The Journal of Animal & Plant Sciences, 25(3): 2015, Page: 844-850
ISSN: 1018-7081

TOXICITY OF AVERMECTIN B1b TO EARTHWORM AND COCKROACHES

S. Siddique[1,2], Q. Syed[1], Y. Saleem[1]✉, A. Adnan[2] and F. A. Qureshi[3]

[1]Food and Biotechnology Research Center, PCSIR Laboratories Complex, Ferozepur Road Lahore, Pakistan.
[2]Department of Chemistry, Government College University, Lahore, Pakistan.
[3]Office of Research, Innovation and Commercialization, COMSATS Institute of Information and Technology, Chak Shahzad, Park Road, Islamabad, Pakistan.
✉Corresponding author E-mail: ysaleem73@hotmail.com

ABSTRACT

The main objective of the present study was to investigate the effect of avermectin B1b on nematodes and arthropods. Avermectin B1b, a component of commercially available abamectin, was obtained as fermentation product of *S. avermitilis* 41445 and used after purification. In the case of earthworm, a representative nematode, the LC_{50} values determined for contact filter paper test from Probit's analysis after 48 and 72 h were 500µg/cm^2 and 300µg/cm^2 respectively. The mortality increased as the concentration of the applied substance increased. The LC_{50} values calculated after 7, 14 and 28 days from Probit's analysis were 712.5248 mg/Kg, 382.6 mg/Kg and 74.6 mg/Kg respectively showing a clear concentration-mortality relationship. For cockroach, a representative arthropod, the LC_{50} values for mortality were lower for oral exposure as compared to the dermal. The no observed effective concentration (NOEC) and lowest observed effective concentration (LOEC) values determined for earthworm mortality calculated on the basis of student's *t-test* were 0.1 mg/Kg and 1.0 mg/Kg respectively. At lower concentrations the cocoon formation was observed with subsequent elimination at 1000 mg /kg of avermectin B1b. The results of present study revealed that avermectin B1b is highly effective against nematodes and arthropods.

Key words: Earthworms, cockroaches, avermectin B1b, Probit's analysis, NOEC, LOEC, LC_{50} determination.

INTRODUCTION

Avermectins mainly the abamectin are the secondary metabolites derived as fermentation product of *Streptomyces avermitilis* and have been used extensively to control both the endo- and ectoparasites (Diao *et al.* 2007). Being broad spectrum in effectiveness, convenient to use and wide margin of safety to target animals they have been used by the farmers all over the world. They are used as active components of many insecticidal and nematocidal products in agriculture (Kolar *et al.* 2006) and a part of veterinary medicines to control and prevent the parasitic diseases (Kolar *et al.* 2006; Floate *et al.* 2005; Kovecses and Marcogliese, 2005). Even a very small dose is very effective (Burg *et al.* 1979; Diao *et al.* 2007). The veterinary medicines can be degraded, transported and distributed among different soil compartments when released to the environment. However, very slow degradation of avermectins and their insolubility in water makes them lesser distributed in the soil (Steel and Wardhaugh, 2002; Sun *et al.* 2005) and also allows them to be retained unaffected in the feces of effected animals and it is possible to recover more than 90% of the total drug used. Abamectin is easily photo degraded however it disappears from the soil slowly and has a half life of about 2-8 weeks (Erzen *et al.* 2005). Different studies revealing the effect of abamectin on flies and beetles assaulting freshly deposited feces in the field have been demonstrated, however, dose-response relationship studies has not been carried out except for earthworms (Sun *et al.* 2005). Only a few studies addressed the effect of avermectin mainly the ivermectin on soil dwelling organisms (Jensen *et al.* 2003) and the toxicity of abamectin to earthworm (Sun *et al.* 2005).

Earthworms make up about 60-80% of the total animal volume in soil (Ouellet *et al.* 2008; Jouquet *et al.* 2010). Their Presence in soil is very important in order to retain soil porosity, the fertility and improved soil structure, physical and chemical alteration of soil organic matter , configuration and stabilization of soil aggregates (Bartlett, *et al.* 2010; Lavelle and Spain 2001; Yanhua *et al.* 2011). Being susceptible to soil chemicals andtheir thick cuticle (Nahmani *et al.* 2007), insecticidal bioaccumulation will not directly affect them however it will cause severe smash up to higher tropical levels (van Gestel *et al.* 2011). Being apposite bioindicators of soil contamination earthworms can be used to impart safety sill for insecticidal treatments (Suthar *et al.* 2008). Although beneficial to the soil, the presence of earthworms resulted in reduction of biomass and thickness of litter layer (Nunes and Espindola, 2012). Forest decline, loss of native plant species, soil erosion, increased humification and decomposition is resulted as litter layer depleted (Frelich *et al.* 2006; Cindy *et al*, 2008).

