academicJournals

Vol. 6(11), pp. 150-153, December 2014
DOI: 10.5897/JEN2013. 0086
Article Number: F0192EC48736
ISSN 2006-9855

http://www.academicjournals.org/JEN

Journal of Entomology and Nematology

Full Length Research Paper

Evaluation of the efficiency of *Gaeolaelaps aculeifer* in control of plant parasitic nematode *Tylenchulus semipenetrans* under greenhouse conditions

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Received 18 December, 2013; Accepted 29 April, 2014

Biological control of plant parasitic nematode *Tylenchulus semipenetrans* was studied under greenhouse conditions. In the present study, the effect of the soil-dwelling predatory mite, *Gaeolaelaps aculeifer* (Acari: Laelapidae), on the population development of citrus nematode was examined. Compared to the nematode-alone, all mite treatments significantly restricted reproduction of citrus nematode. Nematode population ranged from 126 to 161 J2/100 cm^3 soil for the mite-treated plants compared to 398.25 J2/100 cm^3 soil for the nematode untreated plant. As a result, *G. aculeifer* significantly reduced citrus nematode *T. semipenetrans* populations under greenhouse conditions.

Key words: Acari, Biological control, Laelapidae, Predatory mite.

INTRODUCTION

Plant parasitic nematodes are widespread and cause serious losses to most agricultural crops (Al-Rehiayani and Fouly, 2005). Nematodes are often managed with chemical nematicides which can contaminate agro-ecosystems. Natural antagonistic of nematodes and biocontrol agents may provide an alternative to the use of pesticides for nematode management. Numerous nematode species are associated with the citrus rhizosphere; however few species are known to be of economic importance (El-Banhawy et al., 1997). Many nematode species have been reported to be parasiting the citrus but *Tylenchulus semipenetrans* (Cobb, 1913) was the most important on worldwide basis (Safdar et al., 2010).Citrus nematode is one of the most important root nematodes of plant trees that have worldwide distribution and cause reduction of crop production and vegetative growth. In addition, this nematode creates slow decline of citrus trees (Ayazpour et al., 2010). Yield reduction by citrus nematode depending of the infection rate, but on average is 10 to 30% (Verdego-Lucas and McKenry, 2004).

Methods commonly employed to control *T. semipenetrans* depend on local conditions and focus on excluding the pest, minimizing losses through crop management and reducing population of the parasite using nematicides or resistant root stock (El-Banhawy et al., 1997). Considerable information available in the literature has documented the effectiveness of several

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biological agents to manage plant parasitic nematodes (Al-Rehiayani and Fouly, 2005). The majority of mesostigmatid mites in soils are general predators which prey on a range of invertebrates including nematodes (Walter and Lindquist, 1989), although, the minority, are specialized predators feeding on nematodes (Sharma 1971; Habersaat, 1989). Previously, it was found that nymphs and adults of *Lasioseiuss scapulatus* (Kennet) had the ability to capture, consume and complete its entire life cycle on the root knot nematode *Meloidogyne incognita* (Kofoid and White Chitwood) (Imbriani and Mankau, 1983).

Also, the suitability of egg masses of *Meloidogyne* spp. As food source of the ascid mite species *Lasioseius dentatus* (Fox) was studied where it was found that the predatory mite successfully completed its whole life span on egg masses (Fouly, 1997). Al-Rehiayni and Fouly (2005) was studied effect of adding *Cosmolaelaps simplex* (Fox) or aldicarb for control of *T. semipenetrans* on citrus seedlings in greenhouse.

On the other hand, the mesostigmatid mites family Laelapidae Berlese is considered one of the most important groups of soil predators, where it usually feeds on nematodes (Muma, 1975). Predatory laelapids tend to be voracious, polyphagous predators that reproduce quickly and can be reared easily (Beaulieu, 2009). This makes them good candidates for biological control of pests that spend time in the soil or in other plant growing media. The genus *Gaeolaelaps* Evans and Till is currently one of the largest genera of the family Laelapidae*.* Some species of this genus, such as *Gaeolaelaps aculeifer* (Canestrini), *G. oreithyiae* (Walter and Oliver (1989), and *G. gillespiei* Beaulieu, are aggressive predators of nematodes and immature arthropods (Kavianpour et al., 2013).

