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Der Pharmacia Lettre, 2016, 8 (13):311-315
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Compatibility and efficacy of entomopathogenic nematode, *Steinernema carpocapsae* all alone and in combination with some insecticides against *Tuta absoluta*

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ABSTRACT

Compatibility of entomopathogenic nematode, Steinernema carpocapsae All with four recommended insecticides; fipronil, flonicamid, indoxacarb and spinetoram was studied. The entomopathogenic nematode was mixed with all tested insecticides (half of recommended field rate used) and investigated with the wax moth larvae (Galleria mellonella) under laboratory condition. The obtained results cleared that the entomopathogenic nematode, S. carpocapsae All was compatible with all tested insecticides. Ten treatments were used against Tuta absoluta under green house conditions; S. carpocapsae all (250 IJs/ml) all alone, recommended field rate of each insecticide alone, S. carpocapsae (250 IJs/ml) All combined with half of recommended field rate of each insecticide and water only as a control. The obtained results showed that spinetoram alone was the most toxic insecticides against T. absoluta followed by indoxacarb alone with the percent of mine reduction 99.3 and 80%, respectively, after the third treatment. S. carpocapsae All alone was the least toxic with the percent of mine reduction 12.9%. This result confirmed that S. carpocapsae All was not effected against T. absoluta.

Key word: *Steinernema carpocapsae* All, fipronil, flonicamid, indoxacarb, spinetoram and compatibility, *Tuta absoluta*

INTRODUCTION

Entomopathogenic nematodes (EPNs) in general are considered effective agents in controlling agricultural pests [1-2]. *Steinernema carpocapsae* All, occur naturally in the environment as parasites of many insect larvae. The mass rearing and release of these nematodes gives an effective control against many insect pests in a wide range of crops. The first time of their efficacy as a biological control agent was discovered in the United States of America in the thirties of past century [3]. Once released, entomopathogenic nematodes seek out their insect hosts. After that, the entomopathogenic nematodes penetrate the insect cuticle through body openings and release symbiotic bacteria that multiply and rapidly kill the insect. Subsequently, entomopathogenic nematodes feed upon the host and mature into adults, which mate and produce the next generation. The life cycle is completed within a few weeks and hundreds of thousands of entomopathogenic nematodes emerge in search of new hosts.

The tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera, Gelechiidae) is considered one of the most deleterious pests on tomato in South America [4]. In Egypt the tomato leafminer is first recorded in Marsa Mtrooh Governorate in 2009 and by 2010 it had reached Giza, coming well established in all Governorates of Egypt [5]. Tomato plants can be infested from seedlings to mature plants. *T. absoluta* reduces yield and fruit quality, causing up to 100% yield losses in severely infested tomato crops. The effectiveness of entomopathogenic nematodes against *T. absoluta* is poorly documented. In addition, the entomopathogenic nematodes *Steinernema carpocapsae*, *S. feltiae* and *Heterorhabditis bacteriophora* have proved to be capable of infecting late larval instars of *T. absoluta*.

Chemical control has been the main control agent used against *T. absoluta* since it was discovered in South America. Chemical control using synthetic insecticides is the primary method to manage the pest, but it has serious side effects, including the high costs of insecticide, side effects on natural enemy enemies [6], insecticide residues in tomato fruits [7] and building up of insecticide resistance.

The aim of this work is investigate of the possibility of mixing of entomopathogenic nematode *Steinernema carpocapsae* All and some insecticides. Also, comparison of entomopathogenic nematode alone as a biocontrol agent in *T. absoluta* control and/or use it in combination with some insecticides to reduce the side effects of synthetic insecticides

MATERIALS AND METHODS

Insect

This experiment was carried out under green house condition in Central Laboratory for Agriculture Climate, Agricultural Research Center, Dokki, Giza, Egypt. Tomatoes seeds, Newcastle Varity were sown in seedling trays consisting of 120 small holes, filled with compost. After three weeks, the seedlings were transferred into the greenhouse soil. This experiment was designed as plots area (6 x 7 m). The tomato leaf miners, *Tuta absoluta* were invaded the plants naturally.