In terms of biomass, cockroaches are of utmost importance because of large individual body size as compared to other detritivores. In this way cockroaches

constitutes about 24.3% of invertebrates biomass (Basset, 2001). Being highly resistant to insecticides, it is very difficult to control the cockroaches in the soil. The most resistant cockroach pest is the *Blattella germanica*. Therefore it is required to develop new insecticides (Scott, 1991).

Abamectin is a mixture of approximately 80% B1a and 20% B1b. Bioaccumulation and elimination of avermectin B1a in earthworm is reported already (Sun *et al.* 2005) however no data is available showing the effect of avermectin B1b on earthworms and cockroaches. The present study was aimed to reveal the toxicity effects of avermectin B1b on earthworms and cockroaches.

MATERIALS AND METHODS

Test organisms: Cockroaches with weight range between 4-5g and adult earthworms having well developed clitella and weight range of 400-500mg were taken from University of Veterinary and Animals Sciences, Lahore, Pakistan. The culturing of earthworms in the laboratory was done according to the guidelines specified by OECD 2004.

Test chemical: For all the experiments conducted, the avermectin B1b was obtained as fermentation product of S.avermitilis 41445 in SM2 medium by a method as described earlier by Siddique *et al.* (2013) and used in the present study after purification by lyophilization. It is the minor component of commercially available abamectin. It is macrocyclic lactone containing an isopropyl reside in the 25-position showing broad spectrum of anthelmintic activity. The physiochemical properties of avermectin B1b are given in table 1 (Wolstenholme *et al.* 2005).

Table 1. Physiochemical properties of avermectin B1b

Molecular mass	859.1
Physical appearance	White
Molecular formula	$C_{47}H_{70}O_{14}$
Solubility	Ethanol, Methanol, DMF, DMSO
Form	Solid

Test Performance

Contact filter paper test for earthworms: Contact filter paper test was performed according to the method described by (Yanhua *et al.* 2011). At the end of the test the mortality was recorded after 48 and 72h. The earthworms were considered as dead if failed to retort to placid mechanical touch at their front end.

A preliminary test was performed to find out the concentration range in which 0-100% mortality was observed by using the test chemical. About six concentrations i.e.$1x10^{-4}$-$6x10^{-4}$ g/cm^2 and a control were used to find out the concentration range with each experiment performed in replicates of ten. Methanol was used as control and treated earthworms were maintained at 20±1 °C.

Soil tests for earthworms: Soil from the area around PCSIR Labs Complex Lahore, Pakistan was taken to conduct the soil test. Soil test was performed using a range of concentrations of test chemical, 0 mg/Kg, 0.1 mg/Kg, 1.0 mg/Kg, 10.0 mg/Kg, 50.0 mg/Kg, 100.0 mg/Kg, 500.0 mg/Kg and 1000.0 mg/Kg of dry soil. The rest of the test was performed according to the method described by Rombke *et al.* (2010) . Mortality, biomass, number of cocoon formation were accessed after 7, 14 and 28 days of application. Biomass was determined by using a method described earlier by Diao *et al.* (2007).

Laboratory evaluation for Cockroaches: A previously reported method of Strong *et al.* (1993) with minor modification was employed for determining the oral toxicity of avermectin B1b against Cockroaches. Cockroaches were reared in capped glass jars and were maintained at 25±2°C for a12/12 day and dark period. Cockroaches were kept in glass jars with a moistened cotton wick and petroleum jelly applied at upper side of the jar to prevent their escape from jar. Oral toxicity of avermectin B1b was estimated after 3, 7 and 14 days of exposure by providing a piece of bread in each jar soaked with avermectin B1b. For determination of dermal toxicity, aforementioned test chemical was sprayed on the cockroaches and the mortality was observed after 3, 7 and 14 days.

