ISJ 14: 375-378, 2017 ISSN 1824-307X

SHORT COMMUNICATION

Evaluation of the effects of some insecticides based on neonicotinoids on entomopathogenic nematodes, *Steinernema feltiae* and *S. carpocapsae*

V Kwizera, IA Susurluk

Uludag University, Faculty of Agriculture, Department of Plant Protection, 16059 Nilufer-Bursa, Turkey

Accepted September 21, 2017

Abstract

Used of entomopathogenic nematodes (EPNs) are one biological control way against some insect pests, which especially pass one stage of their life in the soil. There have been a lot of developments and new discoveries in the field of entomopathogenic nematology. In spite of developments in the field of biological control, pesticides are still widely used for plant protection. These pesticides have side effects on soil biotop such as EPN species. In this study, we evaluated the effects of two insecticides (acetamiprid and imidacloprid) belong to neonicotinoid group on two EPNs (*Steinernema carpocapsae* and *Steinernema feltiae*). The present experiments were carried out in the laboratory. Mortality rate of the EPN species was performed at 24, 48 and 96 h, after treatment. Inspired by the compatibility observed between the neonicotinoid insecticides and EPNs against insect pest larvae, it was evaluated the direct effects of those insecticides on EPNs. Our results showed less mortality by imidacloprid and mortality rates are a little higher by acetamiprid. These results confirm the high compatibility of imidacloprid with EPNs and the low synergism with acetamiprid. Despite their effects on pollinators and other useful organisms of the biotop, these insecticides can be used effectively against soil dwelling insect pests without high risk on EPNs.

Key Words: entomopathogenic nematodes; neonicotinoid; *Steinernema feltiae*; *Steinernema carpocapsae*

Introduction

Entomopathogenic nematodes (EPNs) are natural agents that control some pest insects (Gaugler and Kaya, 1990). It was first discovered in 1923 and since then the domain of EPN has made great progress (Thomas and Poinar, 1979; Poinar and Grewal, 2012). EPNs in the future may have great potential for farmers to control many harmful insects.

EPNs are a group of nematodes used to control insect pests (Tomalak, 2004, 2005; Jacobson and Martin, 2011). EPNs of the families Steinernematidae and Heterorhabditidae are symbiotically associated with bacteria in the genera *Xenorhabdus* and *Photorhabdus*, respectively (Griffin *et al.*, 2005). When an infective juvenile (IJ) of EPNs enters the body cavity of a susceptible host, the bacteria are released, multiply and host death occurs within two days, hence the term, entomopathogenic (Webster *et al.*, 2002). The nematodes develop and reproduce within the insect cadaver, feeding on the symbiotic bacteria and degraded host tissues (Forst *et al.*, 2002; Poinar and Grewal, 2012). EPNs are a potential alternative to the use of insecticides, which have side effects on the soil biotope. Since their discovery in 1923, researches on EPNs have been intense and a lot of progress have been made like mass production and shipment of IJs (Ehlers and Shapiro-Ilan, 2005; Poinar and Grewal, 2012). Despite the promising sustainability of biological control, insecticides are largely used to control insect pests. Beside pest control, these insecticides have side effects on useful insects. This is the case for the bees, which populations are dangerously reduced by nicotinoid insecticides (Woodcock *et al.*, 2016).

Altought there are many studied on effects of pesticides that largely used in agriculture on EPNs, the studies on neonicotinoids is very limited (Ulu *et al*., 2016). The 20th century was going to be a century of neonicotinoids, this is after the discover of nicotinoids, their wide range use and their substitution to many insecticides as they are successfully applied to control pests in a variety of agricultural crops (Yamamoto and Casida, 1999). The first nicotinoid to be on the market was imidacloprid (Thyssen and Machemer, 1999). Neonicotinoids are a class of insecticides chemically

Corresponding author:
I. Alper Susurluk
Uludag University
Faculty of Agriculture
Department of Plant Protection
E-mail: susurluk@uludag.edu.tr

