ACTA ZOOLOGICA BULGARICA

Acta Zool. Bulg., Supplement 15, August 2020: 71-77

Proceedings of the Third International Conference on Zoology, Zoonoses and Epidemiology, Hissar, Bulgaria, October 21-23, 2019

http://www.acta-zoologica-bulgarica.eu/Suppl_15_14

Biodiversity, Ecology and Conservation
Research Article

Effects of the Modern Biorational Insecticide Spinosad on the Earthworm *Eisenia fetida* (Savigny, 1826) (Annelida: Clitellata)

Jovana Sekulić[1], Mirjana Stojanović[2], Tanja Trakić[2], Filip Popović[2] & Ralitsa Tsekova[3]*

[1] Department of Science, Institute for Information Technologies – Kragujevac, University of Kragujevac, Jovana Cvijića bb, 34000 Kragujevac, Serbia

[2] Institute of Biology and Ecology, Faculty of Science, University of Kragujevac, Radoja Domanovića 12, 34000 Kragujevac, Serbia

[3] South West University "Neofit Rilski", 66 Ivan Michailov Street, 2700 Blagoevgrad, Bulgaria

Abstract: Effects of the biorational insecticide spinosad on the life-cycle parameters (survival, growth, cocoon production and hatching juveniles) of a non-target organism, the earthworm *Eisenia fetida*, were examined. An artificial soil supplemented with different concentrations of the examined insecticide based on the recommended agricultural doses (RAD) was used. The laboratory test was conducted according to the OECD guidelines. Our results showed that the insecticide spinosad had no impact on the earthworm mortality. However, for four weeks assessment, the insecticide effects on weight were significant when applying 4×RAD. At the sixth week of the assessment, the insecticide effects were significant in RAD, 2×RAD and 4×RAD. After eight weeks, statistical analyses revealed a significant difference in all concentrations (except in the lowest concentration ¼RAD) compared with the control. A negative growth inhibition was observed in the ¼RAD, ½RAD and RAD during the four-week experiment. A positive growth inhibition was observed in all other weeks and concentrations. The results of cocoon production and hatching juveniles showed no significant difference between the control and the treatments. The results showed statistically significant impact on the weight after a long-term exposure, even in a concentration smaller than recommended.

Keywords: growth, mortality, reproduction, Lumbricidae, effects on non-target organisms

Introduction

Insecticides are claimed to be a major factor behind the increase in the 20th-century's agricultural productivity (Pimentel 2005). Despite their benefits, insecticides may also have negative consequences. One of them is the impact on non-target organisms, mainly due to physiological similarities between target and non-target organisms (Santos et al. 2010, Walker et al. 2010, Wang et al. 2012). For that reason, the assessment of the risks associated with their use is done on many soil and aquatic organisms (Yasmin & D'Souza 2007). Among soil organisms, the focus is on earthworms because they represent more than 80% of the total biomass of soil invertebrates in many temperate ecosys-

*Corresponding author: ralvir@abv.bg

tems. Also, as ecosystem engineers, they influence on soil structure and aeration, redistribution of organic matter and microbial community (Blouin et al. 2013). On other hand, their protection may provide a margin of safety for other members of the fauna because they can be more sensitive to the contaminants than the other animals (Reinecke et al. 1997) and they may prevent an increase in the pesticide concentration through the food chain (Kizilkaya 2005).

Although nu erous ecotoxicity studies used earthworms in recent years, the majority of them were focused on conventional insecticides (Espinoza-Navarro & Bustos-Obregón 2004, Reinecke & Reinecke 2007, Reddy & Rao 2008, Bansiwal & Rai 2010). Little is known about the impact of modern pesticides on earthworms using the standard test method as described in the guidelines of the OECD (Organisation for Economic Co-operation and Development) (Wang et al. 2012).