Statistical analysis: In case of earthworms or contact filter paper method, a Probit's analysis was performed to find out the LC_{50} values and the toxicity level was determined as described earlier (Finney, 1971). Concentration was classified as super toxic (< 1.0 µg/cm^2), extremely toxic (1-10 µg/cm^2), very toxic (10-100 µg/cm^2), moderately toxic (100-1000 µg/cm^2), and relatively non toxic (> 1000 µg/cm^2) according to Robert and Dorough (1984). In soil test, Probit analysis was used to find out the LC_{50} values and also for the calculation of NOEC and LOEC, student's t-test and p-test were performed. Same statistical approach was applied for determining the oral and dermal toxicity of avermectin B1b against cockroaches.

RESULTS

Contact filter paper toxicity of earthworms: The results of contact filter paper assay for earthworms revealed that the effectiveness of avermectin B1b varied with time of exposure. The LC_{50} values determined from Probit's analysis after 48 and 72h were 500 µg/cm^2 and 300 µg/cm^2 respectively. Difference in mortality recorded after 48 and 72h is shown is Fig. 1. Mortality increased as the concentration of avermectin B1b increased.

Soil toxicity of earthworms: Soil toxicity test of avermectin B1b showed a clear concentration dependent relationship. The mortality increased with concentration and exposure time as is shown in Fig 2.

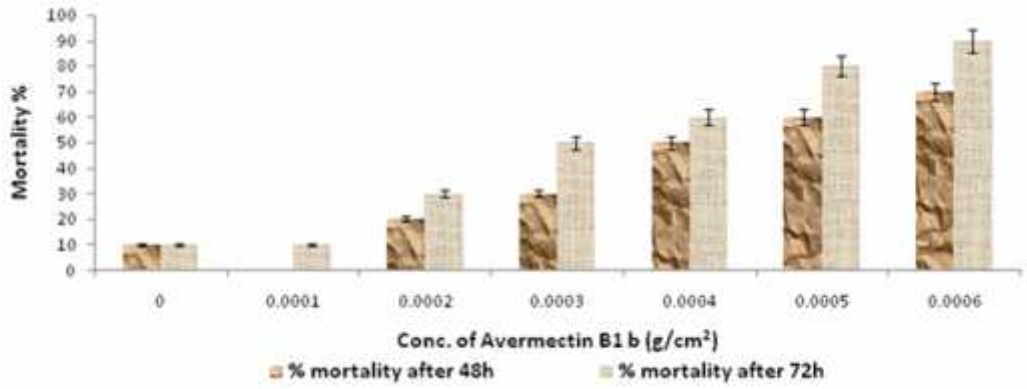

Figure 1: Contact filter paper test. Effect of avermectin B1b on Earthworm mortality. Mortality increased as the exposure time of avermectin B1b increased. Similarly the fig represents that by increasing the concentration of test chemical during contact filter paper, the rate of mortality increased.

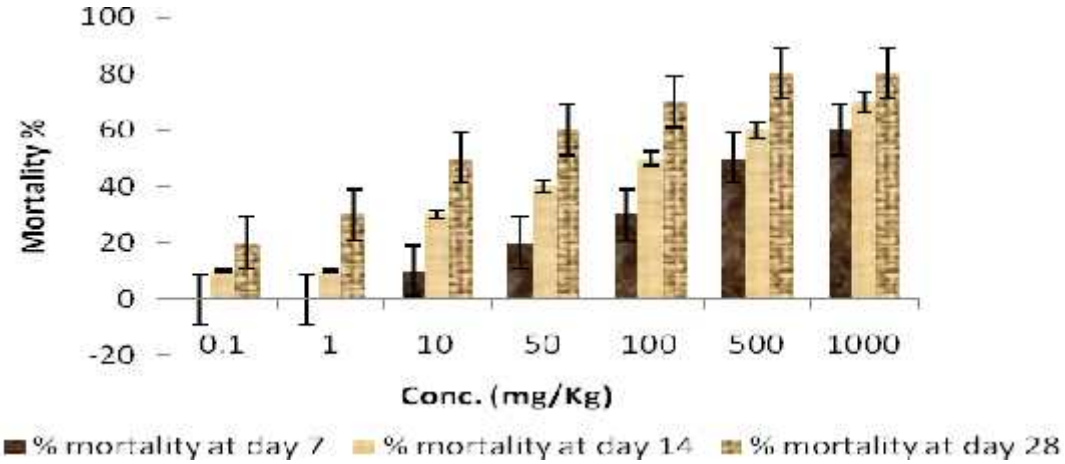

Figure 2: Soil toxicity of earthworm. Toxicity of avermectin B1b in soil against earthworms. The figure showed that with increasing the concentration of avermectin B1b upto 1000 mg/Kg, the mortality increased to 80% after 28 days of exposure. No mortality observed at low doses and less exposure of test chemical

The LC_{50} values calculated after 7, 14 and 28 days from Probit's analysis were 712.5248 mg/Kg, 382.6 mg/Kg and 74.6 mg/Kg respectively. No observed effective concentration (NOEC) and lowest observed effective concentration (LOEC) for mortality calculated on the basis of student's t-*test* were 0.1 mg/Kg and 1.0 mg/Kg respectively.

