



Some Insights on the Effect of Pesticides on Earthworms

Angshu Dutta^{1*} and Himangshu Dutta²

¹Department of Life Sciences, Dibrugarh University, Dibrugarh, Assam, India

²Department of Ecology and Environmental Science, Assam University, Silchar, Assam, India
himangshu.dibru@gmail.com

Available online at: www.isca.in, www.isca.me

Received 23rd January 2016, revised 28th February 2016, accepted 10th March 2016

Abstract

Agricultural expansion has led to an accelerated use of pesticides. However, apart from target pests, non-target organisms are also exposed to such agrochemicals. Several such non-target organisms help in the proper functioning of the soil ecosystem. Among these, earthworms need a special mention on account of the important ecosystem functions which they provide. At the same time, as earthworms always remain in close contact with the soil, they are also highly exposed to the toxicities of applied chemicals. These chemicals have numerous biochemical, physiological and morphological consequences on these organisms. But during the application of pesticides, their effects on earthworms are completely overlooked. However, if an earthworm population breaks down in a particular soil ecosystem, it is difficult to develop it again. Through the present review the hazardous impact of some commonly used pesticides on earthworms have been study elucidated and documented. It was found that chemical pesticides have several harmful impacts on these organisms.

Keywords: Earthworms, Effects, Pesticides, Soil.

Introduction

There has been a dramatic increment of pesticide application in tropical regions due to the agricultural expansion¹. As a result, soil contamination arising due to the application of agrochemicals is a major issue in tropical ecosystems². For example, Monocrotophos which is a widely used insecticide in the cultivation of vegetables, paddy, potatoes and sugarcane etc.³ is likely to exert result in harmful impact on the invertebrate fauna of the soil. This might result in serious consequences as soil invertebrates, which are crucial for decomposition and cycling of nutrients, play an important role in sustaining soil qualities¹. However, much less attention has been given to study the detrimental effects of these chemicals on such non-specific organisms in tropical regions, although much priority has been given to the same in temperate regions².

Pesticides have been found to induce morphological, behavioural and physiological changes in reproductive, nervous, respiratory and osmoregulatory organs of different animals⁴. Earthworms are vulnerable to such chemicals and therefore act as model organisms in the evaluation of the impact of the pesticides⁵. These are a vital component of soil biomass and act as an important indicator of total soil metabolism as well as soil pollution and toxicity⁶⁻¹¹. In fact, several earthworm species have been used as bio-indicators¹² and hence these are used in the evaluation of chemical environmental pollution¹³. Several experiments such as ring testing and standardization of toxicity test using earthworm as the test organisms¹⁴ have contributed immensely towards toxicology studies. Earthworms have also been used as indicator species for eco-toxicological investigations, risk evaluation and scrutinizing the environment

quality^{15,16} and are key organisms in environmental toxicology¹⁷. However, the earthworm which has an unavoidable beneficial role in maintaining soil health is often neglected during application of these chemicals which could cause ceasing of their activity¹⁸.

Therefore, the present review has been attempted to investigate the unfavorable effects of some of the commercially available pesticides on various earthworm species.

Role of earthworms in enhancing soil fertility and structure

Earthworms are a major constituent of soil invertebrate fauna and improve soil structure by breaking organic matter and releasing plant nutrient^{19,20}. These add significantly towards nutrient cycling as well as in enhancing biological activity of the soil ecosystem²¹. The activities of the earthworms influence the cycling of the soil nutrients via their influence on biological and physical attributes of the soil. But it must be noted that various collateral factors such as the flora and fauna of the soil, nature of the soil and climate dictates the degree of this influence²². Moreover, they maintain soil status through their burrowing and casting activities²³. Through their burrowing activities, the earthworms construct labyrinth of tunnels which enhances the soil's capacity to take up water and hence, the dearth of earthworm population necessities the application of costly materials such as gypsum or calcium to order to increase soil permeability. Besides, the action of the calciferous gland of the earthworms along with the buffering action of carbonic acid, helps in the maintenance of soil pH¹⁸. Thus, it is well

understood that earthworms have an important impact on soil fertility and soil permeability²⁴.

