Hellenic Plant Protection Journal **4:** 21-24, 2011

SHORT COMMUNICATION

The effect of a garlic essential oil component and entomopathogenic nematodes on the suppression of *Meloidogyne javanica* on tomato

I. Anastasiadis[1], A.C. Kimbaris[2], M. Kormpi[1], M.G. Polissiou[3] and E. Karanastasi[1]

Summary Root-knot nematodes are worldwide distributed plant pests with a wide range of hosts that cause downgrading and unmarketability of produce, significant yield decrease, or even total failure of various crops. The entomopathogenic nematodes have extensively been studied as a potential alternative method for the control of plant parasitic nematodes. In addition, the essential oil of garlic and its volatile components that possess fumigant properties against several plant pests and pathogens have also been shown to suppress plant parasitic nematodes. The present work is a pilot study examining the possibility of a combined action of *Steinernema carpocapsae* and diallyl disulfide, a volatile component of garlic essential oil, against *Meloidogyne javanica*. The results of the study showed that the combined use of *S. carpocapsae* and diallyl disulfide significantly reduced the population of *M. javanica* on tomato.

Additional keywords: diallyl disulfide, root-knot nematodes, *Steinernema carpocapsae*

Root-knot nematodes (*Meloidogyne* Goldi 1892 - RKN) are obligate parasites of higher plants distributed worldwide causing considerable yield losses and reduction of product quality on almost every plant species. Garlic essential oil and its volatile components have repeatedly been studied and it is now commonly accepted that they possess fumigant properties against several plant pests and pathogens, including plant parasitic nematodes (PPN) (5, 7). Diallyl disulfide, used in the present study, is one of the garlic essential oil volatile components that accounts for 30-50% of the total sulphide mixture (13). Entomopathogenic nematodes (EPN) (*Heterorhabditis* and *Steinernema* species) are obligate parasites of insects that kill their hosts by introducing their bacterial symbionts (*Photorhabdus* and *Xenorhabdus* species, respectively) into the insect's haemocoel (2, 4). Surprisingly, some 25 years ago, it was shown that the co-existence of PPN and EPN causes a reduction in PPN populations (3, 6, 10). The objective of the present study was to examine whether there is a possibility of a combined action of EPN and diallyl disulfide in suppressing RKN.

The *Meloidogyne javanica* (*Mj*) inoculum was produced on tomato plants cv. 'Belladona', maintained in a growth chamber at 25°C for two months. Egg masses of *Mj* were randomly hand-picked from the infected tomato roots and used immediately for soil inoculation.

Steinernema carpocapsae (Sc) (Koppert B.V. Berkel eb Rodenrijs, The Netherlands®) was reared on *Galleria melonella* (Lepidoptera: Pyralidae) at 25°C (8). Infective juveniles were recovered using White traps

[1] Laboratory of Nematology, Department of Entomology and Agricultural Zoology, Benaki Phytopathological Insitute, 8 St. Delta Str., GR-145 61, Kifissia (Athens), Greece
[2] Faculty of Agricultural Development, Democritus University of Thrace, 193, Ath. Pantazidou Str., GR-628 00, Orestiada, Greece
[3] Laboratory of Chemistry, Agricultural University of Athens, 75 Iera Odos Str., GR-118 55, Athens, Greece
Corresponing author: e.karanastasi@bpi.gr

(14) and stored in 1 l flasks filled with tap water at 4°C (approximately for 5-7 days) until further use. For the trials with live infective juveniles (IJ) and prior to the soil inoculation, the nematodes were left for 30-60 min at room temperature in order to recover. For the trials with dead IJ, the nematodes were heat-killed prior to soil inoculation on a heat block at 70°C for 15 min.

Diallyl disulfide (purity 70%) was purchased from Across Organics (New Jersey, USA). Laboratory-based gas chromatography analysis revealed two other main components: diallyl sulfide (15%) and diallyl trisulfide (12%).

Tomato seedlings (cv. 'Belladona') with three pairs of leaves were transplanted in 250 cm^3 plastic transparent pots containing commercial compost soil. After two days, groups of five egg masses (about 2,000 eggs, as estimated after dissolving the egg masses with sodium chloride) were added to the pots. All EPN treatments were added simultaneously with *Mj* at a rate of 7,000 live *S. carpocapsae* (Sc) or 7,000 heat-killed. Each pot received 20 ml of 2 µl/ml diallyl disulfide solution (Dd) in a single or a double application, i.e. concurrent with *Mj* inoculation (single application) or concurrent with and one week after *Mj* inoculation (double application). Control pots received 20 ml of distilled water.

The experiment consisted of eight treatments (plus control) and each treatment was replicated five times in a completely randomized experimental design.