Effect of avermectin B1b on cocoon formation: The concentration-response relationship was also demonstrated in the form of cocoon formation. No. of cocoon formed after 7, 14 and 28 days were calculated. Results revealed that on increasing avermectin B1b concentration, no. of cocoon formation decreased. Also, exposure time affected cocoon-formation in a linear manner as is shown in Fig. 3. Maximum cocoon formation occurred at lowest test concentration of 0.1 mg/kg of avermectin B1b. No cocoon formation was observed at concentration of 1000 mg/kg. Also, cocoon-formation was found to be the maximum after 28 days.

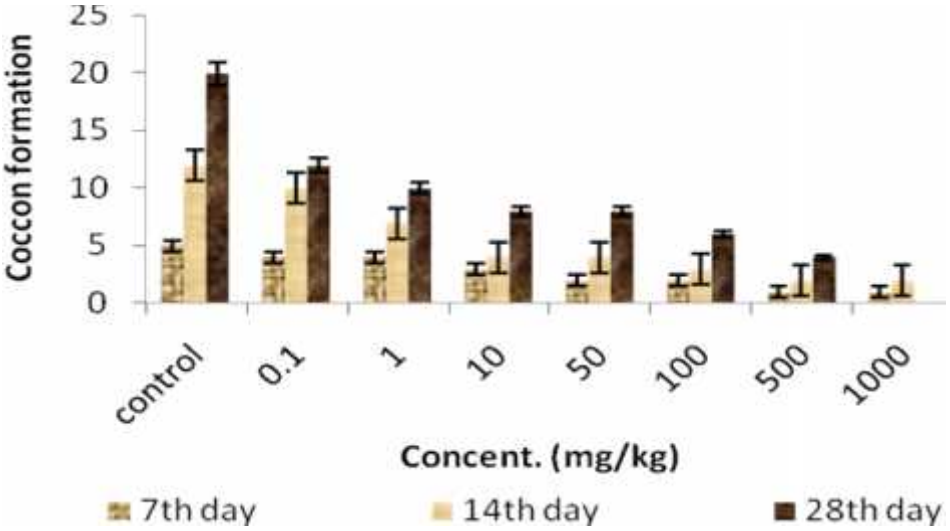

Figure 3: Effect of avermectin B1b on cocoon formation. Avermectin B1b on cocoon formation. The Effect of during soil toxicity of avermectin B1b on earthworm, the cocoon formation as found to decrease as the concentration of test chemical increased. Also the exposure time of test chemical and cocoon formation showed indirect relationship. Cocoon formation depleted at highest 1000 mg / Kg concentration of avermectin B1b.

The effect of concentration and exposure time on biomass of earthworm resulted in the maximum reduction in weight loss at highest concentration of test chemical at 14th and 28th day of exposure when compared to the control. Very little difference in biomass between treated and non-treated test organisms was observed after 7th day of exposure (Data not shown in this manuscript).

Laboratory evaluation for Cockroaches: In the case of cockroaches, a representative arthropod, mortality increased with exposure-time of avermectin B1b. As a general rule in toxicity assays, greater the toxicity of test substance, lower the LC_{50} value and vice versa. In case of both oral and dermal exposures, the oral and the dermal toxicity, maximum mortality was observed after 14 days of exposure followed by 7th and 3rd day of exposure respectively as is shown in Table 2.

Table 2. Oral and dermal exposure of Cockroaches with avermectin B1b

Oral exposure			Dermal exposure		
(Days)	**LC_{50} (95% Cl) (µg/g)**	**Slope ± SEM**	**(Days)**	**LC_{50} (95% Cl) (µg/g)**	**Slope ± SEM**
3	937.60	2.6±0.04	3	1239.10	5.2±0.04
7	668.5	2.18±0.31	7	944.5	7.9±0.2
14	489.4	4.5±0.29	14	784.5	8.5±0.1