However, what determines the strength of earthworm population as well their diversity and how this diversity influences various soil processes is still an enigma²⁵. It is also a fact that once an earthworm population breaks down it becomes difficult to transfer earthworms into the soil and re-establish their population²⁶.

Some earthworm species like *Eisenia fetida* (Oligochaeta, Lumbricidae) are used for cow dung vermin-composting²⁷. This is because these are easily found, have short life span and wide temperature and moisture tolerance ranges. In addition, these are less sensitive earthworms and can be handled without much difficulty²⁸. As a result, *Eisenia fetida* is extensively used as test organism for ecotoxicological, physiological and genetical research²⁸.

Effects of pesticides on earthworms

Earthworms that remain in direct contact with the ground are at a high risk of absorbing applied agro-chemicals¹⁹. In fact, in addition to ingestion, earthworms could also absorb chemicals from pore water through skin²⁹. Pesticides negatively affect earthworm growth and reproduction (number of cocoons produced, hatchlings arising from the cocoons etc)³⁰. Pesticides also lead to cuticle rupture resulting in the release of coelomic fluid and may cause the swelling of body tissues of the earthworms³¹.

The combination of different pesticides at various concentrations results in neurotoxic consequences³². Increased exposure period to elevated dosage of these chemicals may induce anomalies at the cellular level and result in incessant breakdown of proteins³².

An organophosphate pesticide dimethoate has been found to have toxic effects upon the testicular histomorphology of earthworms³³ as well as their protein content and enzyme activities³⁴. There are also many other reports on the toxic effects of pesticides on earthworms. Hans *et al.* (1993) found that three organochlorine insecticides (aldrin, endosulfan and lindane) have negative effects on the earthworm *Pheretima posthuma* which varied according to the duration of exposure³⁵. Callahan *et al.* (1994) observed the mortality with respect to concentration of four different earthworms species - *Allolobophora tuberculata*, *Eisenia fetida*, *Perionyx excavates* and *Eudrilus eugeniae*³⁶. They tested 62 pesticides and reported that chemical impact on organisms which indicated that all the species had similar tolerance.

Kuo reported that benomyl, hymexazol, metalaxyl, methasulfocarb and carbofuran pesticides have lethal effects on the earthworm *Bimastus parvus* (Eisen)³⁷. They reported that carbofuran and methasulfocarb were more toxic. Lethal effect of

benomyl varied with the time of feeding. Mortality of the earthworms decreased as the time of feeding delayed.

Likewise, Mosleh and coworkers reported the effects on the soluble protein of the LC₂₅ treated earthworm due to the exposure to cypermethrin, chlorfluazuron, profenofos, aldicarb, metalaxyl and atrazine³⁴. Among these chemicals, aldicarb was found to be the most toxic. Spurgeon *et al* have stated that copper, zinc, lead and cadmium have impacts on mortality, growth and cocoon production of *Eisenia fetida*³⁸. For all the metals, mortality was comparatively less affected than cocoon production. Organophosphate pesticides such as diazinon and chlorpyrifos had been previously documented to exert toxic effects on earthworm *Aporrectodea caliginosato*³⁹.

However, chlorpyrifos, carbofuran and mancozeb have been found to be more toxic to *Perionyx excavates* than *Eisenia andrei* under tropical conditions². Carbamates have been reported to have toxic effects on earthworms while organophosphates cause the breakdown of earthworm population⁴⁰. Extensive exposure of earthworms to chlorpyrifos and azinphos has been found to drastically affect on them⁴¹.

Research on earthworm

The innate capability of a toxicant to produce detrimental effect on an organism resulting in the impairment of its normal activity or function is called toxicity and one of the apropos ways to study the toxicity of a substance for the organism is by evaluation of the LC₅₀ value⁴². A useful method to calculate The LC₅₀ is by using 'probit' analysis⁴². A host of research have been conducted to study the toxic impact of different chemicals on earthworm activity⁴³⁻⁴⁸. Majority of the works were carried out to assess the potential risk of organophosphate pesticides like malathion, phorate, monocrotophos, fenitrothion by using earthworm as the test organism^{49,50}.

