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BIOLOGICAL CONTROL OF *TYLENCHULUS SEMIPENETRANS* AND *PRATYLENCHUS PENETRANS* INFECTING CITRUS TREES BY THE PREDACEOUS MITE, *MACROCHELES MATRIUS*

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ABSTRACT: *Pot experiments were carried out to investigate the predation efficiency of Macrocheles matrius (Hull) on the two parasitic nematode species infected sour orange, i.e. Citrus nematode, Tylenchulus semipenetrans and Lesion nematode, Pratylenchus penetrans under greenhouse conditions. All applied treatments significantly reduced soil nematode population of T. semipenetrans and P. penetrans. Predation efficiency of M. matrius predator on T. semipenetrans was higher than that occurred on P. penetrans. Treatment of M. matrius gave the highest reduction percentage (77.5%) on T. semipenetrans nematode compared with (76.9%) at the treatment of the nematicide, Carbofuran. In addition, effectiveness of the previous treatments gave a better enhancement of various citrus growth parameters such as: plant height (135.0 cm) ; shoot weight (86.5 g) and root weight (23.5 g) with increment for each by (+64.6; +37.7 and +95.8 %) compared with control values, respectively. Macrocheles matrius gave a higher reduction percentage than the nematicide (Carbofuran) especially against T. semipenetrans. So, it could be encouraging the use of the predator mites as the Macrochelids group in the biological control programs of plant-parasitic nematode.*

Key words: *Bio- control, citrus nematode, lesion nematode, Macrocheles matrius, citrus, mites.*

INTRODUCTION

Citrus is suggested to be one of the most important fruit crops all over the world. It ranks the third position between fruit crops and only preceded with grape and apple (FAO, 2010). The sour orange group (*Citrus aurantium* L.) object is grown throughout the world and provides the greatest citrus rootstocks of the other citrus groups. In Egypt, citrus plantation is nearly 420.678 feddans which represents about 33.08% of the total area of fruit trees. It is the most important fruit crop as for as its acreage, production and exportation potentialities are concerned (Elhagen, 2010).

The parasitic nematode *Tylenchulus semipenetrans* Cobb., consider one of the most serious pest of citrus trees is capable of damaging mature trees and down grading fruit quality. It is a parasite of several woody plant species, and aptly named because it is ubiquitous in the citrus producing regions of the world (Sorribas *et al.,* 2008). *T. semipenetrans* nematode is well-adapted to citrus, with very high numbers required to significantly affect the growth and health of its host (Maafi and Damadzadeh, 2008).

The root lesion nematode (RLN) is a migratory endoparasite that moves between soil and roots, but feeds on and migrates in root cortical cells, can cause economic levels of damage to many crop species. Feeding by the nematode kills tissues in the root cortex, which appear as necrotic lesions or spots on roots. Citrus *Pratylenchus penetrans* feeding on roots and can be reduce the capacity of the plant roots to uptake nutrients and water (Thompson, *et al.,* 2008).

Biological control of plant pests using predatory mites has been extensively practiced in many countries, especially in

protected crops. Several mite species belonging to Macrochelidae have been reported to feed on vermiform nematode. Macrochelidae as a predatory mites are prosperous predaceous mite species which have a potential role as biological control agents on vermiform nematodes and other organisms (Beaulieu and Weeks 2007).

Therefore, the aim of the present investigation led to a new tool for using soil macrochelid mite species*, M. matrius* as an effective biological control agent on the two parasitic nematode species, *T. semipenetrans* and *P. penetrans* infected sour orange comparing with Carbofuran nematicide in management of these creatures.

MATERIALS AND METHODS

Nematode culture:

Juveniles of the citrus parasitic nematode, *Tylenchulus semipenetrans* were obtained from the pure culture reared on seedlings sour orange (*Citrus aurantium*). Lesion nematode, *Pratylenchus penetrans* was obtained from the pure culture reared on tomato plants, in Nematode laboratory of the Entomology and Zoology Department, Faculty of Agriculture, Menoufia University.