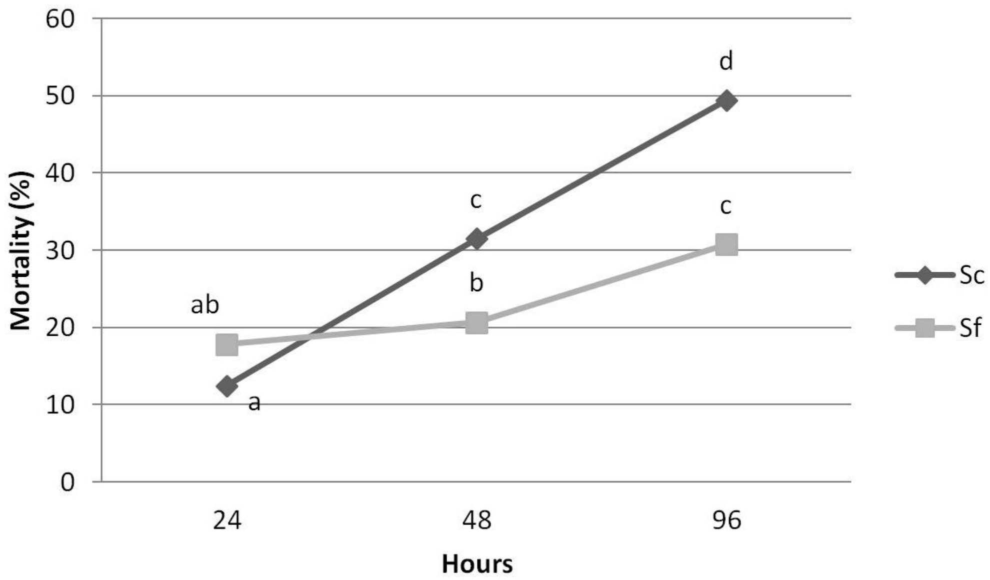

Fig. 1 Effects of acetamiprid on *Steinernema carpocapsae* (Sc) and *Steinernema feltiae* (Sf) (F = 37,4948; df = 5, 18; $p = < 0,0001$).

related to nicotine. The name literally means "new nicotine-like insecticides". Initially neonicotinoids were praised for their low-toxicity to many beneficial insects, including bees; however this claim has come into question. Researches point to potential toxicity to bees and other beneficial insects (Penelope *et al.*, 2012; Tjeerd Blacquière *et al.*, 2012; Gennaro Di Prisco *et al.*, 2013). Neonicotinoids ere today recognized as the first bee killer (Jennifer *et al.,* 2012).

Despite their side effects on bees and other beneficial insects, there are some researches showing synergism of neonicotinoid insecticides with EPNs (Pisa *et al*, 2014). But no previous research has evaluated the direct effects of neonicotinoids on EPNs. This paper will evaluate the direct effects of two neonicotinoids (imidacloprid and acetamiprid) on IJs of two EPNs (*Steinernema carpocapsae* and *Steinernema feltiae*).

Materials and Methods

Imidacloprid and acetamiprid (neonicotinoid insecticides) and two EPNs *Steinernema carpocapsae* and *Steinernema feltiae* were used. The first step was to multiply the EPN samples on *Galleria mellonella*.

Multiplication of EPNs

Twenty-four well cell culture dishes were used for the multiplication of EPNs. A larva was placed in each well and 10 % moistened sand was added. After filling the sand, 2 µl of IJ nematodes were given. It was then incubated at 27 °C. The infected larvae are removed after 48 h and placed in larger wells. Wells were left at room temperature and new IJs were obtained after 10 days (Kaya and Stock, 1997).

Infection

Twenty-four well cell culture vessels which we used for the replication of EPNs were also used here. 70-100 IJ micropipettes were placed in each well and the drug was applied. Four replications were used for each drug. In addition to these, 4 controlled recurrences were applied.

Doses

Field dose of each insecticide was applied. In the fields, doses are normally diluted in 100 liters. In this application, the dose is calculated and diluted in 20 ml: 1.2 mg acetamiprid and 3 µl imidacloprid in 1 ml, respectively. Tap water is used in control wells.

Dead EPNs counting

Counts were made at 24, 48 and 96 h. Live IJs are moving. Dead IJs are still and flat (Ulu *et al*., 2016). Two watches were used to count the dead-living distinction. After counting, the IJs in each well were recorded.

Statistical analysis

The ANOVA test was used to compare the mortality rates of the species with those of each other and themselves in drug efficacy trials. ANOVA test and LSD were performed in the JMP® 7.0 program.