Even though biopesticides are considered to be an alternative to conventional pesticides, they have a completely different mode of action. Apart from the need for specific knowledge about the use of these compounds, also knowledge of their effect on non-target organisms and the environment in general is required (Sporleder et al. 2013). Spinosad (naturally derived insecticide) belongs to biorational insecticides (Sporleder et al. 2013) and also it is a neurotoxic insecticide produced by fermentation by the soil-dwelling actinomycete *Saccharopolyspora spinose* Mertz & Yao (Mertz & Yao 1990). Spinosad is a naturally occurring mixture of two active components, spinosyn A and spinosyn D, and can be described as a macrocyclic lactone containing tetracyclic ring for which two different sugars are bound (Badawy et al. 2016). It is structurally unique and easily degraded to its natural components by a combination of photodegradation and microbial degradation (Thompson et al. 2000). It is known to have excellent insecticidal activity, especially against lepidopteran species (Thompson et al. 2000). Also, it has been studied on mosquitoes (Bond et al. 2004) and spiders (Benamú et al. 2013). According to numerous studies on the impact of spinosad on species of the family Chrysopidae (Miles 2006, Mandour 2006, Maroufpoor et al. 2010, 2015, Sabry & El-Sayed 2011, Hussain et al. 2012), spinosad has been evaluated as slightly toxic or moderately toxic compared to conventional pesticides. In particular, very little is known about the impact of spinosad on earthworms. The only one study has been done on this subject (Karanjkar & Naik 2010), examining the acute toxicity of spinosad on *E. fetida*. On the other hand, findings on the impact of spinosad on soil invertebrates were based mainly on observations on arthropods (Durkin 2016). Therefore, the main aims of this ecotoxicology study are to obtain a more comprehensive understanding on the effects of the spinosad on the non-target earthworm species *E. fetida* as well as to provide the necessary information to be used in ecological risk assessment in terrestrial ecosystems.

Materials and Methods

Insecticide

The insecticide Laser 240 SC (purchased from Dow AgroSciences) has been tested in this experiment. Insecticide was used in a commercial suspension comprising 240 g active ingredient (a.i.)/L and is a mixture of spinosyn A and spinosyn D. In the laboratory experiment, different concentrations of the insecticide based on their recommended agricultural doses (200 ml/ha) (RAD) were used: ¼RAD, ½RAD, RAD, 2×RAD and 4×RAD. The amount of insecticide required was determined by the total area of the experimental box (100 cm^2). For the control treatment, distilled water was used. Each concentration and the control were tested with four replicates.

Earthworms

Specimens of *Eisenia fetida* were cultured in the laboratory in a medium as recommended by OECD (2004). The earthworms selected for the test were acclimatised to the test soil under test conditions for at least 24 h before use. The earthworms used in these experiments were adults with well-developed clitella and weighed between 300 mg and 460 mg.

Artificial test soil

The test soil was the OECD artificial soil (OECD 1984). The soil consisted of 70% quartz sand, 20% kaolin clay, 10% sphagnum peat and calcium carbonate to adjust pH 6.0±0.5. The dry components of the artificial soils had been mixed thoroughly before distilled water was added in order to achieve the desired moisture content of about 35% dry weight. The experimental soils were prepared by adding different concentrations of insecticide (dry weight basis) as appropriate.

Technical test performance

The earthworms were washed, dried on filter paper and weighed. Ten earthworms were placed per plastic container (10.5 x 9.6 x 7 cm) on the soil surface. Test containers were covered with perforated plastic lids and were kept at a temperature of 20±2ºC. The test was carried out under light-dark cycles (16:8). The earthworms were fed with 5 g ground cattle dung and the soil moisture tested once per week. Their mortality and weight were monitored weekly, cocoons are counted after 28 and 56 days, and hatching juveniles after 56 days. The earthworms were considered dead when they did not respond to the gentle mechanical prodding at the anterior of the body. Before weighing, all of the earthworms were sorted, washed with tap water, and blotted with filter paper. Then the earthworms were weighed using an electro-balance and after that returned to the soil. The weights of earthworms in each concentration reported from the various exposure periods were then used to calculate the growth inhibition as follows (Shi et al. 2007):

$$GIn = \frac{Wo - Wt}{Wo} \times 100\%$$

where GIn is the growth inhibition for concentration n, W_0 is the weight on day 0 and Wt is the weight after t days of exposure.