Entomopathogenic nematodes (EPNs)

The entomopathogenic nematode, *Steinernema carpocapsae* All (nematoda: Steinernematidae) was imported from Kiel University, Germany. These nematodes were maintained in the laboratory 25^{o}C ± 2 on *Galleria mellonella* larvae as described by Woodering and Kaya (1988)[8]. The greater wax moth, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) culture was maintained with a methodology adapted by Dutky, *et al* (1964)[9] using an artificial diet modified by Metwally *et al.* (2012)[10].

Chemicals

1- Fipronil (Fipris 5% SC), produced by Anhui Huaxing Chemical industry Co. China. Fipronil a member of the phenyl pyrazole class of pesticides, which are principally chemicals with a herbicidal effect. Fipronil, however, acts as an insecticide with contact and stomach action. Fipronil is an extremely active molecule and is a potent disruptor of the insect central nervous system via the (-aminobutyric acid (GABA) regulated chloride channel. Despite the fact that the GABA channel is important in nerve transmission in both vertebrate and invertebrate animals, and that fipronil does bind to the GABA receptor in vertebrates, the binding is ‘less tight” which offers a degree of selectivity. The recommended field rate is 80 ml/feddan (4200 m^2)

2- Flonicamid (Teppeki 50% WD), produced by ISK Bioscience Europe. Flonicamid belongs to a new class of chemistry called pyridinecarboxamide. The mode of action has not been known, but it is different from all major insecticide classes. Flonicamid controls target pests by contact and ingestion provoking rapid and irreversible feeding cessation. The recommended field rate is 80 g/feddan (4200 m^2)

3- Indoxacarb (Avaunt 15% EC), produced by Du Pont De Nemours. Indoxacarb belong to a new class of insecticides called oxadiazine and it works as a sodium channel blocker. The recommended field rate is 100 ml/feddan (4200 m^2)

4- Spinetoram (Redianet 24%), produced by Dow Agroscience, England. The commercial product is a mixture of chemically modified spinosyn J and spinosyn L. Both compounds are derived from the soil Actinomycete, *Saccharopolyspora spinosa*. Spinetoram effects on target insects are consistent with the activation of the nicotinic acetylcholine receptor, but at a different site than nicotine or the neonicotinoids. Spinetoram also affects GABA (γ-aminobutyric acid) receptors, but their role in the overall activity is unclear. The recommended field rate is 100 ml/feddan (4200 m^2)

Bioassay

Steinernema carpocapsae All suspension was maxed with all tested insecticides and treated of the greater wax moth larvae, *Galleria mellonella* (1 ml of nematode infective juveniles (IJs) suspension that contain 500 IJs/ml were mixed with 1 ml of recommended field concentration of each tested insecticides) evenly distributed with a pipette over a piece of filter paper and placed inside 9 cm diameter Petri dishes. The final concentration of entomopathogenic nematode is 250 IJs/ ml. Each treatment has three replicates and other three replicates treated with water as a control. Four individuals of the last instar larvae of *G. mellonella* were placed in Petri dish in each replicate. Efficacy was evaluated by counting dead larvae every 24 hours. Cadavers of dead insects were placed in white traps [11]. The results showed that the entomopathogenic nematode, *S. carpocapsae* All was not effective in all treatments.

Ten treatments were designed, suspension of *S. carpocapsae* All (250 IJs/ml) alone, *S. carpocapsae* (250 IJs/ml) combined with half of recommended field rate of fipronil, *S. carpocapsae* All (250 IJs/ml) combined with half of recommended field rate of flonicamid, *S. carpocapsae* All (250 IJs/ml) combined with half of recommended field rate of indoxacarb, *S. carpocapsae* All (250 IJs/ml) combined with half of recommended field rate of spinetoram, fipronil with the recommended field rate, flonicamid with the recommended field rate, indoxacarb with the recommended field rate, spinetoram with the recommended field rate and water only as a control. Each treatment has three replicates (6x7 m). Other three replicates were used as a control (treated with water). Plants were fertilized and all agriculture practices carried out. All plots were treated by tested insecticides three time and one week interval. A ten-litre knapsack sprayer was used in insecticides treatment.

The numbers of mines per 10 leaflets were counted after and before seven days of the application and also, the percent of mine reduction calculated by the original number of mine – the new number of mine / the original number of mine x 100.