The toxic effects of imidacloprid, a common neonicotinoid insecticide, on earthworms have also been studied^{51,52} in the tropical agro-ecosystem. In fact, a number of investigations have been undertaken to monitor the effect of some pesticides on earthworms⁵³.

Dimethoate is an extensively used organophosphate insecticide in India^{54,55}. Some of the widely used earthworm species for toxicological studies are - *Eisenia andrei*, *Eisenia fetida*, *Lampito mauritii*, *Eudrilus eugeniae* and *Pontoscolex corethrurus*².

Need for further study on earthworms

Although literature has reported the investigations on the effects various pesticides, there are several which have not been. In this regard an important example is dimethoate-30% EC (Rogor), which is an extensively used systemic organophosphate insecticide with toxicity varying with purity⁵⁶. At this point it

can be reminded that the term organophosphate is used to cover all the toxic compounds which contain a phosphorous atom⁵⁷. Most of the toxic organophosphates are esters of phosphoric, phosphorothioic or phosphonic acids or of their anhydrides, halides or amides⁵⁸. According to Kulkarni *et al*, the organophosphate compounds can be represented with help of the following formula (Figure-1)⁵⁹:

Dimethoate can have numerous toxicological and ecological impacts⁶⁰. It has been found to exert several toxic effects on mammals (FAO) and fishes⁶¹. Dimethoate leads to the decrease of protein content in *Labeo rohita*⁶², *Puntius ticto*⁶³ and *Heteropneustes fossilis*⁶⁴. This is because free amino acids serve as supplementary energy source during stress and hence under such circumstances, protein metabolism is likely to increase⁶². The decline in the amount of protein content is indicative of the probable fact that the pesticide treatment causes the exhaustion of the organism's metabolic reserves resulting in the energy deficiency and in order to compensate this loss the proteins undergo catabolism leading to the production of required amount of energy⁶⁵.

Dimethoate is likely to be toxic to other organophosphates such as malathion⁶⁶ and parathion¹³.

Conclusion

Pesticides have the potential to severely damage beneficial non-targeted soil fauna leading to the breakdown of their population and causing unwanted shifts of the community². Earthworms, which are one of the important soil fauna, are extremely vulnerable to the adverse effects of pesticides and the consequences are evident at the species, population as well as community levels⁵. Such sensitivity of earthworms to pollutants has been well documented and it has been found that physiological, morphological and ecological characters are its important determinants².

However, there are a number of external factors that determine pesticide toxicity; among them are soil properties which determine the availability chemicals to soil invertebrates and in this way determine their toxicity⁶⁷. In addition, higher temperatures elevate microbial activity and degradation of chemical compounds, leading to reduced toxicity².

Subjecting the test organisms to sub-lethal dose of the pesticide can be considered as a pragmatic approach to forecast the potential environmental consequences as in the field the organisms are usually exposed or come in contact with a lower concentration of the pesticides⁶⁸. The harmful impacts of pesticides have also been also documented in case of other invertebrates like bees which are important pollinators⁶⁹.