Statistical analysis

LC_{50} (the concentration that is lethal to 50% of individuals), as following 95% confidence interval, was calculated using the program CalcuSyn. Statistical analysis was performed using the SPSS software (SPSS 16.0 for Windows). The Shapiro-Wilk test was used to ensure the normality assumption. On the basis that they are used one-way ANOVA ($p < 0.05$) or Kruskal-Wallis H test for assessing the effects of contaminants on growth. With post hoc, in comparison of means (growth, reproduction), Dunnet t-test was applied. The data for growth inhibition was subjected to ANOVA using the Student Newman Keuls (S-N-K). Data are presented as the mean ± standard deviation (SD).

Results

One hundred percent survival of earthworms in the control treatment was recorded at the end of the test. In the first week, mortality only occurred in the highest concentration. In the third week of experiment, the number of dead individuals in this concentration increased slightly (average 12.5%), and the situation remained unchanged until the end of the experiment. Therefore, the LC_{50} value (3.62 $mg \cdot kg^{-1}$) was much higher than the highest concentrations that we used (4×RAD).

The earthworms treated with ½RAD, ¼RAD and RAD increased weight during the two weeks compared with their initial weight in these same treatments (Table 1). A decrease in weight was recorded when applying higher concentrations. During the first two weeks there was no statistically significant difference between treatment and control. In the fourth week, where the mean biomass was between 345 mg and 400 mg, there was a statistically significant difference ($p < 0.05$) between 4×RAD and the control. At the sixth week of the assessment, the earthworms treated with the insecticide had a mean biomass between 283 mg and 343 mg. The insecticide effects were significant ($p < 0.05$) in RAD, 2×RAD and 4×RAD. After eight weeks, the earthworms had the mean biomass between 273 mg and 320 mg. Statistical analyses have shown a significant difference in all concentrations, except in the lowest concentration (¼ AD) compared with the control (Table 1).

The growth inhibition (Fig. 1) was negative in the control, which means that the earthworms gained weight. Negative growth inhibition was observed in the ¼RAD, ½RAD and RAD during the four-week experiment. Positive growth inhibition was observed in all other weeks and concentrations. Statistical analysis showed significances in concentrations 2×RAD and 4×RAD. The results of cocoon

Table 1. Growth of the earthworm *Eisenia fetida* exposed to spinosad during eight weeks.

Spinosad	Mean weight per earthworm (mg)				
	0 week	**2 weeks**	**4 weeks**	**6 weeks**	**8 weeks**
Control	395±21	423±21	423±22	413±33	400±17
¼RAD	330±55	348±48	345±57	330±42	293±25
½RAD	355±48	383±39	380±14	343±39	313±26 [a]
RAD	373±39	395±26	380±36	320±47 [a]	305±21 [a]
2×RAD	405±39	375±33	375±26	323±30 [a]	320±22 [a]
4×RAD	388±56	363±57	358±50 [a]	283±61 [a]	273±48 [a]

RAD - recommended agricultural doses.

[a] Significant differences ($p < 0.05$) between treatment and control are indicated for each week.