Experimental preparation and design:

Potted experiment was carried out under greenhouse conditions at the Experimental Farm of the Faculty of Agriculture, Menoufia University, Shibin El-Kom, Egypt. The experiment layout was randomized complete block design. Each treatment was represented with three replicates. Three seedlings of the sour orange, *Citrus aurantium* L. one year and a half old, were planted in plastic pot 25 cm in diameter filled with 4 kg sterilized clay-sand mixed soil (1:1, v/v). After one week for seedlings adaptation, 1000 J_2 of both of *T. semipenetrans* and *P. penetrans* per 1kg were added by pipette into three holes around each seedling; 100 individuals of the soil predaceous mite, *M. matrius* (Acarina: Machrochelidae) were added to the soil of each pot combined with each of the two nematode species , in addition , nematode species were added singly for a comparison.

Material preparation:

Mite individuals were collected from chicken and farmyard manures, taken by an iron sampler vol, 1000 cm^3 and extracted by using modified Tullgren funnels for 72 hours (Lindquest *et al,.*1979). The extracted mites were received in distilled water and then transferred onto plastic rearing units (Fouly, 1996). Examination of soil predator *M. matrius* mite was carried out twice daily by a stereomicroscope to identify and count all the individuals.

Nematicide Carbofuran, its trade name is (Carburan); common name is Carbofuran; mole Formula is $C_{12}H_{15}NO_3$; chemical Name is 2, 3-di hydro-2, 2-dimethyl-7-benzofuranyl methyl carbamate; formulation is 10% G and applied as soil treatment at the rate of 6 kg / feddan (20 g per pot).

Nematode extraction and counts:

Each composite soil sample was carefully mixed, and an aliquot of 100 cm^3 was processed for nematode extraction according to methods described by (Southey, 1970); each treatment was replicated three times. An aliquant of 1 ml each of nematode suspensions were pipetted off, placed in a Hawksley counting slide and examined by using a stereomicroscope.

Nematode counts were done after 30, 60, 90 and 120 days of application, and the identification to generic level were based on morphology of the adult and larval forms, according to the description of (Mai and Lyon, 1975). At the end of the experiment, roots & shoots fresh weight and plant height were recorded.

Statistical analysis:

The obtained data were subjected to analysis of variance (ANOVA) using CoStat

Software, Version 6.4 (2008). The mean differences were compared by Least Significant Difference (L.S.D. 5%)

Reduction percentages were computed according to Abbott formula (1925).

Increase or decrease %= Control – treatment / Control x 100.

RESULTS AND DISCUSSION

Data presented in Table (1) showed that, the average numbers of *T. semipenetrans* and *P. penetrans* juveniles per 100 g soil, 30, 60, 90 and 120 days after the application of *M. matrius* solely or combined compared with a nematicide (Carbofuran) for controlling *T. semipenetrans* and *P. penetrans* infected citrus plants under shield conditions.

Statistical analysis indicated that all treatments significantly suppressed the nematode population in the soil treated after 30, 60, 90 and 120 days compared with control treatment.

Combined treatment, of (*M. matrius* + *T. semipenetrans*) yielded through four months the least mean of nematode species (276.0 ind's), while (*M. matrius* + *P. penetrans*) gave the higher mean (388.1 ind's). A nematicide (Carbofuran) when added to each parasitic nematode sp as comperator with a predaceous mite species resulted mean of (280.5 and 248.6 ind's) through the same period, respectively, i.e. with slightly effectiveness on *T. semipenetrans*. These results may be due to preferentialism of *M. matrius* for certain nematode prey to feed on it; propagate and carrying out its activities.

The highest reduction percentages of the nematode population in the soil, were recorded by application of combined treatment (*M. matrius* + *T. semipenetrans*) compared with (Carbofuran + *T. semipenetrans*) with (77.5 and 76.9 %), respectively. But this treatment of (*M. matrius*) recorded (66.3%) reduction percentage on *P. penetrans* in the four months compared with a nematicide (Carbofuran) which recorded (78.2%).

Results showed that, the predator mite (*M. matrius*) considered more efficient on the prey *T. semipenetrans*, than *P. penetrans*. This may can be explained to the probability that, *T. semipenetrans* consider semi-endoparasitic on roots, thus facilitating predator to eat females easily, in contrast to the *P. penetrans* which consider endoparasitic in roots.