Statistical analysis

Data were subjected to the analysis of variance test (ANOVA) via Randomized Complete Block Design (RCBD) (F. test) and analysis of variance (one ways classification ANOVA) followed by a least significant difference (LSD) at 5% (Costat Statistical Software, 1990)[12].

RESULTS AND DISCUSSION

The obtained data in Table 1 are show that after the first treatment, the numbers of mines per 10 leaves by *Tuta absoluta* reduced sharply in spinetoram application to 20.7 compared with 40.7 of mines per 10 leaflets before treatment. The percentage of mines reduction is 49.1%. Statistical analysis shows that there are significant differences between all treatments. Spinetoram alone is the most toxic insecticides after the first treatment followed by the mixture of entomopathogenic nematode *Steinernema carpocapsae* All and the half recommended field rate of spinetoram.

Table 1. Effect of entomopathogenic nematode, *Steinernema carpocapsae* All alone and in combination with some insecticides on tomato leafminer, *T. absoluta* under green house conditions

Tested insecticides	Mean No. of mine / 10 leaflets before treatment	Mean No. of mine / 10 leaflets after 1^{st} treatment	Percent of reduction %	Mean No. of mine / 10 leaflets after 2^{nd} treatment	Percent of reduction %	Mean No. of mine / 10 leaflets after 3^{rd} treatment	Percent of reduction %
All+ Fipronil	37.7 ± 2.9	34.3 ± 1.5^{b}	9.1	30.3 ± 0.6^{bc}	19.6	26.3 ± 2.5^{c}	29.5
All+ Flonicamid	39.3 ± 2.5	35.3 ± 2.5^{b}	10.2	32.7 ± 1.5^{b}	16.8	29.3 ± 2.5^{c}	25.4
All + Indoxacarb	39.3 ± 1.5	33.3 ± 2.5^{b}	15.3	30.0 ± 2.6^{bc}	23.7	26.3 ± 4.2^{c}	33.1
All + Spinetoram	39.3 ± 3.2	26.0 ± 4.6^{c}	33.8	18.0 ± 2.0^{e}	54.2	16.3 ± 1.5^{d}	58.5
All	41.0 ± 2.6	36.7 ± 2.1^{b}	10.5	33.7 ± 1.2^{b}	17.8	35.7 ± 4.2^{b}	12.9
Fipronil	40.7 ± 2.5	30.7 ± 2.1^{b}	24.6	24.0 ± 4.4^{d}	41.1	14.7 ± 3.8^{d}	63.9
Flonicamid	40.0 ± 3.6	34.0 ± 1.0^{b}	15	26.0 ± 3.6^{cd}	35.0	23.3 ± 1.5^{c}	41.8
Indoxacarb	40.0 ± 2.0	31.7 ± 2.1^{b}	20.8	14.7 ± 3.5^{e}	63.3	8.0 ± 2.0^{e}	80.0
Spinetoram	40.7 ± 3.5	20.7 ± 2.1^{d}	49.1	6.3 ± 1.5^{f}	84.5	0.3 ± 0.6^{f}	99.3
Control	40.0 ± 3.0	43.7 ± 2.5^{a}	---	43.0 ± 3.5^{a}	---	45.3 ± 3.8^{a}	---
F – values		18.8^{***}		45.1^{***}		61.7^{***}	
LSD 5%		4.2		4.7		4.9	

After the second treatment the number of mine per 10 leaflets is reduced to 6.3 mines / 10 leaflets in spinetoram application, with the reduction percent 84.5% followed by indoxacarb treatment (63.3%). The numbers of mines per 10 leaflets is reduced to 33.7 in the plots treated by entomopathogenic nematode *S. carpocapsae* All alone compared with 36.7 mines per 10 leaflets after the first application with the percent of reduction reached at 17.8%. The mixture of entomopathogenic nematode *S. carpocapsae* All and the half recommended field rate of spinetoram reduced the mines per 10 leaflets to 18 after the second application compared with 39.3 mine per 10 leaflets before the first application. The statistical analysis shows that there is a significant difference between spinetoram and other treatments. After the third application the leaflet mines approximately not found in spinetoram application and the percent of mines reduction reached to 99.3%, while in entomopathogenic nematode *S. carpocapsae* All only treatment the numbers of mines per 10 leaflets are 35.7 with 12.9% reduction compared to before the first application.