Results and Discussion

Effects of acetamiprid

According to the results, the mortality rate increases with time (24, 48 and 96 h) (Fig. 1). The lowest mortality rate was significantly detected at 24 hours. Time-dependent differences were observed.

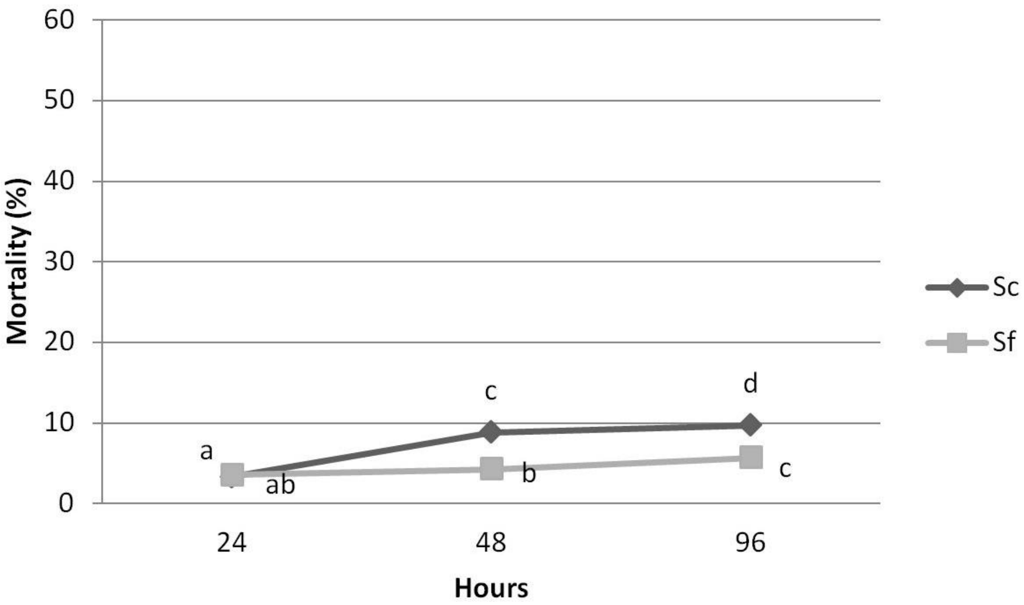

Fig. 2 Effects of imidacloprid on *Steinernema carpocapsae* (Sc) and *Steinernema feltiae* (Sf) (F= 2,6041; df = 5, 18; p = 0,0610)

Effects of imidacloprid

According to the results obtained, the mortality rate increases with time and the lowest mortality was observed at 24 h (Fig. 2).

It was determined that the mortality rate changed with time and dose. The lowest mortality rates were observed at 24 h, high mortality at 96 h. There is no direct numerical correlation between doses and mortality rates (Fig. 2). Mortality increases with time without direct numerical correlation with dose variation.

Since there were no previous studies evaluating the direct effects of insecticides on EPNs, this study focuses on the effects of the two insecticides on EPNs.

Comparing these results, it is observed that the acetamiprid has a higher mortality than imidacloprid. This confirms existing research evaluating the compatibility of EPNs and imidacloprid and acetamiprid to control of pests. According to those researchers, compatibility with imidacloprid is high. Acetamiprid is less compatible. These results were reported by Koppenhöfer *et al.* (2003). In previous green houses and field studies on control of five scarab larvae, neonicotinoid insecticide imidacloprid has been shown to interact synergistically with five entomopathogenic nematode species. Two other neonicotinoids, thiamethoxamine and acetamiprid showed weaker interaction with nematodes in the scarab larvae.

Imidacloprid and EPNs have shown good synergy against harmful insects (Farkhanda, 2012; Sheykhnejad *et al*., 2024), and are consistent with this research; imidacloprid was found to have a mortality rate of 0,122 % to 14,25 % in this study.

Compared to the mortality of insects by these three insecticides which are 7.1 µg - 8.09 µg/bee in contact application and 8.85 µg - 14.52 µg in oral application (acetamiprid) and 0.0179 µg - 0.243 µg/bee in contact application and 0.0037 - 0.081 µg in oral application (imidacloprid) (Jennifer *et al*., 2012), these neonicotinoid insecticides proved to be a lower EPN killer. These differences can be assessed in terms of physiological and nutritional aspects. The physiology of the insects on one side and the EPN on the other side are very different (Wharton, 1986; Klowden, 2013). It is proposed to investigate the contribution of nematode physiology to resistance to different pesticides.

