

## REVIEW ARTICLE

# World of earthworms with pesticides and insecticides

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## ABSTRACT

Earthworms are important organisms in soil communities and are known for sustaining the life of the soil. They are used as a model organism in environmental risk assessment of chemicals and soil toxicology. Soil provides physical and nutritive support to agriculture system by regulating biogeochemical cycles, nutrient cycle, waste degradation, organic matter degradation etc. The biggest threat to soil health are pesticides and synthetic chemicals including fertilizers. Earthworms are most severely hit by these xenobiotic compounds leading to a sizeable reduction of their population and adversely affecting soil fertility. Earthworms are incredible soil organisms playing a crucial role in maintaining soil health. Pesticides used in crop management are known to be most over-purchased and irrationally used soil toxicants, simultaneously, used insecticides contribute to a quantum of damage to earthworms and other non-target organisms. LC<sub>50</sub> and LD<sub>50</sub> studies revealed that earthworms are highly susceptible to insecticides causing immobility, rigidity and also show a significant effect on biomass reduction, growth and reproduction by disrupting various physiological activities leading to loss of earthworm population and soil biodiversity.

**KEY WORDS:** earthworms; insecticide; non-target organism; soil macrofauna; xenobiotics

## ABBREVIATIONS:

**LC:** Lethal Concentration; **LD:** Lethal Dose; **2,4-D:** 2,4-Dichlorophenoxyacetic acid; **2,4,5-T:** 2,4,5-Trichlorophenoxyacetic acid; **IRAC:** Insecticide Resistance Action Committee; **PAN:** Pesticide Action Network; **CCOHS:** Canadian center for occupational health and safety; **EPA:** Environment Protection Agency; **DDT:** Dichloro Diphenyl Trichloroethane; **B.C.:** Before Christ; **AChE:** Acetylcholineesterase; **nAChR:** Nicotinic Acetylcholine Receptor; **GABA:** gamma-Aminobutyric acid; **USD:** United States Dollar; **FICCI:** Federation of Indian chambers of commerce and Industry; **IOBC:** International Organization for Biological Control; **OECD:** Organization for Economic Cooperation and Development; **ISO:** International Standards Organizations; **GST:** Glutathione-S-transferase; **IPM:** Integrated Pest Management; **IGR:** Insect Growth Regulator

## Introduction

Agricultural expansion and indiscriminate use of pesticides often lead to affect soil ecosystem causing heavy population damage, toxicity and soil pollution (Hole *et al.*, 2005; Mangala *et al.*, 2009). An estimate has been made globally that \$38 billion are spent on pesticides each year (Pan-Germany, 2012). The pesticides applied to the agricultural field should only be toxic to the target organisms, biodegradable and eco-friendly to some extent (Rosell *et al.*, 2008). But unfortunately most of the pesticides are non-specific and kill organisms that are harmless and very useful to the various ecosystems. This concern got attention just after the publication of Silent Spring by Rachel Carson in 1962, which brought environmental issues to concern to the general public. Along the developmental scale, the advance farming practices caused bioaccumulation in humans as well as in many other animals.

The pesticides used in agriculture land cause morphological, behavioral and physiological changes in reproductive, nervous, respiratory and osmoregulatory organs of many soil organisms and contaminate the soil which exerts a harmful impact on various invertebrates (Fingerman, 1984; Mangala *et al.*, 2009., De Silva P.M.C.S. 2009). Depending upon the chemical nature of pesticides and soil properties organs undergo a series of chemical pathways, transport, adsorption and desorption processes (Thapar *et al.*, 2015, Baishya, 2015). Among

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the different classes of pesticides, insecticides are found to be most lethal toxic class of pesticides and pose risk to non-target organisms (Aktar *et al.*, 2009, Mahmood, 2016) The insecticide residues have been reported from agriculture systems along with many other ecotypes such as cropping fields, estuaries, oceans and even in the many urban settlements (Sánchez-Bayo, 2011; Guruge & Tanabe, 2001).