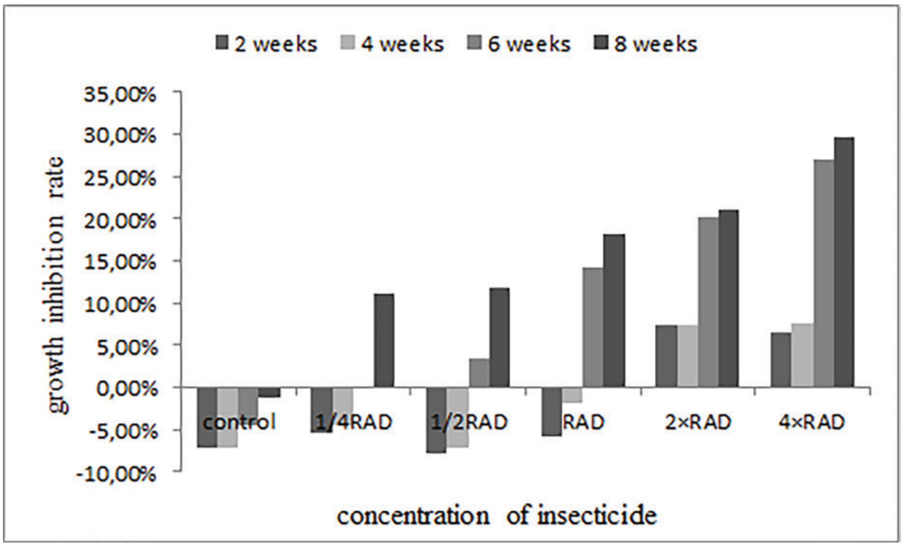

Fig. 1. Growth inhibition rates of the earthworm *Eisenia fetida* after exposure to spinosad under testing conditions

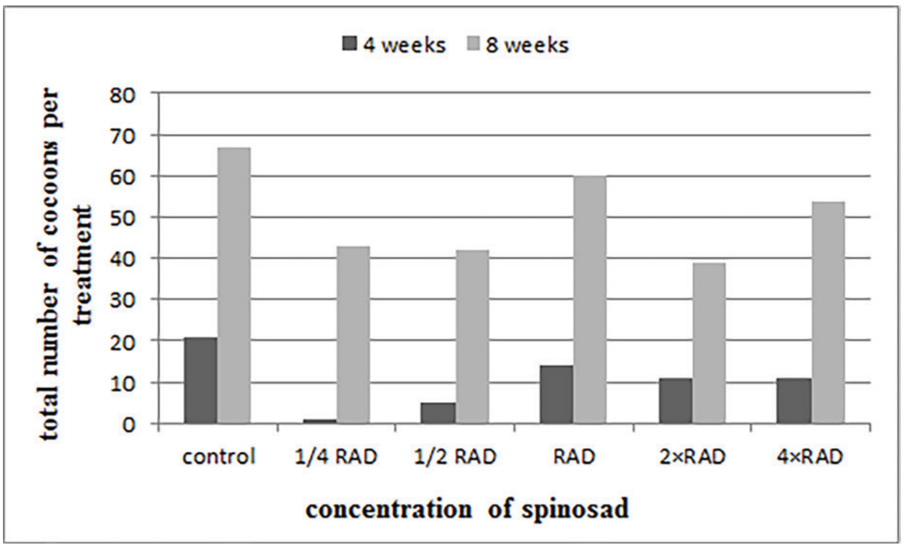

Fig. 2. Production of cocoons of *Eisenia fetida* after exposure to different concentrations of spinosad.

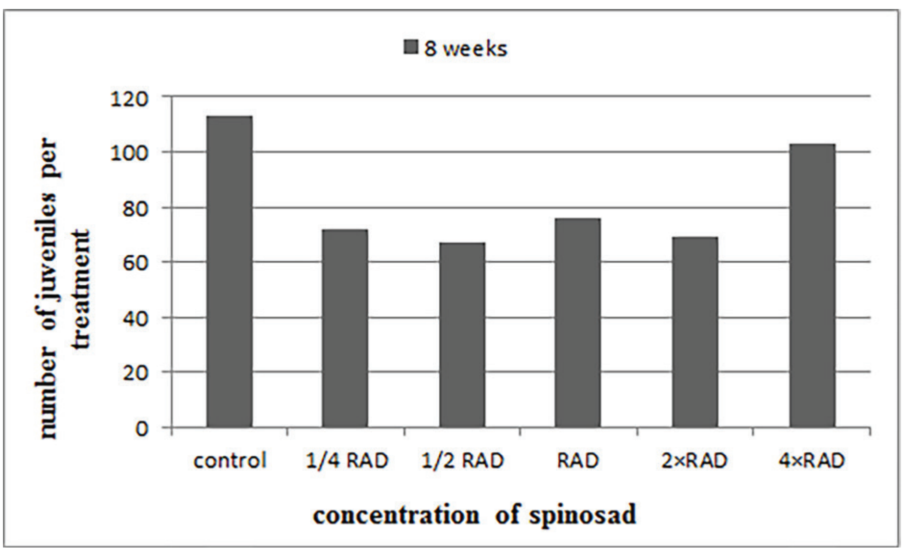

Fig. 3. Numbers of juveniles of *Eisenia fetida* after exposure to different concentrations of spinosad.

production (Fig. 2) and hatching juveniles (Fig. 3) showed no significant difference ($p < 0.05$) between the control and the treatments.