References

Ehlers R-U, Shapiro-Ilan DI. Mass Production: Nematodes as biological control agents. CABI Publishing, Oxfordshire OX10 8DE UK, pp 65-78, 2005.

Farkhanda M. Synergism of imidacloprid and entomopathogenic nematodes for the control of eastern subterranean termite, reticulitermes flavipes (Isoptera: Rhinotermitidae). Pakistan J. Zool. 44: 1397-1403, 2012.

Forst S, Clarke D. Bacteria-Nematode symbiosis: entomopathogenic nematology. CABI Publishing. CAB Int. Wallingford, Oxon OX10 8DE UK, pp 57-77, 2002.

Goulson D, Kreutzweiser DP, Krupke C, Liess M, McField M, Morrissey C, *et al.* Effects of neonicotinoids and fipronil on non-target invertebrates. Environ. Sci. Pollut. Res. 22: 68-102, 2014.

Griffin CT, Boemare NE, Lewis EE. Biology and behaviour: nematodes as biological control agents. CABI Publishing, Oxfordshire OX10 8DE UK, pp 47-64, 2005.

Jacobson R, Martin G. A potential role for entomopathogenic nematodes within IPM of *Tuta absoluta* (Meyrick) on organic tomato crops. IOBC/WPRS Bulletin, 68: 79-83, 2011.

Jennifer H, Mace V, Schepherd M, Biddinger D, Mader E, Black SH, et al. Are nicotinoids killing bees? The xerces society for invertebrate conservation. www.xerces.org. Portland, 2012.

Kaya, HK, Stock, SP. Techniques in insect nematology. In: LACEY, L.A, 17 editor. Techniques in insect pathology. London, UK: Academic Press, pp 18 281-324, 1997.

Koppenhöfer AM, Grewal PS, Kaya HK. 2000. Synergism of imidacloprid and entomopathogenic nematodes against white grubs: the mechanism. Entomol. Exp. et Appl. 94: 283-293, 2000.

Pisa LW, Amaral-Rogers V, Belzunces LP, Bonmatin JM, Downs CA, Poinar GOJr, et al. History of entomopathogenic nematology. J. Nematol. 44: 153-161, 2012.

Sheykhnejad H, Ghadamyari M, Koppenhöfer AM, Karimi J. Interactions between entomopathogenic nematodes and imidacloprid for rose sawfly control. Biocontrol Sci. Technol. 24: 1481-1486, 2014.

Thyssen J, Machemer L. 1999. Imidacloprid: Toxicology and Metabolism. In: Yamamoto I, Casida JE (eds), Nicotinoid insecticides and the nicotinic acetylcholine receptor, pp 213-222, 1999.

Tomalak M. Infectivity of entomopathogenic nematodes to soil-dwelling developmental stages of the tree leaf beetles *Altica quercetorum* and *Agelastica alni*. Entomol. Exp. et Appl. 110: 125-133, 2004.

Tomalak M. Potentials of entomopathogenic nematodes for biological control of selected pests insects infesting urban trees. Bulletin OILB/SCROP 28: 3-7, 2005.

Webster JM, Chen G, HU K, Li J. Bacterial metabolites: entomopathogenic nematology. CABI Publishing. CAB Int., Wallingford, Oxon OX10 8DE, UK pp 99-114, 2002.

Woodcock BA, Isaac NJB, Bullock JM, Roy DB, Garthwaite DG, Crowe A, *et al*. Impacts of neonicotinoid use on long-term population changes in wild bees in England. *Nature Communıcatıons* 7 (12459) | DOI: 10.1038/ncomms12459 | www.nature.com/naturecommunications, 2016.

Ulu TC, Sadic B, Susurluk IA. Effects of different pesticides on virulence and mortality of some entomopathogenic nematodes. Inv. Surv. J. 13: 111-115, 2016.

Yamamoto I, Casida JE. Nicotinoid insecticides and the nicotinic acetylcholine receptor. 299 p., 1999.