There are more than 8300 species in Oligochaetes, out of which more than half are terrestrial earthworms (Reynolds & Wetzel, 2004). The earthworm diversity of India represent 11.1% out of total earthworm diversity in the world. There are more than 505 species and sub-species of earthworms belonging to 67 genera and 10 families (Julka, 2001; Kathireswari, 2016). Earthworms are the supreme component of soil macrofauna and are the most important soil invertebrates responsible for developing and maintaining the nutritive value of soil by converting biodegradable material and organic waste into nutrient-rich vermicast (Kaushal *et al.*, 1995).

Vermicast obtained by modulation of organic waste through earthworm gut is different from its parental waste material and popularly known as black gold (Lim *et al.*, 2015b; Patangray, 2014). Earthworms are acknowledged as ‘ecosystem engineers’ as they extensively influence physical, chemical and biological properties of soil (Pelosi *et al.*, 2014). Earthworms boost soil physical properties such as hydraulic conductivity, porosity, bulk density, infiltrability, aggregate stability etc. (Devkota *et al.*, 2014). Earthworms improve nutrient availability by ingesting organic residues of different C:N ratios (Patnaik & Dash, 1990). Activities of earthworms also help in enhancing beneficial soil microbes. The gut mucus secretion and excretion from earthworm are known to enhance the activity of microorganisms (Bhaduria & Saxena 2010). The incredible services provided by the earthworms to the ecosystem are somehow at risk and recent research findings are now mainly focused on understanding earthworms and their responses to different pesticides.

## World of pesticides

Historians have traced the use of pesticides to the time of Homer around 1000 B.C. but the earliest records of insecticides are associated with the burning of brimstone (Sulfur) as a fumigant. The insecticide selection was limited during the onset of World War II and by the end of it, it got a new concept of insect control with the modern era of chemicals. The first synthetic organic insecticide introduced was DDT. Traditionally, insecticides are chemical or biological agents meant for the control the insects. The control may be by killing of the insects or by preventing them from engaging in their destructive activities. Insecticides may be natural or manmade and applied to target pests by applying with various delivery systems such as spray, baits, slow-release, diffusion etc. (Ware & Whitacre, 2004).

### Classification of pesticides

Pesticides are classified as insecticides, fungicides, herbicides, rodenticides, nematicides, molluscicides and plant growth regulators. Each group is specifically designed to target pests, but they put undesired toxic effects on non-target organisms. (Cortet *et al.*, 2002; Jänsch *et al.*, 2005; Lo, 2010; Zhang *et al.*, 2010; Yasmin & D’Souza, 2010; Wang *et al.*, 2012a; Milanovic *et al.*, 2014).

The major classes of pesticides are summarized in Table 1. Among the different classes of pesticides, insecticides are known as one of the major class that contributes greatly to pest control and are further divided into different groups. The insecticide groups are classified on the basis of their chemical nature as per Insecticide Resistance Action Committee IRAC 2016 (Table 2).

### Toxicity and lethality of pesticides

The effect of toxic chemicals in a biological system is dose-related. The LC<sub>50</sub> (Fifty percent lethal concentration) is the amount of pesticide dispersed in the air and the value is measured in milligrams per liter. The lower the LC<sub>50</sub> value, the more lethal is the pesticide. Whereas LD<sub>50</sub>

**Table 1.** Major classes of pesticides\*.

Types of Pesticides	Use and Action	Examples
Insecticides	A substance used to control or eliminate or to prevent the attack of the insects that destroys/kill/mitigate plant/ animal.	DDT, Methyl Parathion, Phorate, Chloropyrifos, Imidacloprid, Cypermethrin, Dimethoate
Herbicides	Substances which are used to control the noxious weed and other vegetation that is growing with the desired species causing poor plant growth.	Acetochlor, Butachlor, Terbis, Glyphosate, 2,4-D, and 2,4,5-T.
Fungicides	Substances used to destroy or inhibit the growth of fungi/diseases that infect plants/animal.	Carbendazim, Ampropylfos, Carboxin
Rodenticides	Chemicals used to kill rodents i.e. mice, rat etc.	Warfarin, Arsenous oxide
Nematicides	Substances used to repel or inhibit the nematodes damaging various crops.	Aldicarb, Carbofuran
Molluscicides	Substances used to inhibit the growth and kills snails and slugs and small black sans-culottes.	Gardene, Fentin, Copper sulfate.
Plant growth regulators	A substance that causes the retardation or accelerates the rate of growth or rate of maturation.	Acibenzolar, Probenazole