The obtained results are sameness with those obtained by (Mcsorley and Koon, 2009), who ascertained that, the predatory mites worthy of as a bio control agents on plant-parasitic nematodes.

Regarding to the influence of treatments solely or combined on some citrus growth characters, such as plant height; shoot weight and root weight. Statistical analysis of the obtained data in Table (2) indicated that there are significant differences between all tested measurements and control treatment.

It was clearly shown that all treatments led to considerable increments of plant height; shoot weight and root weight compared with control (pathogenic treatment). The highest plant height recorded that, (135.0 cm); shoot weight (86.5 g) and root weight (23.5 g) were obtained by treatment application with (*M. matrius*) on *T. semipenetrans*. While these parameters in Carbofuran treatment are lower than that found in previously treatment.

T. semipenetrans can be controlled by predaceous mites, particularly in soil free of agriculture chemicals like fertilizers or pesticides, so predaceous mites are important agents for the control of nematodes, the number of predaceous mites greatly increased and significantly correlated with populations of *T. semipenetrans* (El-banhawy, *et al.,* 1997).

Table (1) : Impact of *M. matrius* on the population density of *T. semipenetrans* and *P. penetrans* infected citrus plants and reduction % , under shield conditions.

Treatments	Aver. no. of *T. semipenetrans* & *P. penetrans* juveniles/ 100 g soil					Reduction %				
	Days post-treatments									
	30 Days	60 Days	90 Days	120 Days	Overall mean	30 Days	60 Days	90 Days	120 Days	overall mean
T. semip-enetrans + M. matrius	481.5 f	299.0 e	217.5 d	106.0 d	276.0 d	56.5	76.7	84.1	92.5	77.5
T. semip-enetrans alone	1108.0 a	1284.0 a	1370.0 a	1421.0 a	1295.8 a	-	-	-	-	-
P. penetrans + M. matrius	632.0 c	473.0 c	306.5 c	141.0 c	388.1 c	41.8	59.4	74.9	89.1	66.3
P. penetrans alone	1086.0 b	1165.0 b	1221.0 b	1297.0 b	1192.3 b	-	-	-	-	-
Carbofuran + *T. semip-enetrans*	507.5 d	341.0 d	182.0 e	91.5 e	280.5 d	54.2	73.4	86.7	93.5	76.9
Carbofuran + *P. penetrans*	492.0 e	270.5 f	146.0 f	86.0 e	248.6 e	54.7	76.8	88.0	93.3	78.2
Check	0	0	0	0	0	0	0	0	0	0
LSD 5%	8.8	7.1	5.3	8.9	5.3	-	-	-	-	-

Means in each column followed by the same letter (s) are not significantly different at 5% level.

Table (2) : Influence of treatments on some citrus plant characters

Treatments	Plant height (cm)	Shoot weight (g)	Root weight (g)
T. semipenetrans + *M. matrius*	135.0 b	86.5 b	23.5 b
T. semipenetrans alone	82.0 g	63.0 f	12.0 f
P. penetrans + *M. matrius*	115.0 e	79.7 c	16.0 e
P. penetrans alone	90.0 f	67.5 e	10.0 g
Carbofuran + *T. semipenetrans*	131.0 c	81.0 c	19.5 d
Carbofuran + *P. penetrans*	126.0 d	77.4 d	21.0 c
Check	151.0 a	102.0 a	26.0 a
LSD 5%	3.5	1.7	0.8

Means in each column followed by the same letter (s) are not significantly different at 5%

Predaciousness potentiality of predatory mites mainly depended on lumped of their densities, over 300 ind's and their feed prefer ability of preys occurred on more active juveniles than fixed egg masses (Chen *et al.,* 2013).

As for the increase or decrease of tested plants characters Table (3), the obtained results indicated that, all plant treatments caused increments of plant height; shoot weight and root weight. The highest increments were recorded in treatment (*M. matrius* + *T. semipenetrans*), plant height (+64.6%); shoot weight (+37.7%) and root weight (+95.8%). At higher rates compared with a nematicide (Carbofuran).

The obtained results are in agreements with (Manwaring *et al.,* 2015) who ascertained that, preferentialism of predatory mites not only to feed on plant-parasitic nematodes, but also caused enhancement plant characters.