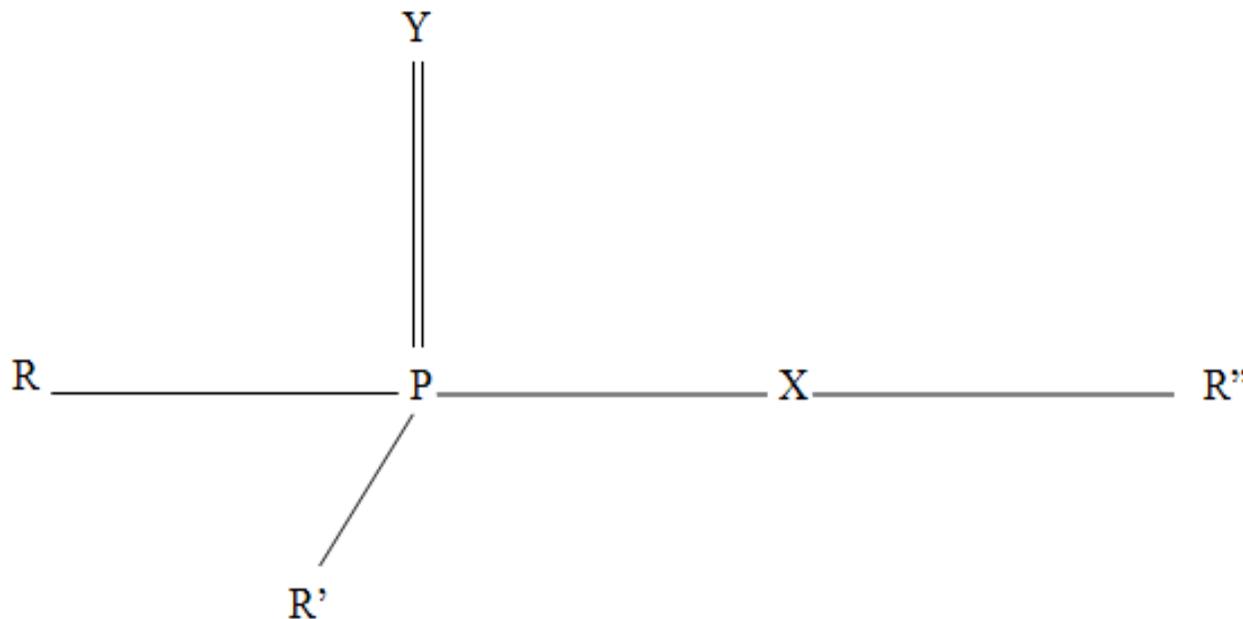


Figure-1

Structure of organophosphate compound (R, R' stands for the short hydrocarbon and oxygen groups respectively; X and Y can be either sulphur or oxygen whereas the group that is metabolized by the contact organism is represented by R''). Phosphorous, which is highly electrophilic, forms the central atom to which all these groups are attached and play a critical role leading to physiological effects.)