Gaeolaelaps was considered at different taxonomic levels by authors: as a species group (Van Aswegen and Loots, 1970); or as a subgenus of *Hypoaspis*sens. lat. (Karg 1989; Faraji et al., 2008), and as a distinct genus (Lapina, 1976; Hyatt, 1964).

In the present study, we investigated the efficiency of soil-dwelling predatory mite *G. aculeifer* in biological control of citrus nematode in greenhouse and the effect of adding mite individuals for reduce *T. semipenetrans*on citrus seedlings.

MATERIALS AND METHODS

Rearing of predatory mite in laboratory

The predatory laelapid mite *G. aculeifer* was isolated from soil samples under shrubs of hopbush, *Dodonaea viscose (L.) Jgacq.* in Fars Science and Research University. After identification, isolated mites were reared in glass jars filled with damp sawdust. Mite samples have been maintained on the acarid mite species *Rhizoglyphus robini* Claparede, as a food source in rearing units. The acarid mite was maintained in laboratory on onion. All units were kept under normal room temperature at 25 ± 1°C and 65 ± 5% relative humidity.

Extracting nematode *T. semipenetrans* in laboratory

Nematodes were extracted from soil samples taken with a hand trowel at 15-30 cm beneath the tree canopy in citrus orchard. In this study, Baermann's technique was used to separating nematode of soil suspention (Goody, 1957).

Greenhouse experiments

A group of 20 plastic pots (25 cm in diameter) containing sand loam sterilized soil and previously transplanted with single 3-month seedlings of key lime (*Citrus aurantifolia).* The transplanted pots were divided into five groups, four pots each, where the first group was infected with approximately 100 juvenile stages of *T. semipenetrans*/pot. The second group received the mites at the same time of nematode inoculation, while the third one received 20 individuals of *G. aculeifer*/pot 15 days after nematode inoculation. The fourth group was infected with nematodes 15 days after adding 20 individuals of *G. aculeifer*/pot and the fifth one was left without any nematode inoculation and mites. All pots were kept under greenhouse conditions at 27 ± 1°C and 65 ± 5 % relative humidity.

Ninety days after nematode inoculation, citrus seedling that were carefully under consideration where data dealing with shoot length and fresh weight for root system were recorded. Moreover, the second juvenile stage (J2) found in 100 mL of soil samples was estimated for each nematode treatment after extraction by sieving using modified Baermann-funnel method (Goody, 1957). On the other hand, soil samples of about 250 g each were also subjected for mite extraction by the aid of the modified Berlese's (Tullgern's) funnels (Berlese, 1905). Where, the average number of mite/250 g of soil was calculated.

In all cases, data were subsequently analyzed by least significance difference (LSD), Duncan's multiple rang and analysis of variance (ANOVA) testes, where the reproduction index of predatory mite = final mite population (PF)/ initial mite population (PI).

RESULTS AND DISCUSSION

In the study of plant growth, the mite-treated citrus plants showed the better growth as compared to the untreated plants. Significantly enhanced shoot growth was observed in all the mite-treated citrus plants as compared to the *T. semipenetrans* untreated control (35.70 cm) (Table 1).

Root weight was slightly enhanced in all mite-treated plants but this response was not significant compared to the plants with nematodes only. The citrus plants without nematodes had the greatest shoot length (58.1 cm). Seedlings with nematode alone as compared to the citrus plants without nematodes showed symptoms such as small and yellowing leaves, low growth, and death of top of branches and defoliate.

Based on the fact that the *T. semipenetrans* is semi-parasitic nematode; therefore predatory mite feeds on egg masses and juvenile stages of *T. semipenetrans*. Similarly, it was found before that addition of the predatory mite species *L. dentatus* either at the same time or 40 days after root-knot nematode inoculation had significant improvement in shoot length, weight and root weight of tomatoes seedlings under greenhouse condition (Mostafa et al., 1997).

Table 1. Plant response after the infection of citrus nematode *Tylenchulus semipenetrans* in the presence of the predatory mite *Gaeolaelaps aculeifer* under greenhouse conditions.

