminated 8 days after introduced with exudates to eggs because, in eighth days, there was no newly hatch juveniles were observed for all populations. It was found out that, non-diluted tomato root exudates influenced positively the number of hatched second stage juveniles. The total number of egg hatching was the highest in treatments of non-diluted tomato root exudates followed diluted-tomato root exudates, *R. sativus* and the least egg hatching was found in water as considered control (Fig. 1). But, the log cumulative hatches of all population were not significantly different for exudates (Table 1). Also, there is no statistically a difference between populations (Table 2) response to different exudates. When non diluted and diluted tomato root exudates applied, NY population has the least hatching eggs, but MI was the first. When OSRC root exudates applied, MH population has the most second stage juveniles, although MI has the least. When water applied to the eggs, almost all population has similar hatching egg numbers. So, localities may have an important role of this kind of response.

Oil seed radish has been known as a maintenance host for *Meloidogyne chitwoodi* (Rf = 2.58) (Ferris *et al.*, 1993; Al-Rehiyani and Hafez, 1998) for *M. hapla* (Faulkner and McElroy, 1964; Gaskin and Crittenden, 1956), for *M. incognita* race 1 and 3 and for *M. javanica* (McSorley and Frederick, 1995) and susceptible host for *M. incognita* and *M. javanica* (Gardner and Caswell-Chen, 1994; Anonymous, 2006). The main aspect of OSRC is that it has been used for the management of plant-parasitic nematodes (Zasada and Ferris, 2004; Smtih *et al.*, 2004) intelligently use this cover crop for pest management, it is necessary

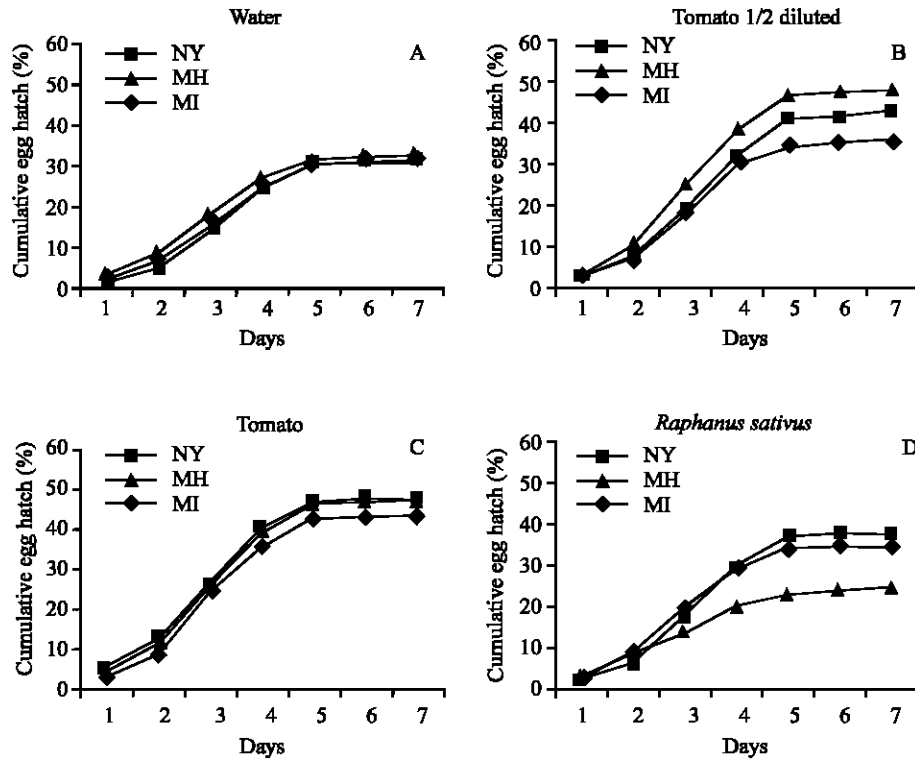


Fig. 1: Effects of different exudates A. Water, B. Tomato 1/2 diluted, C. Tomato and D. *Raphanus sativus* on percentage of cumulative egg hatching of NY, MH, MI populations of *Meloidogyne hapla*

Table 1: Effects of different root exudates (Water, Tomato 1/2 diluted, Tomato, *Raphanus sativus*) on Log cumulative egg hatch of *Meloidogyne hapla* populations

Treatments	Log cumulative egg hatching, days after introducing root exudates and water to eggs							
	1	2	3	4	5	6	7	8
Water	1.36A*	1.83A	2.20A	2.40A	2.49A	2.50A	2.50A	2.50A
Tomato 1/2 diluted	1.46A	1.93A	2.31A	2.52A	2.60A	2.61A	2.62A	2.62A
Tomato	1.60A	2.03A	2.39A	2.58A	2.65A	2.65A	2.66A	2.66A
<i>R. sativus</i>	1.47A	1.92A	2.23A	2.41A	2.49A	2.50A	2.50A	2.50A