References

1. Mangala P., De Silva C.S., Pathiratne A. and Van Gestel C.A.M. (2009). Influence of Temperature and Soil Type on the Toxicity of Three Pesticides to *Eisenia Andrei*. *Chemosphere*, 76, 1410-1415.
2. De silva P.M.C.S. (2009). Pesticide effects on earthworms: A tropical perspective, Ph.D. Thesis. Department of Ecological Science, VU University, Amsterdam, The Netherlands .
3. Arnaud C., Saint-Denis M., Narbonne N.F., Soler P. and Ribera D. (2000). Influences of different standardized test methods on biochemical responses in the earthworm *Eisenia fetida Andrei*. *Soil Biol. Biochem.*, 32, 67-73.
4. Fingerman M. (1984). Pollution our enemy. *Proc. Symp. Physiol. Resp. Anim. Poll.*, I-VI.
5. Edwards C.A. and Bohlen P.J. (1993). The effects of toxic chemicals on earthworms. *Rev. Environ. Contam. Toxicol.*, 125, 23-99.
6. Peakall D.B. (1994). The role of biomarkers in environmental assessment (1) Introduction. *Ecotoxicol.*, 3(3), 157-160.
7. Van Straalen N.M. and Van Gestel C.A.M. (1998). Soil Invertebrates and Micro-Organisms. Blackwell Science, Oxford.
8. Paoletti M.G. (1999). The role of earthworms for assessment of sustainability and as bioindicators. *Agri. Ecosys. Env.*, 74, 137-155.
9. Spurgeon D.J. and Hopkin S.P. (1999). Seasonal variations in the abundance, biomass and biodiversity of earthworm in soil contaminated with metal emissions from a primary smelting works. *J. Appl. Ecol.*, 36, 173-183.
10. Lock K. and Janssen C.R. (2003). Effect of new soil metal immobilizing agents on metal toxicity to terrestrial invertebrates. *Env. Poll.*, 121, 123-127.
11. Brady N.C. and Weil R.R. (2004). Elements of the Nature and Properties of Soil (Second Edition). Pearson Prentice Hall Publ., Upper Saddle River, New Jersey, USA.
12. Scott-Fordsmann J.J. and Weeks J.M. (2000). Biomarkers in earthworms. *Rev. Environ. Contam. Toxicol.*, 165, 117-159.
13. Bustos-Oberg E. and Goicochea R.I. (2002). Pesticide soil contamination mainly affects earthworm male reproductive parameters. *Asian J. Andro.*, 4, 195-199.
14. OECD (1984). Guidelines for testing of chemicals. Test 207: Earthworm Acute Toxicity Test. Organization for Economic Co-operation and Development (OECD), Paris.
15. Curry J.P., Doherty P., Purvis G. and Schmidt O. (2008). Relationship between earthworm populations and management intensity in cattle-grazed pastures in Ireland. *Appl. Soil Ecol.*, 39(1), 58-64.
16. Mahajan S., Kanwar S.S. and Sharma S.P. (2007). Longterm effect of mineral fertilizers and amendements on microbial dynamics in an alfisol of western Himalayas. *Indian J. Microbiol.*, 47(1), 86-89.
17. Spurgeon D.J. and Hopkin S.P. (1993). Extrapolation of the laboratory based OECD earthworm toxicity test to metal-contaminated field sites. Ecotoxicological Group, School of Animal and Microbial Sciences. University of Reading, UK.
18. Lawrence F. and London J.R. (1997). The role of earthworms in healthy soils. *Venaura Farm- producer of Naturally Grown Vegetables*, 21, 40-44.
19. Edwards C.A. and Bohlen P.J. (1996). Biology and ecology of earthworms. Chapman and Hall, New York, USA.
20. Bartlett M.D., Briones M.J.I., Neilson R., Schmidt O., Spurgeon D. and Creamer R.E. (2010). A critical review of current methods in earthworm ecology: from individuals to populations. *European J. Soil Biol.*, 46, 67-73.
21. Parmelee R.W., Bohlen P.J. and Blair J.M. (Eds). (1998). Earthworm Ecology. St Lucie Press, 123-143.
22. Zhang W.X., Chen D.M. and Zhao C.C. (2007). Functions of earthworm in ecosystem. *Biodiver. Sci.*, 15, 142-153.
23. Panda S. and Sahu S.K. (1997). Recovery of respiratory and excretory activity of *Drawida willsi* (Oligochaeta) following application of malathion in soil. *J. Ecobiol.*, 9(2), 97-102.
24. Wang X., Li H.X., Ru F. and Wang D.D. (2004). Effects of earthworms on nitrogen leaching in wheat field agroecosyste. *Acta Pedologica Sinica.*, 41, 987-990.
25. Bohlen P.J., Edwards W.M. and Edwards C.A. (1995). Earthworm community structure and diversity in experimental agricultural watershed in Northeastern Ohio. *J. Plant and Soil*, 170(1), 233-239.
26. Tisdall J.M. and Mckenzie B.M. (1999). Correlation between earthworm and plant growth. *Biol. Fertility Soils*, 30, 1-2.
27. Garg V.K., Chand S., Chhillar A. and Yadav A. (2005). Growth and reproduction of *Eisenia foetida* in various animal wastes during vermicomposting. *Appl. Ecol. Env. Res.*, 3(2), 51-59.
28. Domínguez J. (2004). State of the art and new perspectives on vermicomposting research, In Earthworm Ecology (Second Edition) (Ed. C.A. Edwards). CRC