DISCUSSION

Abamectin, a mixture of avermectin B1a and B1b, is very efficient against parasites and potentially perilous for invertebrates in the soil (Bueno and Freitas, 2004). Yanhua *et al.* (2011) reported that toxicity inference of an insecticide deceptively fluctuates when different test methods are adopted for testing. Spraying of avermectins on test animals resulted in muscle paralysis along with non-specific effects on metabolism (Zidar *et al.* 2004). The paralysis of muscles occurs due to the suppression of electrical activity which is caused by the activation of irreversible chlorine permeability (Ding *et al.* 2001). The activation of irreversible permeability results in behavioral changes, mortality, reduction in biomass and cocoon formation (Thain *et al.* 1997). The present study was conducted to test the effectiveness of avermectin B1b produced as a fermentation product of *Streptomyces avermitilis* 41445. Two species selected for toxicity determination of avermectin B1b were earthworm and cockroach. Contact filter paper and soil toxicity assays were performed for earthworm. In each case the test chemical was proved toxic for earthworms and cockroaches.

During contact filter paper test, insecticide was absorbed through skin. The absorbed quantity was

measured to determine relative toxicity of insecticides to earthworms. This test is one of the common preliminary screening techniques; however in case of soil ecosystem this technique does not give fruitful result (Miyazaki *et al.* 2002; Tripathi *et al.* 2010). In the present study when contact filter paper test was used for the earthworms, avermectin B1b proved to be moderately toxic at a concentration of 500µg / cm^2 and 300µg / cm^2 after 48 and 72h respectively. From the LC_{50} values calculated using Probit's analysis, it was estimated that avermectin B1b was most effective on earthworms after 72h of exposure. Roberts and Dorough, (1984) classified the chemical as super toxic at LC_{50} <1.0 µg / cm^2, extremely toxic at LC_{50} = 1-10 µg / cm^2, very toxic at LC_{50} = 10-100 µg / cm^2, moderately toxic at LC_{50} = 100-1000 µg / cm^2 and relatively non toxic at LC_{50} >1000 µg/cm^2. In a study conducted by Yanhua *et al.* (2011), they reported intermediate toxicity response of antibiotics, carbamates and organophosphate against *E. fetida* in contact filter paper assay. The LC_{50} value for ivermectin in their study was 23.08 µg / cm^2 claiming it to be very toxic. However in present study the avermectin B1b showed moderate toxicity against the tested organism.

It is reported that toxicity of insecticides to earthworms can be assessed through artificial soil toxicity test. (Udovic and Lestan, 2010). In a study conducted by Yanhua *et al.* (2011), the LC_{50} value for ivermectin after 7 and 14 days of exposure were 31.05 mg / kg and 27.86 mg / kg which showed that LC_{50} values decreased when the exposure time was increased.. Mortality increased with time and concentration of test material; which exhibited intermediate toxicity level. In the present study, on performing soil test to evaluate the toxicity of avermectin B1b against earthworms, a decrease in the LC_{50} values with increasing the exposure time was observed. The estimated LC_{50} values for avermectin B1b found in the present research were 712.5248 mg / Kg, 382.6 mg / Kg and 74.6 mg / Kg after 7, 14 and 28 days of exposure respectively. The higher concentrations of avermectin B1b observed in this study might be due to the reason that only one abamectin component is influencing the target animals. Diao *et al.* (2007) mentioned in their study the values of NOEC and LOEC to be 5.00mg / Kg and >5.00mg / Kg for earthworm survival against abamectin. NOEC and LOEC for mortality of target species in the present study were 0.1mg / Kg and 1.0 mg / Kg respectively. The EC_{50} value estimated after 48h of exposure of abamectin in a research conducted by Nunes and Espindola, (2012) against *Eisenia andei* in soil test was 3.92 mg / kg with NOEC and LOEC values 0.85 mg / kg and 1.75 mg / kg respectively. Diao *et al.* (2007) reported the potential risks of abamectin against earthworms confirming the abamectin to be toxic against them. Gunn and Sadd, (1994) performed a research work on the effect of ivermectin on earthworm survival rate. Theyreported that there was no survival observed at above 20 mg / kg in OECD artificial soil. The LC_{50} value reported by Halley *et al.* (1989) for ivermectin in OECD artificial soil against *E. fetida* was 314 mg / kg. In another study conducted by Wislocki *et al.* (1989), the LC_{50} value for abamectin against earthworms after 28 days of exposure was 28 mg / kg of soil dry weight. On 14^{th} day of exposure, the calculated value of LC_{50} for abamectin against earthworm was 17.1 mg / kg in OECD artificial soil which represents acute toxicity of the abamectin (Sun *et al.* 2005). In a study conducted by Kolar *et al.* (2010), the LC_{50} determined for abamectin against isopod survival was 71 mg / kg of dry soil. In their study the values for NOEC and LOEC were 3 mg / kg and 10 mg / kg respectively.