Experimental plants were incubated in a growth chamber at 25°C. After 28 days, the roots were submerged in water to gently rinse away the soil. The roots were dried off on tissue paper and their fresh weight was measured. Subsequently, the roots were cut into 1-2 cm pieces and females of *Mj* were teased from the roots and counted at 17.5x magnification. Statistical analysis was performed with SAS software (SAS Institute, Cary, NC) and mean separation was conducted using the Tukey's test (12).

The number of female *Mj* counted in roots treated with live or dead Sc and/or Dd was significantly lower than that in the untreated control ($P<0.001$). The greatest disparity was observed when Dd was applied in a double application (at 0 and 7 days post-*Mj* inoculation) to soil previously treated with dead or live Sc. No statistically significant ($P<0.001$) differences were noted between the treatments with live and dead Sc. However, in both treatments, the number of *Mj* females was reduced by 32% and 45%, respectively, compared to the control. The total numbers of *Mj* females counted in tomato roots treated with Dd in a single or a double application, were significantly ($P<0.001$) lower than those in the control. In treatments with dead Sc + Dd, the number of *Mj* females that developed from egg masses in the Dd double application was significantly ($P<0.001$) lower than that in the single application. In treatments with live Sc + Dd, the number of *Mj* females developed from egg masses in the Dd double application was not significantly ($P<0.001$) different to that of the single application (Table 1). No statistically significant differences were noted with regard to root weight ($P>0.05$). The results of the present study showed that both live and dead IJ of Sc suppressed *Mj* on tomato plants, which is in accordance with the findings of Lewis *et al.* (9). In contrast to these results, Grewal *et al.* (6) found no effect of live IJ, which may indicate that these nematodes act by a slower release of the intestinal bacterial agents induced by their natural death. Bird and Bird (3) also suggested that PPN suppression may be due to a competition for habitat and space. These factors may influence the effectiveness of live IJ, depending on the environmental conditions, the plant parasitic and entomopathogenic nematode species, the soil type and the host plant or the presence/absence of insect hosts. The results of the present study are in agreement with previous research on nematode suppression by garlic seeds and bulbs, garlic essential oil and garlic essential oil components (1, 6). Diallyl disulfide was more effective when it was used in two applications, one concurrent with *Mj* inoculation and a second one 7 days later. It is likely that some eggs that

Table 1. Effects of diallyl disulfide (Dd), used alone or in combination with dead or live *Steinernema carpocapsae* (Sc), on the number of female *Meloidogyne javanica* (*Mj*), 28 days after inoculation of potted tomato plants (cv. 'Belladona').

Treatment	Rate/dose	Time of application (dpi)[1]	Nematodes per root [2]
Control	0	0	243 a[3]
Dd	2 µl/ml	0	112.8 cd
Dd	2 & 2 µl/ml	0 & 7	76.4 def
Live Sc	7,000 IJ	0	165.4 b
Dead Sc	7,000 IJ	0	133 bc
Live Sc + Dd	7,000 IJ + 2 µl/ml	0	104.2 cde
Live Sc + Dd	7,000 IJ + (2 & 2 µl/ml)	0 + (0 & 7)	71.2 ef
Dead Sc + Dd	7,000 IJ + 2 µl/ml	0	101 cde
Dead Sc + Dd	7,000 IJ + (2 & 2 µl/ml)	0 + (0 & 7)	59.4 f
LSD = 40			

[1]dpi: days post-*Mj* inoculation
[2]Mean of five replicates
[3]Numbers followed by the same letter do not differ significantly at P<0.001

survive the first application hatch into infective juveniles, which are subsequently killed by the second application. Alternatively, due to the high volatility of the diallyl disulfide and the protective nature of the egg mass (11), repeated applications are required to achieve higher effectiveness. It is also worth mentioning that neither the single nor the double application of diallyl disulfide caused any phytotoxicity.

It can be concluded that both *S. carpocapsae* and diallyl disulfide exhibit significant nematicidal or nematostatic properties and have the potential for nematode control. However, more parameters should be studied, such as plant parasitic nematode initial infestation density, soil type, application time, dosage and repeated applications of diallyl disulfide.