- Press, Boca Raton, Florida, 401-424.
29. Belfroid A., Meiling J., Sijm D., Hermens J., Seinen W. and Van Gestel K. (1994). Uptake of hydrophobic halogenated aromatic hydrocarbons from food by earthworms (*Eisenia andrei*). *Arch. Environ. Contam. Toxicol.*, 27, 260-265.
 30. Yasmin S. and D'Souza D. (2010). Effects of pesticides on the growth and development of earthworm: A review. *Appl. Env. Soil Sci.*, 20, 1-9.
 31. Solaimalai A., Ramesh R.T. and Baskar M. (2004). Pesticides and environment. *Env. Contam. Bioreclam.*, 7, 345-382.
 32. Schreck E., Geret F., Gontier L. and Treihou M. (2008). Neurotoxic effect and metabolic responses induced by a mixture of six pesticides on the earthworm *Aporrectodea caliginosa nocturna*. *Chemosphere*, 71, 1832-1839.
 33. Lakhani L., Khatri A. and Choudhary P. (2012). Effect of dimethoate on testicular histomorphology of the earthworm *Eudichogaster kinneari* (Stephenson). *Int. Res. J. Biol. Sc.*, 1(4), 77-80.
 34. Mosleh Y.Y. and Paris-Palacios S. (2003). Couderchet M. and Vernet G., Acute and sublethal effects of two insecticides on earthworm (*Lumbricus terrestris* L.) under conditions. *Env. Toxicol.*, 18, 20-26.
 35. Hans R.K., Chan M.A., Farooq M. and Beg M.U. (1993). Glutathione-S-transferase activity in the earthworms, *Pheretima posthuma* exposed to three insecticides. *Soil Biol. Biochem.*, 25(4), 509-511.
 36. Callahan C.A., Shirazi M.A. and Neuhauser E.F. (1994). Comparative toxicity of chemicals to earthworms. *Eisenia fetida*. *Environ. Toxicol.*, 13(2), 291-298.
 37. Kuo T. and Huang Y.K. (1993). Lethal effects of five commonly used pesticides on the earthworm *Bimastus parvus eiser*. *J. Agri. Assoc. China New Ser.*, No. 162, 33-42.
 38. Spurgeon D.J., Hopkin S.P. and Jones D.T. (1994). Effects of cadmium copper, lead and zinc on growth, reproduction and survival of the earthworm *Eisenia foetida* (Savigny). *Environ. Poll.*, 84(2), 123-130.
 39. Booth L.H., Heppelthwaite V.J. and O'Halloran K. (2000). Growth, development and fecundity of the earthworm *Aporrectodea caliginosa* after exposure to two organophosphates. *New Zealand Plant Protection*, 53, 221-225.
 40. Edwards C.A. (1987). The environmental impact of insecticides. In Integrated pest management (Ed. V. Delucchi). International Perspective Parasitology, Geneva, Switzerland, 309-329.
 41. Reinecke S.A. and Reinecke A.J. (2007). The impact of organophosphate pesticides in orchards on earthworms in the Western Cape, South Africa. *Ecotoxicity and Env. Safety*, 66, 244-251.
 42. Finney D.J. (1971). Probit Analysis (Third Edition). Cambridge University Press, London.
 43. Rallmbke J., Jaonsch S., Junker T., Pohl B., Scheffczyk A. and Schallnaay H.J. (2007). The effect of tributyltin-oxide on earthworm, springtails and plants in artificial and natural soils. *Ach. Env. Contam. Toxicol.*, 52(4), 525-534.
 44. Langan A.M. and Shaw E.M. (2006). Responses of the earthworm *Lumbricus terrestris* (L.) to iron phosphate and metaldehyde slug pellet formulations. *Appl. Soil Ecol.*, 34 (2-3), 184-189.
 45. Lydy M.J. and Linck S.L. (2003). Assessing the impact of triazine herbicide on organophosphate insecticide toxicity to the earthworm *Eisenia fetida*. *Arch. Env. Contam. Toxicol.*, 45(3), 343-349.
 46. Kalka J., Miksch K., Grabinska-Sota E. and Zbrog A. (2002). The effects of pyrethroid insecticides on earthworm *Eisenia fetida*. *Fresenius Env. Bull.*, 11(2), 114-117.
 47. Ribera D., Narbonne J.F., Arnaud C. and Saint-Denis M. (2001). Biochemical responses of the earthworm *Eisenia fetida andrei* exposed to contaminated artificial soil, effect of carbaryl. *Soil Biol. Biochem.*, 33(7-8), 1123-1130.
 48. Morowati M. (2000). Histochemical and histopathological study of the intestine of the earthworm (*Pheretima elongate*) exposed to a field dose of the herbicide glyphosate. *The Environmentalist.*, 20(2), 105-111.
 49. Panda S. and Sahu S.K. (1999). Effects of malathion on the growth and reproduction of *Drawida willsi* (Oligochaeta) under laboratory conditions. *Soil Biol. Biochem.*, 31, 363-366.
 50. Patnaik H.K. and Dash M.C. (1990). Toxicity of monocrotophos and fenitrothion to four common Indian earthworm species. *Poll. Residue*, 9, 95-99.
 51. Capowiez Y., Rault M., Costagliolia G. and Mazzia C. (2005). Lethal and sublethal effects of imidacloprid on two earthworm species (*Aporrectodea nocturna* and *Allolobophora icterica*). *Biol. Fertility Soils*. 41(3), 135-143.
 52. Capowiez Y., Bastardie F. and Costagliolia G. (2006). Sublethal effects of Imidacloprid on burrowing behaviour of two earthworm species: Modification of the 3D burrow systems in artificial cores and consequences on gas diffusion in soil. *Soil Biol. Biochem.*, 38(2), 285-293.
 53. Potter D.A. (1990). Toxicity of pesticides to earthworms and effect on thatch degradation in Kentucky Blue grass turf. *J. Economic Entomol.*, 3, 2362-2369.