In case of cockroaches, the LC_{50} value decreased from 3^{rd} to 14^{th} day of oral as well as dermal exposure, as calculated by Probit' analysis. In a previous study conducted by Koehler *et al.* (1991), it is reported that toxicity of abamectin increased as the exposure time is increased because abamectin is a slow reacting toxicant. In their study 31-75% mortality was observed for German Cockroaches after 9 days of exposure (Koehler *et al.* 1991). In present research work, 40-80% and 40-90% mortality observed from day 3 to 14 for oral and dermal toxicity respectively.

Exposure time and concentration of test chemical also affects the biomass significantly. In a study conducted by Diao *et al.* (2007), it was observed that there was a significant reduction in the biomass of earthworms after 14 and 28 days at 5.0 mg / kg abamectin. However after 14 days of 2.5 mg / kg abamectin exposure, the difference between the control and the experimental species was less significant. In the present study the maximum reduction in the biomass of the exposed species was after 14 and 28 days of exposure at highest concentration (1000.0 mg / Kg) of the avermectin B1b. In a study conducted by Kolar *et al.* (2008), it was reported that earthworms showed a dose related biomass reduction. In their study the values of NOEC and LOEC calculated for biomass reduction of earthworms in OECD artificial soil were 9.8 mg / kg and 29 mg / kg respectively. In another experiments performed by Kolar *et al.* (2010), it is reported that difference in biomass between treated and non-treated isopods was less significant when fed with 10, 20, 100 and 300 mg abamectin per kg of dry soil. Jensen *et al.* (2007) reported that EC_{50} value for abamectin was 0.46 mg / kg against biomass of earthworms with 0.25 mg / kg NOEC.

Diao *et al.* (2007) reported that number of cocoon formation is directly influenced by concentration of abamectin. Significant reduction in cocoon formation was observed at 0.25mg / kg of abamectin. In the present study the similar dose response relationship was observed for avermectin B1b for earthworms. Gunn and Sadd,

(1994) reported that at a concentration of 4mg / kg of ivermectin there was a momentous reduction in the cocoon formation. Our study is in-line with previously reported studies and clearly indicate that avermectin B1b is highly effective against nematodes and arthropods.

Acknowledgements: The present research work was conducted in PCSIR Labs Complex, Lahore, and was funded by Higher Education Commission, Pakistan.

REFERENCES

Bartlett, M. D., M. J. I. Briones, R. Neilson, O. Schmidt, D. Spurgeon, and R. E. Creamer (2010). A critical review of current methods in earthworm ecology: from individuals to populations. Eur. J. Soil Biol. 46: 67–73.

Basset, Y. (2001). Invertebrates in the canopy of tropical rain forests. How much do we really know? Plant Ecolo. 153: 87-107.

Bueno, A. F. and S. Freitas (2004). Effect of the insecticides abamectin and lufenuron on eggs and larvae of Chrysoperla externa under laboratory conditions. Bio Control. 49: 277-283.

Burg, R.W., B. M. Miller, E. E. Baker, J. Birnbaum, S. A. Currie, R. Hartman, Y. L. Kong, R.L. Monaghan, G. Olson, and I. Putter (1979). Avermectins, new family of potent anthelmintic agents: producing organisms and fermentation. J. Antimicrob. Agents Chemother. 15**:** 361-367.

Cindy, M.H., E. F. Lee, B. R. Peter, and P. John (2008). Exotic earthworm effects on hardwood forest floor, nutrient availability and native plants: a mesocosm study. Oecologia. 155: 509–518.

De Silva, P. M. C. S. and C. A. M. van Gestel (2009). Comparative sensitivity of *Eisenia andrei* and *Perionyx excavatus* in earthworm avoidance tests using two soil types in the tropics. Chemosphere 77: 1609–1613.

Diao, X., Jensen, J. and A. D. Hansen (2007). Toxicity of anthelmintic abamectin to four species of soil invertebrates. Environ. Pollution 148: 514-519.

Ding, J., C. D. Drewes, and W. H. Hsu (2001). Behavioral effects of ivermectin in a freshwater oligocheate, Lumbriculus variegates. Environ. Toxicol. Chem. 20: 1584–1590.