Literature Cited

1. Agbenin, N.O., Emechebe, A.M., Marley, P.S. and Akpa, A.D. 2005. Evaluation of nematicidal action of some botanicals on *Meloidogyne incognita* in vivo and in vitro. *Journal of Agriculture and Rural Development in the Tropics and Subtropics*, 106: 29-39.
2. Akhurst, R. and Boemare, N.E. 1990. Biology and taxonomy of *Xenorhabdus*. In Gaugler, R. and Kaya, H.K. (eds) *Entomopathogenic nematodes in biological control*, CRC Press, Boca Raton, FL, pp. 75-87.
3. Bird, A.F. and Bird, J. 1986. Observations on the use of insect parasitic nematodes as means of biological control of root-knot nematodes. *International Journal of Parasitology,* 26: 511-516.
4. Burman, M. 1982. *Neoplectena carpocapsae*: A toxin production by axenic insect parasitic nematodes. *Nematologica,* 28: 62-70.
5. Cetintas, R. and Yarba, M.M. 2010. Nematicidal effects of five plant essential oils on the Southern Root-Knot nematode, *Meloidogyne incognita* Race 2. *Journal of Animal and Veterinary Advances*, 9: 222-225.
6. Grewal, P.S., Lewis, E.E. and Venkatachari, S. 1999. Allelopathy: a possible mechanism of suppression of plant-parasitic nematodes by entomopathogenic nematodes. *Nematology,* 1: 735-743.
7. Gupta, R. and Sharma, N.K. 1993. A study of the nematicidal activity of allicin – an active principle in garlic, *Allium sativum* L., against root-knot nematode, *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949. *International Journal of Pest Management,* 39: 390-392.
8. Kaya, H.K. and Stock, S.P. 1997. Techniques in insect nematology. In Lacey, L. (ed.) *Manual of techniques in insect pathology.* Academic Press, New York, pp. 281-322.
9. Lewis, E.E., Grewal, P.S. and Sardanelli, S. 2001. Interactions between the *Steinernema feltiae, Xenorhabdus bovienii* insect pathogen complex and the Root-Knot nematode *Meloidogyne incognita*. *Biological Control*, 21: 55-62.
10. Molina, J.P., Dolinski, C., Souza, R.M. and Lew-

is, E.E. 2007. Effect of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterhabditidae) on *Meloidogyne mayaguensis* Rammah and Hirshmann (Tylenchida: Meloidoginidae) infection in tomato plants. *Journal of Nematology*, 39: 338-342.

11. Orion, D., Kritzman, G, Meyer, S.L.F., Erbe, E.F and Chitwood, D.J. 2001. A role of the gelatinous matrix in the resistance of root-knot nematode (*Meloidogne* spp.) eggs to microorganisms *Journal of Nematology*, 33: 203-207.
12. SAS Institute. 1997. SAS/STAT software changes and enhancements through release 6.12. SAS Institute, Cary, NC, USA.
13. Vernin, G. and Metzger, J. 1991. Gc-Ms (EI, PCI, NCI, SIM) SPECMA bank analysis of volatile sulfur compounds in garlic essential oils. In Linskens, H.F. and Jackson, J.F. (eds), *Essential oils and waxes*. Springer-Verlag, Berlin, Germany, pp. 99-130
14. White, G.F. 1927. A method for obtaining infective nematode larvae from cultures. *Science*, 66: 302-303.

Received: 12 May 2010; Accepted: 8 July 2011

ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Επίδραση ενός αιθερίου ελαίου του σκόρδου και εντομοπαθογόνων νηματωδών στην καταστολή του *Meloidogyne javanica* στην τομάτα

Ι. Αναστασιάδης, Α.Κ. Κυμπάρης, Μ. Κορμπή, Μ.Γ. Πολυσίου και Ε. Καραναστάση

Περίληψη Οι νηματώδεις του γένους *Meloidogyne* έχουν παγκόσμια εξάπλωση και πολύ μεγάλο εύρος ξενιστών, στους οποίους μπορεί να προκαλέσουν υποβάθμιση της εμπορευσιμότητας του παραγόμενου προϊόντος, σημαντική μείωση της παραγωγής, και σε πολλές περιπτώσεις ακόμη και την ολική καταστροφή της καλλιέργειας. Οι εντομοπαθογόνοι νηματώδεις είναι οργανισμοί που έχουν μελετηθεί εκτενώς ως προς τη δυνατότητα χρησιμοποίησής τους ως εναλλακτικών μεθόδων αντιμετώπισης των φυτοπαρασιτικών νηματωδών. Επίσης, το αιθέριο έλαιο του σκόρδου, το οποίο διαθέτει απολυμαντικές ιδιότητες εναντίον πολυάριθμων εχθρών και ασθενειών των φυτών, έχει ήδη αποδειχτεί ότι μπορεί να καταστέλλει και τη δράση των νηματωδών. Η παρούσα εργασία αποτελεί μια πιλοτική μελέτη που σκοπό έχει να διερευνήσει την πιθανότητα συνδυαστικής δράσης του εντομοπαθογόνου νηματώδη *Steinernema carpocapsae* και του διάλλυλο δισουλφιδίου, ενός πτητικού συστατικού του αιθερίου ελαίου του σκόρδου, για την αντιμετώπιση του *Meloidogyne javanica*.

Hellenic Plant Protection Journal **4:** 21-24, 2011