Discussion

In our study, no significant mortality has been recorded. Frampton et al. (2006) consider that acute mortality is not the most sensitive endpoint, which is in agreement with our results. However, the value for LC_{50} calculated for 28 days is higher than the highest concentration used in the experiment.

According to the results (Table 1) for the RAD and for the lower concentrations, it has been noticed that earthworms increase their weight during the first two weeks. A decrease in weight is immediately registered when using higher concentrations. Statistical analyses have shown a significant difference between control and the higher concentration (4×RAD). However, after six and eight weeks, there is a significant difference even in a concentration that is lower than the recommended agricultural dose (½RAD). Similar data have been obtained for growth inhibition (Fig. 1). These results are in accordance with the data of Yasmin & D'Souza (2010) outlining that the weight loss is an indicator of a physiological stress, which depends on the concentration of pesticides and exposure time. Mosleh et al. (2003) assume that this may also be a result of the energy mobilising the body's defence against pesticides and, therefore, no energy for growth is available.

On the other hand, Zhou et al. (2007) have considered that the reproduction parameters are clearly more sensitive test endpoints for risk assessment than others. Among others, the reproduction may be inhibited or stopped at concentrations well below the lethal concentration (Neuhauser et al. 1985). When it comes to our results of cocoon production and hatching juveniles, there was no statistically significant difference among the control and any of the treatments. Often, endpoints such as growth and reproduction are considered separately. However, according to Jager et al. (2006), these endpoints are closely related and reproduction generally starts at certain minimum body size. Actually, in our study, spinosad does not affect the weight on that way to reduce the formation of cocoons. With the passage of time, the number of cocoons has grown, which means that spinosad does not affect the quality of the ova and the number of hatchlings.

Among the pesticides used in agricultural practice, organophosphate insecticides and synthetic pyrethroids are the most commonly used (Espinoza-Navarro & Bustos-Obregón 2004). Furthermore, in addition to agriculture, they are used in veterinary practice as well as for home use. Synthetic pyrethroids, however, are becoming increasingly important, while the use of organophosphate insecticides has been drastically curtailed by the long ago prohibition of the use of almost all products containing chlorpyrifos and diazinon. As the primary replacement, the use of synthetic pyrethroids has increased dramatically in recent years (Wang et al. 2009). Synthetic pyrethroids are neurotoxic, just as spinosad, but they differ in the mechanism of action. Pyrethroids are sodium channel modulators and thus causing rapid paralysis and death in insects (Santos et al. 2007), while spinosad is nicotinic acetylcholine receptor (nAChR) allosteric modulator that is clearly novel and unique among known insect control products (Lumaret et al. 2012). Spinosad activates the nicotinic acetylcholine receptor in insects but at a completely different site from nicotine or imidacloprid. Until now, artificial synthetic pyrethroids were proved to be highly toxic to non-target organisms, especially on earthworms. Results of Wang et al. (2012) have shown toxicity of four types of these insecticides on *E. fetida*: cyhalothrin, cypermethrin, fenpropathrin and lambda-cyhalthrin. Cypermethrin has also been shown to be toxic to the tropical earthworm *Perionyx excavates* (see Gupta et al. 2010). Stäbler (2002) used a 56-days reproduction study that exposed the impact of bifenthrin on individual specimens of *E. fetida*, demonstrating the low value for 56-d NOEC for reproduction equal to 2.13 $mg \cdot kg^{-1}$ in test conditions and the corrected 56-d NOEC for reproduction in standard European soil equal to 0.7242 $mg \cdot kg^{-1}$. Unlike the pyrethroids, on the basis of our results as well as those of Karanjkar & Naik (2010), spinosad does not show influence on earthworms. Also, it has been found that it is not toxic for birds but has relatively low toxicity to mammals and it is only slightly to moderately toxic for aquatic organisms. In addition, chronic toxicological tests on mammals have shown that spinosad is not carcinogenic, teratogenic, mutagenic or neurotoxic (Thompson et al. 2000). Spinosad has proved to be safe for many beneficial insects (Lumaret et al. 2012). Also, spinosad proved to be a very good insecticide for control of insects of the orders Lepidoptera, Diptera and Thysanoptera as well as of some species of Coleoptera and Orthoptera (Thompson et al. 2000). The same authors give comparative data on cypermethrin and spinosad, and clearly noticed the lower activity of spinosad on important beneficial insects, but pointed out that the values generally coincided in terms of harmful insects.