Erzen, N.K., L. Kolar, V. C. Flajs, J. Kuzner, I. Marc, and M. Pogacnik (2005). Degradation of abamectin and doramectin on sheep grazed pasture. Ecotoxicology 14: 627-635.

Finney, D.J. (1971). Probit analysis, 3rd edn. Cambridge University Press, London

Floate, K.D., K. G. Wardhaugh, A. B. Boxall and T. N. Sherratt (2005). Fecal residues of veterinary parasiticides: non target effects in the pasture environment. Annual Review of Entomology 50: 153-179.

Frelich, L.E., C. M. Hale, S. Scheu, A. R. Holdsworth, L. Heneghan, P. J. Bohlen and P.B. Reich (2006). Earthworm invasion into previously earthworm free temperate and boreal forests. Biol. Invasions. 8: 1235–1245.

Gunn, A. and J.W. Sadd (1994). The effect of ivermectin on the survival, behavior and cocoon production of the earthworm *Eisenia fetida*. Pedobiologia 38: 327-333.

Halley, B. A., T. A. Jacob and A. Y. Lu (1989). The environmental impact of the use of ivermectine. Environmental effects and fate. Chemosphere 18: 1543-1563.

Jensen, J., P. H. Krogh and L.E. Sverdrup (2003). Effects of the antibacterial agents tiamulin, olaquindox and metronidazole on the antihelmintic ivermectin on the soil invertebrate species *Folsomia fimetaria* (Collembola) and *Enchytraeus crypticus* (Enchytraeidae). Chemosphere 50: 437-443.

Jensen, J., X. Diao, and J. J. Scott-Fordsmand (2007). Sub-lethal toxicity of the antiparasitic abamectin on earthworms and the application of neutral red retention time as a biomarker. Chemosphere 68: 744–750

Jouquet, P., T. Plumere, T.D. Thu, C. Rumpel, T. T. Duc and D. Orange (2010). The rehabilitation of tropical soils using compost and vermin compost is affected by the presence of endogeic earthworms. Appl. Soil Ecol. 46: 125–133.

Koehler, G.P., T. H. Atkinson, I. Andrichard and S. Patterson (1991). Toxicity of abamectin to cockroaches (Dictyoptera: Blattellidae, Blattidae). J. Econ. Entoml. 84(6): 1758-1762.

Kolar, L., A. Jemec, A., C. A. M. Van Gestel, V. Janez, R. Hrzenjak N. K. Erzen and P. Zidar (2010). Toxicity of abamectin to the terrestrial isopod *Porcellio scaber* (Isopoda, Crustacea) Ecotoxicology 19: 917–927.

Kolar, L., N. K. E. L. Logerwerf and C.A.M. van Gestel (2008). Toxicity of abamectin and doramectin to soil invertebrates. Environ. Pollution 151: 182-189.

Kolar, L., V. C. Flajs, J. Kuzner, I. Marc, M. Pogacnik, A. Bidovec, C. A. M. Van Gestel, and E.N. Kozuh (2006). Time profile of abamectin and doramectin excretion and degradation in sheep faeces. Environ. Pollution 144: 197-202.

Kovecses, J. and D.J. Marcogliese (2005). Avermectins: Potential Environmental Risks and Impacts on Freshwater Ecosystems in Quebec. Scientific and Technical Report ST-233E. Environment CanadaQuebec Region, Environmental Conservation, St. Lawrence Centre, 72 pp.

Lavelle, P. and A. Spain (2001). Soil Ecology. Kluwer Scientific Publications, Amsterdam

Miyazaki, A., T. Amano, H. Saito and Y. Nakano (2002). Acute toxicity of chlorophenols to earthworms using a simple paper contact method and comparison with toxicities to fresh water organisms. Chemosphere 47: 65–69.

Nahmani, J., M. E. Hodson, and S. Black (2007). Effects of metals on life cycle parameters of the earthworm *Eisenia fetida* exposed to field-contaminated, metal-polluted soils. Environ. Pollut. 149: 44–58.

Nunes, M. E. T. and E. G. Espındola (2012). Sensitivity of *Eisenia Andrei* (Annelida, Oligochaeta) to a commercial formulation of abamectin in avoidance tests with artificial substrate and natural soil under tropical conditions. Ecotoxicology 21: 1063–1071.