\*As per Pesticide Action Network 2010 (PAN 2010)

(Fifty percent lethal dose) is calculated under controlled laboratory conditions by administration of the specific dose within a particular time to estimate the toxicity of the pesticides to an organisms (Table 3 and Table 4). The LD<sub>50</sub> values are expressed as milligram per kilogram of body weight (Canadian center for occupational health and safety 2018).

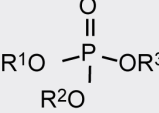
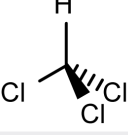
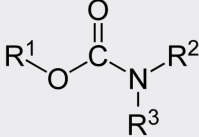
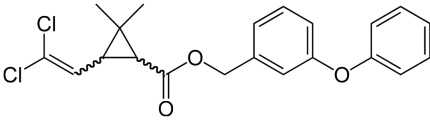
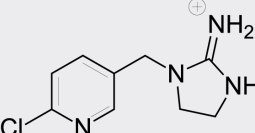
### Production and consumption of pesticides worldwide and Indian scenario

The rise in population has increased the demand for agricultural products and to meet the demand the agriculture practices are commercialized into agribusiness. This practice facilitated the growth of crop protection by formulating agrochemicals on a large scale. The pesticide

market scenario seems to be export-oriented rather than import-oriented (Table 5). The pesticide market of India is expected to grow by 12% to 13% per annum to reach \$6.8 billion by 2017 and export demand by 15% to 16% (Surana *et al.*, 2012). In the year 2018, as per India pesticide industry analysis, the CAGR (compound annual growth rate) observed 14.7% rendering the predicted size of the market at Rs. 2, 29, 800 million whereas on other side the global insecticide market is valued at USD 15.30 billion in 2016 and is likely to reach USD 20.82 Billion by 2022, at a CAGR of 5.27 from 2016 to 2022 respectively (Agro pages 2015).

The Indian pesticide industry is the biggest in Asia and the 12<sup>th</sup> in the world and ranks fourth among global suppliers and it is expected to increase its growth till 2026.

**Table 2.** Classification of insecticides based on their chemical nature (IRAC 2016)\*

Main Groups	Action	Basic Structure	Examples
Organophosphates	Inhibit AChE in nervous system of target organisms		Chlorpyrifos, Dichlorovos, Triazophos, Profenofos, Parathion, Phorate, Diazinon
Organochlorines	Binds at GABA site Inhibit chloride flow in the nervous system of target organisms		Chlordane, Endosulfan
Carbamates	Inhibit AChE in nervous system of target organisms		Aldicarb, Carbaryl, Carbofuran, Isoprocarb
Pyrethroids	Acts on Nervous system which cause changes in nerve membrane permeability to sodium and potassium ions		Acrinathrin, Allethrin, Bioallethrin, Cycloprothrin, beta-Cyfluthrin, Cyhalothrin, lambda-Cyhalothrin, gamma-Cyhalothrin, Cypermethrin, alpha-Cypermethrin, beta-Cypermethrin, theta cypermethrin, zeta-Cypermethrin, Pyrethrins (pyrethrum)
Neonicotinoids	Acts as an agonist of acetylcholine and is therefore effective on many insects		Acetamiprid, Clothianidin, Dinotefuran, Imidacloprid, Nitenpyram, Thiacloprid, Thiamethoxam.