Based on many studies and a favourable ecological profile, spinosad is recommended for use in organic farming (Thompson & Hutchins 1999, Copping & Menn 2000). However, it has been established that some aquatic invertebrates are sensitive to long-term exposure to spinosad (Williams et al. 2003). Also, our results indicate that earthworms are also sensitive to long-term exposure to this pesticide. Namely, in the sixth week of the experiment, spinosad has a statistically significant effect on the weight of earthworm in the recommended agricultural dose, while in the eighth week it has an effect even at a concentration that is twice smaller than the recommended agricultural dose. On the other hand, in our study the number of cocoons is not significantly reduced. However, if we take into account the connection of parameters such as weight and reproduction (Jager et al. 2006), then it is almost certain that the number of cocoons could be also decreased in the following period. Our findings are in accordance with the results of Badawy et al. (2016), which have concluded that spinosad is more active for a long period of time than some other pesticides. Therefore, further research is needed for assessment of the residual toxicity of spinosad, especially when repeated treatments are concerned.

Conclusions

The results obtained in this study indicate that there is no statistically significant mortality of earthworms due to spinosad. Additionally, Thompson et al. (2000) emphasise the positive characteristics of this chemical substance such as unique structure and mode of operation, rapid degradation, efficacy against pests, safe for beneficial organisms. Also, the advantage of spinosad compared to the pyrethroid, especially cypermethrin, is clear. On the other hand, the toxicity spectrum of spinosad, especially negative effects on non-targeted aquatic invertebrates, as well as our results showing statistically significant impact on the weight after a long time exposure, even in a concentration that is less than recommended, strongly warn that the use of these chemical substances must be with maximum responsibility and high precautions.

Acknowledgments: The authors are very grateful to Ivan Dimitrov (registered farm, Dimitrovgrad, Serbia) for providing earthworms for this experiment. This work was supported by the Ministry of Education and Science of the Republic of Serbia (Grant No. 41010).

References

Badawy M., Taktak N., Awad O., Elfiki S. & El-Ela N. 2016. Evaluation of released malathion and spinosad from chitosan/alginate/gelatin capsules against *Culex pipiens* larvae. Research and Reports in Tropical Medicine 7: 23–38.

Bansiwal K. & Rai N. 2010. Assessment of malathion toxicity in certain organs of earthworm, *Eisenia foetida*. Bioscan 5: 473–476.

Benamú M. A., Schneider M. I., González A. & Sánchez N. E. 2013. Short and long-term effects of three neurotoxic insecticides on biological and behavioural attributes of the orb-web spider *Alpaida veniliae* (Araneae, Araneidae): implications for IPM programs. Ecotoxicology 22: 1155–64.

Blouin M., Hodson M.E., Delgado E.A., Baker G. Brussaar L. Butt K.R., Dai J., Dendooven L. Peres G. Tondoh J. E., Cluzeau D. & Brun J.J. 2013. A review of earthworm impact on soil function and ecosystem services. European Journal of Soil Science 64: 161–182.

Bond J. G., Marina C.F. & Williams T. 2004. The naturally derived insecticide spinosad is highly toxic to *Aedes* and *Anopheles* mosquito larvae. Medical and Veterinary Entomology 18: 50–56.