54. Jemec A., Tisler T., Drobne D., Sepcic K., Fournier D. and Trebse P. (2007). Comparative toxicity of imidacloprid, its commercial liquid formulation and of diazinon to non-target arthropod, the microcrustacean *Daphnia magna*. *Chemosphere*, 68(8), 1408-1418.
55. Panda S. and Sahu S.K. (2004). Recovery of acetylcholine esterase activity of *Drawida willsi* (Oligochaeta) following application of three pesticides to soil. *Chemosphere*, 55(2), 283-290.
56. Clarke E.G.C and Clarke M.L. (1975). Veterinary toxicology (First Edition). Cassell and Collier, Macmillan Publishers, London, UK.
57. O'Brien R.D. (1967). Insectides: Action and metabolism. Academic Press, London and New York.
58. O'Brien R.D. (1960). Toxic Phosphorous Esters: Chemistry, Metabolism and Biological effects. Academic Press, New York, USA.
59. Kulkarni S.G. and Wakale A.S. (2012). Determination of LC50 of the Monocrotophos (Organophosphate) Pesticide on Indian Earthworm, *Lampito mauritii*. *Int. J. of Basic and Appl. Res.*, 2, 110-121.
60. Ravensdown Safety Datasheet. (2012). <http://www.ravensdown.co.nz/SafetyDatasheets/rogorsds.pdf>. Accessed on 02/06/2015.
61. Bantu N. and Vakita R. (2013). Effect of Dimethoate on mortality and Biochemical changes of Freshwater fish *Labeo rohita* (Hamilton). *J. Biol. Today's World*, 2(10), 456-470.
62. Nagaraju B. and Rathnamma V. (2013). Effect of dimethoate on mortality and biochemical changes of freshwater fish *Labeo rohita* (Hamilton). *J. Biol. Today's World*, 2(10), 456-470.
63. Ganeshwade R.M. (2012). Biochemical changes induced by dimethoate (Rogor 30% EC) in the gills of fresh water fish *Puntius ticto* (Hamilton). *J. Ecol. Nat. Env.*, 4(7), 181-185.
64. Borah S. and Yadav R.N.S. (1995). Alteration in the protein, free amino acid, nucleic acid and carbohydrate content of muscle and gills in rogor exposed freshwater fish *Heteropneustes fossilis*. *Res. Pol.*, 14(1), 99-103.
65. Orr G.L. and Doner R.G.H. (1982). Effect of lindane (Thexachlorocyclohexane) on carbohydrate and lipid reserve in the American Cockroach, *Periplaneta americana* L. *Pesticide Biochem. Physiol.*, 17, 89-95.
66. Pal A. and Patidar P. (2014). Effect of insecticide malathion on weight of *Eisenia foetida* earthworm. *AISECT Univ. J.*, 3(5), 1-3.
67. Van Gestel C.A.M. and Ma W.C. (1988). Toxicity and bioaccumulation of chlorophenols in earthworms, in relation to bioavailability in soil. *Ecotoxicol. Environ. Safety*, 15, 289-297.
68. Römbke J. (2007). Garcia M.V. and Scheffczyk A., Effects of the fungicide benomyl on earthworms in laboratory tests under tropical and temperate conditions. *Arch. Env. Contam. Toxicol.*, 53(4), 590-598.
69. Hackenberg D. (2007). Letter from David Hackenberg to American growers. Plattform Imkerinnen, Austria.