\* As per Insecticide Resistance Action Committee

**Table 3.** Toxicity range of pesticides (CCOHS 2018).

S.No.	Category	LD <sub>50</sub> oral mg/kg (ppm)	Example
1	Extremely toxic	1 mg/kg(ppm) or less	Parathion, aldicarb
2	Highly toxic	1–50 mg/kg(ppm)	Endrin
3	Moderately toxic	50–500 mg/kg(ppm)	DDT, Carbofuran
4	Slightly toxic	500–1000 mg/kg(ppm)	Malathion
5	Non-toxic (practically)	1–5 gm/kg	–

**Table 4.** Acute toxicity range of pesticides according to the Environment Protection Agency (2009).

Class	Category	Rat as an animal model		
		Oral LD <sub>50</sub> (mg/kg)	Dermal LD <sub>50</sub> (mg/kg)	Inhalation LC <sub>50</sub> (mg/l)
I	Danger	<50	<200	<0.2
II	Warning	50–500	200–2,000	0.2–2.0
III	Caution	500–5000	2,000–20,000	2.0–20
IV	Caution (Optional)	>5,000	>20,000	>20

The pesticide market is likely to display a CAGR of 7.04% in value terms by the year 2026 (Agro pages 2015). Among different classes of pesticides (Figure 1 and Figure 2) the insecticides dominate the other classes of pesticides and accounts for 60% of total market value and are used in major crops like rice and cotton, whereas herbicides and fungicides account for 16% and 18% respectively (FICCI 2015). Globally the consumption of herbicide is found to be highest followed by insecticides, fungicides and other pesticides (Arnab *et al.*, 2014). Consumption of agrochemicals in India is one of the lowest in the world with per hectare consumption of just 0.6kg/ha compared to US (4.5kg/ha) and Japan (11 kg/ha) (FICCI, 2014). This practice of pesticide usage in India needs to focus on high yield of bio-pesticides to promote eco-friendly and sustainable methods of agriculture.

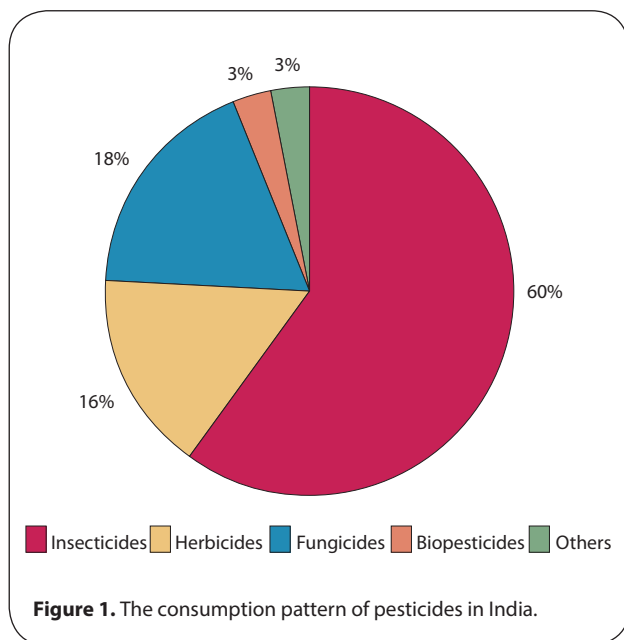


Figure 1. The consumption pattern of pesticides in India.

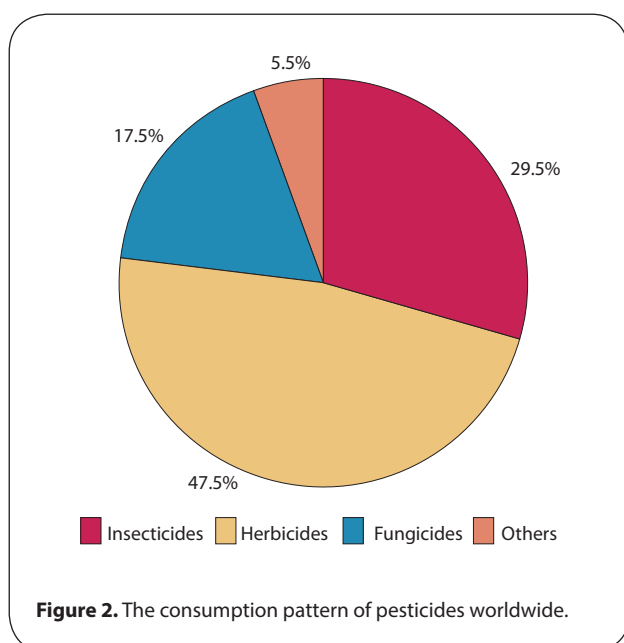


Figure 2. The consumption pattern of pesticides worldwide.