Copping L. G. & Menn J.J 2000. Biopesticides: a review of their action, applications and efficacy. Pest Management Science 56: 651–676.

Durkin P. R. 2016. Spinosad: Human Health and Ecological Risk Assessment. Manlius, New York.

Espinoza-Navarro O. & Bustos-Obregón E. 2004. Sublethal doses of malathion alter male reproductive parameters of *Eisenia foetida*. International Journal of Morphology 22: 297–302.

Frampton G.K., Jänsch S., Scott-Fordsmand J.J., Römbke J. & van den Brink P.J. 2006. Effects of pesticides on soil invertebrates in laboratory studies: A review and analysis using species sensitivity distributions. Environmental Toxicology and Chemistry 9: 2480–2489.

Gupta R.D., Chakravorty P.P. & Kaviraj A. 2010. Studies on relative toxicities of six insecticides on epigeic earthworm, *Perionyx excavates*. Bulletin of Environmental Contamination and Toxicology 85: 83–86.

Hussain D., Ali A., Tariq R., Mushtaq-ul-Hassan M. & Saleem M. 2012. Comparative toxicity of some new chemistry insecticides on *Chrysoperla carnea* (Stephens) under laboratory conditions. Journal of Agricultural Reseach 50: 4.

Jager T., Reinecke S.A. & Reinecke A. J. 2006. Using process-based modelling to analyse earthworm life cycles. Soil Biology and Biochemistry 38: 1–6.

Karanjkar A.S & Naik R.L. 2010. Acute toxicity: novel mode of pesticides on earthworm. International Journal of Plant Protection 2: 182–185.

Kizilkaya R. 2005. The role of different organic wastes on zinc bioaccumulation by earthworm *Lumbricus terrestris* L. (Oligochaeta) in successive Zn added soil. Ecological Engineering 25: 322–331.

Lumaret J. P., Errouissi F., Floate K., Römbke J. & Wardhaugh K. 2012. A review on the toxicity and non-target effects of macrocyclic lactones in terrestrial and aquatic environments. Current Pharmaceutical Biotechnology 13: 1004-1060.

Mandour N. S. 2006. Influence of spinosad on immature and adult stages of *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae). BioControl 54: 93–102.

Maroufpoor M., Aramideh S., Hassan Safaralizadeh M., Asghar Pourmirza A. & Ghasemzadeh S. 2015. Toxicity of spinosad on common green lacewing *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) larvae via ingestion technique under laboratory conditions. Archive of Phytopathology and Plant Protection 48: 412–420.

Maroufpoor M., Hasan Safaralizadeh M., Asghar Pourmirza A., Allahvaisy S. & Ghasemzadeh S. 2010. Lethal effects of spinosad on *Chrysoperla carnea* larvae (Neuroptera: Chrisopidae) under laboratory conditions. Journal of Plant Protection Research 50: 179–183.

Mertz F. P. & Yao R. C. 1990. *Saccharopolyspora spinosa* sp. nov. isolated from soil collected in a sugar mill rum still. International Journal of Systematic Bacteriology 40: 34–39.

Miles M. 2006. The effects of Spinosad on beneficial insects and mites used in integrayed pest management systems in greenhouse. Pesticides and Beneficial Organisms 29: 53–59.

Mosleh Y.Y., Palacios S.P., Couderchet M. & Vernet G. 2003. Acute and sublethal effects of two insecticides on earthworms (*Lumbricus terrestris* L.) under laboratory conditions. Environmental Toxicology 18: 1–8.

Neuhauser E.F., Loehr R.C., Milligan D.L. & Malecki M.R. 1985. Toxicity of metals to the earthworm *Eisenia fetida*. Biology and Fertility of Soils 1: 149–152.

OECD 1984. Guidelines for testing of chemicals, in: Earthworm Acute Toxicity Tests. Paris: vol. 207 OECD.

OECD 2004. Guideline for testing of chemicals, in: Earthworm Reproduction Test (*Eisenia fetida/Eisenia andrei*). Paris: vol. 222OECD.