* Values in the column having same letter do not differ significantly at p = 0.05 level

Table 2: Changes of cumulative egg hatching (percentage) of *M. hapla* populations from New York (NY), Manhattan (MH) and Michigan (MI) applying after root exudates day by day

<i>M. hapla</i> populations	Cumulative egg hatching (%) days after apply of exudates to eggs							
	1	2	3	4	5	6	7	8
NY	2.64A*	7.93A	18.75A	31.36A	38.63A	39.25A	39.78A	39.78A
MH	3.98A	10.36A	21.03A	31.40A	36.94A	37.89A	38.21A	38.21A
MI	2.57A	7.80A	19.44A	29.78A	35.21A	35.66A	36.14A	36.14A

* Values in the column having same letter do not differ significantly at p = 0.05 level

to understand the mechanism(s) by which OSRC suppresses plant-parasitic nematodes. One possible mechanism concerns inhibitory compounds released during decomposition of OSRC residue after incorporation into soil. First recognition between plant-parasitic nematodes and hosts, stimulations of hatching and tends of juvenile through plants involve some signals though plant to nematodes including root

exudates (Perry, 1987; Prot, 1980). From this point of view, determination of exudates derivates from roots must be done for especially management of plant-parasitic nematodes (Zhao *et al.*, 2000). Lots of researches reported that, root exudates derivates from plants roots through soil, could be attractant or repellent or neutral effects on the behavior of nematode (Griffin, 1969; Griffin and Elgin, 1977; Viglierchio, 1961). Therefore,

place, time, conditions must be considered and all combinations must be searched separately. Even though it has been known from 65 years that, some root exudates have a big importance on recognition of nematodes to the hosts, it is difficult to determine these effects separately (Zhao *et al.*, 2000). The effects of host root exudates stimulate egg hatching of cyst nematodes (e.g., *Globodera pallida* Stone, *G. rostochiensis* (Wollenweber) sometimes provides indications of host susceptibility or resistance to these nematodes (Arntzen *et al.*, 1994). Poor stimulation of egg hatch was suggested as a component of host tolerance to potato cyst nematode by potato cultivar (Evans, 1983).

Studies about hatching response of *Meloidogyne* to root exudates are difficult to evaluate because of a variety of experiment protocols. But in general, root exudates have been known not necessary for hatching, although exudates may cause increasing the number of hatching juveniles. In this genus temperature is the key factor for hatching and host availability is achieved with diapause in unhatched juveniles which may be mediated through the host (Perry, 1987). Good or poor host cowpea and maize root leachates has the same type of effects of *M. incognita* egg hatching in the study of Idawu and Fawole (1990) and Inserra *et al.* (1983) also have similar results related with *M. chitwoodi* and *M. hapla*. Thus, it may be accepted that, root exudates have no effects on the hatching of the genus *Meloidogyne* (Perry, 1987). Present results also have a big harmony with those. Our objective was to determine effects of *R. sativus* and compare with tomato root exudates on egg hatching of different populations of *M. hapla* and to understand the way of *R. sativus* in a using of trap crops or green manure for managing *M. hapla* in vegetable production. So, it may be said that, OSRC does not decrease egg hatching level of root-knot nematodes, thus, when OSRC introduced into the agricultural system as trap crops or green manure, it would not be possible to see less second stage juveniles of root-knot nematodes. But OSRC may be effected reproduction of penetrated juveniles or confused to finding host abilities of root-knot nematodes. Being a good host, may be related with the penetration of plant-parasitic nematodes as a big probability that it must need further researches. So, it is clear that, OSRC does not affect the level of second stage juveniles.

The egg is often one of the most resistant stages in the nematode life cycle, possibly due to its three-layer shell. Brassicaceae produce glucosinolates which are β -D-thioglucosides, distinguished from one another by differences in their organic side chains (R groups)

(Zasada and Ferris, 2004). Demonstration of allelopathic and nematicidal activity in OSRC suggests the potential of developing a practical pest management strategy using OSRC as a green manure crop. But determining the toxicity of aqueous extracts involved in plant-parasitic nematode suppression is only part of the information needed to optimize this management tactic. Further research is needed to determine the activity and behavior of OSRC compounds released in soil. The chemistry of OSRC species has been reviewed by Zasada and Ferris (2004). The chemistry of OSRC is complicated and we do not know to which compounds the organisms in this study were exposed. Additional research should be directed toward identifying OSRC-produced specific compounds that are toxic to plant-parasitic nematodes. Further research is needed to understand the fate of compounds produced by OSRC in soil and their use for control of plant-parasitic nematodes.

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