Globally, the pesticides cover only 25% of the cultivated land area and consumption of pesticide worldwide is 2 million tons per year including India while comparing with Korea and Japan where it is 6.6 and 12.0 kg/ha respectively whereas Indian consumption is 0.5 kg/ha.

### Pesticides and soil environment

Soil has the center position for the existence of organisms and ensures their survival, the term soil health and soil environment are used to describe the soil property which holds soil physical, chemical, biological characteristics, those maintain productivity and environment quality which promote the health of plants and animals (Doran.,1994). Soil is a mandatory component for terrestrial environment and is acknowledged as “Biological engine of the earth” (Ritz *et al.*, 2004). Before the era of industrial revolution, i.e. early to mid-1900’s, farming practices were environment-friendly and the connection between agriculture and ecology was very strong. Immediately after this, the ecology and farming linkage was ignored resulting in high productivity at the cost of the environmental quality. Therefore the agro-ecosystem safety becomes a daunting challenge and is adversely affected the soil health.

Use of pesticides has become an integral part of our modern life in order to meet the demand of a growing population which is expected to be 10 billion by 2050 (Saravi & Shokrzadeh, 2011). As per an estimate of the last decade nearly \$38 billion was spent on pesticides globally (Pan-Germany, 2012). The major fraction of pesticides accumulated in the soil and further repeated use of pesticides may cause lethal effects. The accumulation of pesticides in organo-mineral components of complex structures greatly influence the processes like mobilization, immobilization, bioavailability and transport (Gevao *et al.*, 2003; Piccolo *et al.*, 1998). The degraded pesticides alter microbial diversity, biochemical reactions and enzymatic activity (Hussain *et al.*, 2009; Munoz-Leoz *et al.*, 2011). The enzymatic pool of soil comprises of free enzymes, immobilized extracellular enzymes and the enzymes secreted by the microorganism well known

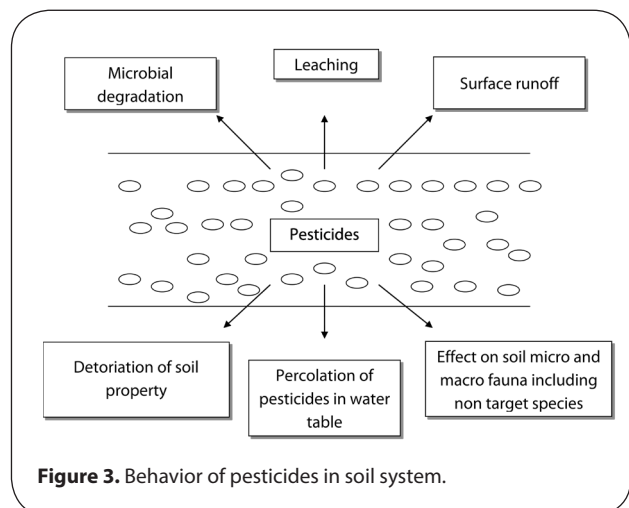


Figure 3. Behavior of pesticides in soil system.



**Table 5.** Import/Export of pesticides in India in recent years (metric tons of active ingredients), according to the Directorate General of Commercial Intelligence and Statistics, Kolkata, WB, Ministry of Commerce (DGCI&S, 2010–2017).

Import/Export	Category	Country	2010–11	2011–12	2012–13	2013–14	2014–15	2015–16	2016–17
Import	Pesticides	India	53996	58647	65018	77375	95361	71029	100238
Export	Pesticides	India	173171	207948	228790	252747	285209	307368	379852

\*Q.T in Metric Tonnes (Technical Grade)

as bioindicators of soil health (Mayanglambam *et al.*, 2005; Hussain *et al.*, 2009). The change in enzymatic activity demonstrates the effect of pesticides on soil biological functions (Garcia *et al.*, 1997; Romero *et al.*, 2010). Pesticides channel themselves through various biophysical pathways in soil ecosystems (Figure 3).