Ouellet, G., D. R. Lapen, E. Topp, M. Sawada and M. Edwards (2008). A heuristic model to predict earthworm biomass in agro ecosystems based on selected management and soil properties. Appl. Soil Ecol. 39: 35–45.

Roberts, B. L. and H. W. Dorough (1984). Relative toxicities of chemicals to the earth-worm *Eisenia foetida*. Environ. Toxicol. Chem. 3: 67–78.

Rombke, J., K. A. Krogh, T. Moser, A. Scheffczyk and M. Liebig (2010). Effects of the Veterinary Pharmaceutical Ivermectin on Soil Invertebrates in Laboratory Tests. Arch. Environ. Contam. Toxicol. 58: 332–340.

Scott, J.G. (1991). Toxicity of abamectin and hydramethylation of insecticide-susceptible and resistant strains of German Cockroaches (Dictyoptera: Blattellidae). Agri. Entomol. 8(2): 77-82.

Siddique, S., Q. Syed, A. Adnan., M. Nadeem, I. Muhammad and F.A. Qureshi (2013). Production of Avermectin B1b from *Streptomyces avermitilis* 41445 by Batch Submerged Fermentation. Jundishapur J. Microbiology 6(8): e7198.

Steel, J.W. and K.G. Wardhaugh (2002). Ecological impact of macrocyclic lactones on dung fauna. In: Vercruysse, J., Rew, R.S. (Eds.), Macrocyclic Lactones and Antiparasitic Therapy. CAB International, Wallingford, pp. 141-162.

Strong, C.A., P.G. Koehler and R.S. Patterson (1993). Oral toxicity and repellency of borates to German cockroaches (Dictyoptera: Blattellidae). J Econ Entomol. 86(5):1458-63.

Suarez, V.H. (2002). Helminthic control on grazing ruminants and environmental risks in South America. Veterinary Research 33: 563-573.

Sun, Y., X. Diao, Q. Zhang and J. Shen (2005). Bioaccumulation and elimination of avermectin B1a in the earthworms (*Eisenia fetida*). Chemosphere 60: 699-704.

Suthar, S., S. Singh and S. Dhawan (2008). Earthworms as bioindicator of metals (Zn, Fe, Mn, Cu, Pb and Cd) in soils: Is metal bioaccumulation affected by their ecological category. Ecol. Eng. 32: 99–107.

Thain, J. E., I. M. Davies, G. H. Rae and Y. T. Allen (1997). Acute toxicity of ivermectin to the lugworm, *Arenicola marina*. Aquaculture 159: 47–52.

Tripathi, G., N. Kachhwaha and I. Dabi (2010). Comparative studies on carbofuran- induced changes in some cytoplasmic and mitochondrial enzymes and proteins of epigeic, anecic and endogeic earthworms. Pestic. Biochem. Physiol. 96: 30–35.

Udovic, M and D. Lestan (2010). Eisenia fetida avoidance behavior as a tool for assessing the efficiency of remediation of Pb, Zn and Cd polluted soil. Environ. Pollut. 158: 2766–2772.

van Gestel, C.A.M., M. D. Ortiz, E. Borgman and R.A. Verweij (2011). The bioaccumulation of Molybdenum in the earthworm *Eisenia andrei*: influence of soil properties and ageing. Chemosphere 82: 1614–1619.

Wironen, M. and T.R. Moore (2006). Exotic earthworm invasion increases soil carbon and nitrogen in an old-growth forest in southern Quebec. Can. J. For. Res. 36: 845–854.

Wislocki, P.G., L. S. Grosso and R.A. Dybas (1989). Environmental aspects of abamectin use in crop protection. In: Campbell, W.C. (Ed.), Ivermectin and Abamectin. Springer Verlag, New York, pp. 182-200

Wolstenholme, A. J., A.T. Rogers (2005). Glutamate-gated chloride channels and the mode of action of the avermectin/milbemycin anthelmintics Parasitology 131: Suppl : S85-95.

Yanhua, W., C. Tao, Z. Xueping, Y. Ruixian, C. Liping, W. Changxing and W. Qiang (2011). Comparative acute toxicity of twenty-four insecticides to earthworm, *Eisenia fetida*. Ecotoxicology and Environ. Safety doi:10.1016/j.ecoenv.2011.12.016

Zidar, P., D. S. Drobne, C. A.M. Van Gestel and M. Donker (2004). Food selection as a means of Cu intake reduction in the terrestrial isopod *Porcellio scaber* (Crustacea, Isopoda). Appl. Soil. Ecol. 25: 257–265.