Treatment		Shoot length (cm)	Root weight (g)
1	Nematode alone	35.70[c]	19.20[ab]
2	Nematode + mite at the same time	42.70[b]	20.21[a]
3	Mites 15 days after nematode inoculation	37.45[b]	18.70[ab]
4	Mites 15 days before nematode inoculation	40.12[b]	19.30[ab]
5	No mite and nematode inoculation	58.10[a]	21.04[a]

*Means in a column followed by the same letter(s) are not significantly different (p=0.05).

Table 2. Citrus nematode *Tylenchulus semipenetrans* development in citrus seedlings in the presence of the predatory mite *Gaeolaelaps aculeifer* under greenhouse conditions.

Treatment		No. nematode/100 cm^3 soil (X)	No. mites/250 cm^3 soil (X)	Reproduction index of mites (PF/PI)
1	Nematode alone	398.25[d]	-	-
2	Nematode + mite at the same time	126[b]	3.12[a]	42.84[b]
3	Mites 15 days after nematode inoculation	161[bc]	2.44[a]	31.51[ab]
4	Mites 15 days before nematode inoculation	133.75[c]	1.97[a]	24.45[a]
5	No mite and nematode inoculation	-	-	-

X= mean of 4 replicates, PF= extracted mite population, PI= initial mite population. *Means in a column followed by the same letter(s) are not significantly different (p=0.01) according to LSD test.

Table 3. Analysis of variance of treatments (ANOVA).

S.O.V	df	Var. of no. nematode	df	Var. of no. mite	Var. of PF/PI
Treatments	3	67465.17**	2	1.3160**	344.3933**
Std. error	12	16.125	9	0.0676	16.5972
cv	-	1.96	-	10.35	12.37

**= The test has been significant at 1% level

The result shows the potential of the predatory mite *G. aculeifer* in repressing *T. semipenetrans* reproduction. Compared to the nematode alone, all mite treatments significantly restricted reproduction of *T. semipenetrans*. Nematode populations ranged from 126 to 161 J2s/100 cm^3 for the mite-treated plants compared to 398 - 25 J2s/100 cm^3 for the nematode untreated control (Table 2). Nematode population significantly was at lowest level when the predatory mites were added to the treatments at the same time of nematode inoculation.

In spite of a difference means of treatments 2 and 4 in LSD test did not have any significant differences, but tow treatments 1 and 3 showed significant difference (p=1%) according to LSD (Table 3).

Concerning the reproduction index of *G. aculeifer*, it was noticed that it was at its highest level when the predatory mites were added to the treatments at the same time of nematode inoculation (PF/PI=42.84) and followed by 31.51 and 24.45 for mites that were added 15 days after and before nematode inoculation, respectively (Table 2).

Also, there were no significant differences between the effect of application time of mites added to citrus seedlings 15 days after nematode inoculation and either mites added at the same time or mites added 15 days before inoculation n the reproduction index of *G. aculeifer*; whereas, there was significant difference between the effect of adding mites at the same time of inoculation and those that were added before inoculation on the reproduction index of *G. aculeifer*.

Similarly, it was previously found that the reproduction index of the ascid predatory mite *L. dentatus* was higher when mite individuals were added to tomato seedlings 40 days after root-knot nematode inoculation (Abou Setta et al., 1986) and similarly, these results significant match with findings (Al-Rehiayani and Fouly, 2005) about reproduction index of the predatory mite species *C. simplex* on *T. semipenetrans* under greenhouse condition. That may be due to mite species and its feeding behavior as well as to the biological aspects of nematodes. Therefore, it can be concluded that *G. aculeifer* had a better response which directly represented by its reproductive potentiality and capability to reducing citrus nematode populations when it was added at the same time of nematode inoculation. In other word, the predatory mite *G. aculeifer* had the chance to search the developmental individuals of citrus nematodes and feed on them before they can reach the root system and become more difficult for the predator. These results are in harmony with the previous findings where it was found that the developmental stages of root-knot nematodes were eaten by *L. scapulatus* under laboratory and greenhouse conditions (Imbriani and Mankau, 1983).

Finally, it can be concluded that the predator mite *G. aculeifer* could be considered as a biological control agent, which may limit populations of citrus nematode. Moreover, mite capability to feed, survive and reproduce on nematodes can be integrated with other control tactics and further field work in this area is highly warranted.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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