Pimentel D. 2005. Environmental and economic costs of the application of pesticides primarily in the United States. Environment, Development and Sustainability 7: 229–252.

Reddy N. C. & Rao J. V. 2008. Biological response of earthworm, *Eisenia foetida* (Savigny) to an organophosphorous pesticide, profenofos. Ecotoxicology and Environmental Safety 71: 574–582.

Reinecke A. J., Reinecke S. A. & Lambrechts H. 1997. Uptake and toxicity of copper and zinc for the African earthworm, *Eudrilus eugeniae* (Oligochaeta). Biology and Fertility of Soils 24: 27–31.

Reinecke S. A. & Reinecke A. J. 2007. The impact of organophosphate pesticides in orchards on earthworms in the Western Cape, South Africa. Ecotoxicology and Environmental Safety 66: 244–251.

Sabry K.H. & El-Sayed A.A. 2011. Biosafety of a biopesticide and some pesticides used on cotton crop against green lacewing, *Chrysoperla carnea* (Stehens) (Neuroptera: Chrysopidae). Journal of Biopesticides 4: 214–218.

Santos M.A.T., Areas M.A. & Reyes F. G. R. 2007. Piretróides – uma visão geral. Alimentos e Nutrição Araraquara 18: 339–349.

Santos M.J.G., Soares A. M.V.M. & Loureiro S. 2010. Joint effects of three plant protection products to the terrestrial isopod *Porcellionides pruinosus* and the collembolan *Folsomia candida*. Chemosphere 80: 1021–1030.

Shi Y., Shi Y., Wang X., Lu Y. &Yan S. 2007. Comparative effects of lindane and deltamethrin on mortality, growth, and cellulose activity in earthworms (*Eisenia fetida*). Pesticide Biochemistry and Physiology 89: 31–38.

Sporleder M. & Lawrence A.L. 2013. Biopesticides. In: Giordanengo P., Vincent C. & Alyokhin A. (Eds.): Insects pests of potato. United States: Global perspectives on biology and management, pp. 463–497.

Stäbler D. 2002. Talstar 8SC assessment of effects on reproduction and growth on *Eisenia foetida* in artificial soil. GAB Biotechnologies – Germany.

Thompson G. & Hutchins S. 1999. Spinosad. Pest Outlook 10: 78–81.

Thompson G. D., Dutton R. & Sparks T. C. 2000. Spinosad – a case study: an example from a natural products discovery programme. Pest Management Science 56: 696–702.

Walker A. N., Golden R. & Hors M. 2010. Morphologic effects of in vivo acute exposure to the pesticide methoprene on the hepatopancreas of a non-target organism, *Homarus americanus*. Ecotoxicology and Environmental Safety 73: 1867–1874.

Wang C., Chen F., Zhang Q. & Fang Z. 2009. Chronic toxicity and cytotoxicity of synthetic pyrethroid insecticide cis-bifenthrin. Journal of Environmental Science 21: 1710–1715.

Wang Y., Cang T., Zhao X., Yu R., Chen C., Wu C. & Wang Q. 2012. Comparative acute toxicity of twenty-four insecticides to earthworm, *Eisenia fetida*. Ecotoxicology and Environmental Safety 79: 122–128.

Williams T., Valle J. & Viñuelas E. 2003. Is the naturally derived insecticide Spinosad® compatible with insect natural enemies? Biocontrol Science and Technology 13: 459–475.

Yasmin S. & D'Souza D. 2007. Effect of pesticides on the reproductive output of *Eisenia fetida*. Bulletin of Environmental Contamination and Toxicology 79: 529–533.

Yasmin S. & D'Souza D. 2010. Effects of pesticides on the growth and reproduction of earthworm: a review. Applied and Environmental Soil Science 2010: 1–9.

Zhou S., Duan C., Fu H., Chen Y., Wang X. & Yu Z. 2007. Toxicity assessment for chlorpyrifos-contaminated soil with three different earthworm test methods. Journal of Environmental Science 19: 854–858.