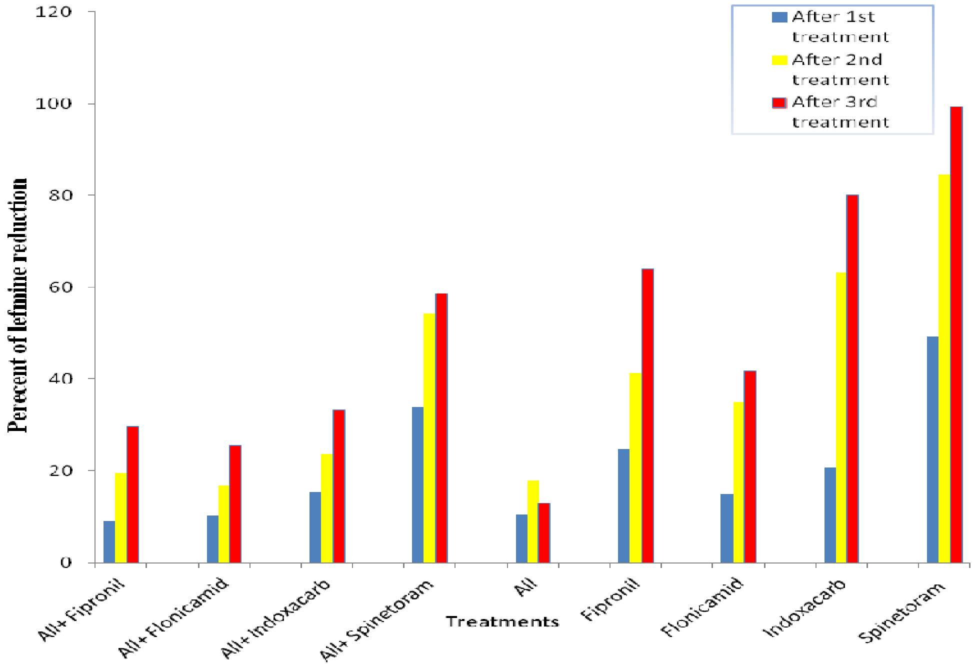

Fig. 1. Effect of entomopathogenic nematodes alon or in combination of some insecticides against *Tuta absoluta*

The obtained results in Table 1 and Figure 1 showed that the percentage of leaflets mines reduced sharply in spinetoram application followed by indoxacarb. It was increased after the first application to 49.1 and 20.8% in spinetoram and indoxacarb, respectively. After the second and third application it was reached at 84.5 and 63.3; and 99.3 and 80%, respectively. When the entomopathogenic nematode *S. carpocapsae* All was used alone against *T. absoluta* infestation the numbers of leaflets mines slightly decreased. The percentages of leaflets mines reduction in the first, second and third application were 10.5, 17.8 and 12.9%, respectively. This means that the entomopathogenic nematode *S. carpocapsae* All was not effective against *T. absoluta*. This may be due to the susceptibility of *S. carpocapsae* All to some a biotic factor such as drought, light, UV radiation and high temperature. Also, the presence of the *T. absoluta* larvae in tunnels reduces the effectiveness of *S. carpocapsae* All. When the entomopathogenic nematode *S. carpocapsae* All was mixed with half recommended field rate of all tested insecticides, the efficacy of these mixtures were not effective against *T. absoluta* infestation. The maximum efficacy was in the mixture of *S. carpocapsae* All and spinetoram after the third application. The percentge of reduction was 58.5%. These results were consistent with Mahmoud *et al.* 2016[13]. The authors found that *S. carpocapsae* All was not effective under greenhouse condition when it combined with any insecticides at concentration of 5000 IJs/25 ml. Shapiro-Ilan *et al.* (2006)[14] found that foliar applications have been less successful than soil applications due to nematode susceptibility to desiccation and UV. Türköz and Kaşkavalci (2016)[15] cleared that entomopathogenic nematodes were effective against *T. absoluta* larvae outside the tomato leaves not in side. Van Damme et al. (2016)[16] stated that *S. carpocapsae* All was effective against *T. absoluta* larvae under laboratory conditions. On the other hand, Batalla-Carrera *et al*. (2010)[17] found that in the pot experiments the entomopathogenic nematode (1,000 IJs/ ml) treatment reduced insect infestation of tomato plants by 87–95%. The authors demonstrated that the suitability of entomopathogenic nematodes for controlling *T. absoluta*. Gözel and Kasap (2015)[18] cleared that entomopathogenic nematode (50 IJs/cm^2) can be effective candidates in control of tomato leafminer, *T. absoluta*, under field conditions by using more than type of these biological agents into the *T. absoluta* management programme. The authors concluded that entomopathogenic nematodes were able to seek out and infect *T. absoluta* larvae whether inside or outside of the tomato leaf. As mentioned in Table (1) spinetoram was the most toxic against *T. absoluta* larvae. This may be due to the systemic efficacy for this insecticide. So, it can be reached at the larvae in leaf tunnel. Ragaei et al. (2013)[19] found that, under laboratory condition the larval mortality of spinetoram against *T. absoluta* was 80%.