Machrochelidae mites have highly predaciousness efficiency as bio control agents on plant-parasitic and soil nematodes, because its ability to feed voraciously and killed numerous of these creatures in both culture and experimental dishes (Bilgrami, 1993).

Predators with high biocontrol effectively are useful aptly, for controlling soil nematodes without any negative impacts on environment and human health. These limitations worthy of to introduce these predators in a successful strategy for controlling nematodes as a substituent long use of chemical pesticides which has brought contrariwise of preceding limitations (Chun *et al.,* 2014).

Finally, prosperity and explicitly of a predatory mite *M. matrius* as a biological control agent on the two citrus parasitic nematodes under study, specially *T. semipenetrans*, sheds a light on its availableness and utilization in strategy of biological control of plant-parasitic nematode program. Its voraciousness against parasitic nematodes which caused a considerable reduction percentage exceeded that occurred by application of a nematicide Carbofuran in this respect.

Preferability of *M. matrius* to feed on *T. semipenetrans* than that occurred on *P. penetrans* may be due to its parasitism nature; semi or endo parasitic on roots, behaviouristic of its J_2 larvae and its ability of root infection to take into a predatory mite *M. matrius* preferred certain prey to feed on it aptly; voraciously; propagate and carrying out its activities, while a nematicide Carbofuran is a toxic; expensive; harmful for human health and causes many problems for both soil and plants properties for these parameters it have not the aforementioned limitations and that because neglected by many researchers for these reasons.

Table (3) : Increase or decrease of some vegetative characters, on citrus as influenced by treatments application

Treatments	Plant height cm	Shoot weight g	Root weight g
T. semipenetrans + *M. matrius*	+64.6	+37.7	+95.8
T. semipenetrans alone	-	-	-
P. penetrans + *M. matrius*	+27.8	+18.1	+60.0
P. penetrans alone	-	-	-
Carbofuran + *T. semipenetrans*	+59.8	+28.8	+21.9
Carbofuran + *P. penetrans*	+40.0	+14.7	+110.0
Check	+84.1	+61.9	+116.7

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المكافحة الحيوية لنيماتودا الموالح ونيماتودا التقرح التي تصيب أشجار الموالح بواسطة الحلم المفترس ماكروكيلس ماترياس *Macrocheles matrius*

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الملخص العربى

أجريت التجارب في أصص على شتلات البرتقال لبحث القدرة الإفتراسية للحلم *Macrocheles matrius* لمقاومة نوعين من نيماتودا الموالح المتطفلة على أشجار البرتقال وهما نيماتودا الموالح *Tylenchulus semipenetrans* ونيماتودا التقرح في الموالح *Pratylenchus penetrans* تحت ظروف الصوبة.

بصفة عامة أدت جميع المعاملات إلى خفض معنوي في الكثافة العددية لنوعي نيماتودا التربة تحت الدراسة وهما كما ذكرنا نيماتودا الموالح ونيماتودا التقرح في الموالح. وكانت الكفاءة الإفتراسية للحلم المفترس لنيماتودا الموالح أعلى مما كان في نيماتودا التقرح وقد بلغت نسبة الموت في نيماتودا الموالح نتيجة إفتراس الحلم لها (77.9٪) بينما وباستخدام المبيد النيماتودي كربوفيوران مع نيماتودا الموالح بلغت نسبة الموت فيها إلى (76.9٪) وذلك أقل قليلاً من المفترس.

يضاف إلى ما سبق أنة كان تأثير المعاملة السابقة مشجعاً على تحسن صفات النمو المختلفة في الموالح مثل طول النبات (135.0 سم) & وزن المجموع الخضري (86.5 جم) ووزن الجذر (23.5 جم) بزيادة في كل منها بمقدار (64.6 & 37.7 & 95.8 ٪) مقارنة بالكنترول على التوالي.

ولما كان إستخدام الحلم المفترس *M. matrius* اعطى تفوقاً في إحداث نسبة موت أعلى مما في حالة إستعمال المبيد النيماتودي فإنه يجب تشجيع كل من الباحثين في هذا المجال وكذلك المزارعين على إستخدام الحلم المفترس خاصة الحلم الماكروكيليدي في برامج المقاومة الحيوية للنيماتودا المتطفلة على النبات.