Finally, the obtained results confirmed that entomopathogenic nematode *S. carpocapsae* All was not effective against *T. absoluta* larvae under greenhouse condition. Using of entomopathogenic nematode *S. carpocapsae* All in combination with the half recommendation rate of some insecticides was moderately effective when it combined with spinetoram after the third application. The percent of infestation reduction reached at 58.5%.

REFERENCES

[1] O.S. Grewal, E.A.B. De Nardo, M.M.Aguillera, *Neotrop. Entomol*., **2001**, 30, 191– 205
[2] S. Hazir, H.K. Kaya, P. Stock, N. Keskin, *Turk. J. Biol*. **2003**, 27, 181–202.
[3] Ž. Laznik, S. Trdan, *Acta agric. Slovenica,* **2008**, 91, 227-237.
[4] M.M.M. Miranda, M. Picanco, J.C. Zanuncio, R.N.C Guedes, *Biocont Sci Technol*., **1998**, 8: 597–606
[5] S.A. Tamerak, Int. Symp. manag. *Tuta absoluta*, pp– 18, 16 November **2011** Agadir, Morocco
[6] C.D. Campbell, J.F. Walgenbach, G.G. Kennedy, *J Econ Entomol*., **1991,** 84: 1662-1667.
[7] J.F. Walgenbach, R.B. Leidy, T. J. Sheets, *J.Econ. Entomol*., **1991**, 84, 978-986.
[8], J.L. Woodering, H.K. Kaya, *Ark. Agric. Exp. St. South. Cooper. Bull*., **1988**, 331: 430.
[9] S.R. Dutky, J.V. Thompson, G.E. Cantwell, *J. Insect Pathol*., **1964**, 6, 417-422.
[10] H.M.S. Metwally, G.A. Hafez, M.A. Hussein, M.A. Hussein, H.A. Salem, M.M.E. Saleh, Egy. *J. Biol. Pest* Cont., **2012**, 22, (1), 15-17.
[11] C.F. White, *Science*, **1927**, 66: 302-303.
[12] Costat Statistical Software, **1990**, 4.20. Berkeley, CA, USA.
[13] M.F. Mahmoud, H.M. Mahfouz, K.M. Mohamed, *Int. J. Res. Environ. Sci*., **2016**, 2, 1: 11-8.
[14] D.I. Shapiro-Ilan, D.H. Gouge, S.J. Piggott, *J P Fife, Biol. Cont*., **2006**, 38, 124–133
[15] S. Türköz, G. Kaşkavalci, *Türk. Entomol. Derg*., **2016**, 40, (2): 175-183
[16] V.M. Van Damme, B. Beck, E Berckmoes, R Moerkens, L Wittemans, R. De Vis, D. Nuyttens, H.F. Casteels, M. Maes, L. Tirryb, P. De Clercq, *Pest Manag Sci*., **2016**, 72: 1702–1709
[17] L. Batalla-Carrera, A. Morton, F. García-del-Pino, *BioControl*, **2010**, 55 (4): 523-530
[18] Ç. Gözel, İ. Kasap, *Türk. Entomol. Derg*., **2015**, 39 (3): 229-237
[19] M Ragaeia, K.H Sabrya and AM El-Rafei.. *Arch. Phytopath. Plant Protec*., **2013**, 46 (20): 2450–2458