Animals thriving in soil are always under the threat of various chemicals used in agricultural practices, more specifically the pesticides. It is well established that these xenobiotic products are usually difficult to degrade by soil microbes therefore there is always a chance of their entry to various food chains and food webs resulting bioaccumulation and bio-concentration (Maurya & Malik 2016; Dureja & Tanwar, 2012; Edward & Bolen, 1992; Paoletti, 1999). Earthworms bio-accumulate organic pollutant (Jager *et al.*, 2005), heavy metals (Nahmani *et al.*, 2007) and nanoparticles (Canesi & Prochazkova, 2014) through skin and via soil ingestion. The effect of these pesticides applied to soil has effect on earthworm mortality (Roberts & Dorough, 1984; Panda S & Sahu, 2002), reproduction (Senapati *et al.*, 1991; Schaefer 2004), metabolism (Brown *et al.*, 2004) and also enhance the mechanism of bio-amplification (Stephenson *et al.*, 1997; Johnson *et al.*, 1999). Earthworms experience inadvertent toxicity from terrestrially applied pesticides (Edward & Bolen, 1992) and this uptake of chemical increases bio-concentration of pesticides in earthworms.

Therefore the knowledge of the toxico-kinetics of terrestrially applied pesticides in earthworms is necessary to predict the risks of bio-concentration and bio-accumulation (Van Gestel & Weeks, 2004) on earthworm populations and ecological communities. The bio-accumulation of insecticides in earthworms may not lead to a significant effect on the animal to that extent but may produce serious damage to higher trophic level, but with long-term exposure to these pesticides, earthworms get acclimatized and accumulated (Huang & Iskandar, 1999). The increase in the concentration of pesticides and their non-biodegradable nature make them persist in the tissue of the organism at each successive level of food chain through the process of bio-amplification which cause greater harm to those of higher trophic level compared to those of lower levels. Several studies have been undertaken and demonstrated that at each trophic level the lethality of these pesticide increases (Gill & Garg, 2014).

#### Pesticide toxicity and non-target organism

The effect of pesticides on non-target organisms has been a matter of debate for researchers worldwide. There are many reports on the non-target killing of various species

(Ware, 1980; Aktar *et al.*, 2009; Datta *et al.*, 2016; Dutta & Dutta, 2016; Stanley *et al.*, 2016). Pesticides show the extreme effect on the aquatic ecosystem, animal and plant biodiversity and terrestrial food webs. It is estimated that less than 0.1% of pesticides applied to crop reach to the target pest (Pimental, 1995) and more than 99% of applied pesticide have the potential to impact non-target organisms and it percolates deep into the soil ecosystems including the water-table.

In India, 76% of the pesticides used are insecticides whereas globally the insecticide consumption is 44% (Mathur, 1999). The insecticidal effects on non-target species are categorized as per Nasreen *et al.* (2000) harmless (<50% mortality), slightly harmful (50–79% mortality), moderately harmful (80–89% mortality) and harmful (>90% mortality) when tested as per the field recommended dose. The categorization standards are used by the International Organization for Biological Control, West Palaearctic Regional Section (IOBC/WPRS) working group, to assess the insecticidal effects on non-target organisms (Hassan, 1989). The insecticides also act as a potential neurotoxicant on non-target species as it inhibits the essential enzyme, acetylcholinesterase (AChE) in the nervous system of insects and other animal species (Gambi *et al.*, 2007; Caselli *et al.*, 2006). There are reports that toxicity of chemical pesticides used not only affects the target pests but also other species in different degrees (Sanchez-Bayo, 2012). Such as, natural insect enemies e.g., parasitoids and predators are most susceptible to insecticides and are severely affected (Aveling, 1981; Vickerman, 1988). Along with natural enemies, the population of soil arthropods is also drastically disturbed because of indiscriminate pesticide application in agricultural systems. Soil invertebrates are essential for the maintenance of soil structure, transformation, nutrient dynamics and mineralization of organic matter severally affecting the food chain and food webs.

In some cases, the concentrations of pesticides residue have been shown to be sufficiently high to affect many non-target species, including very important soil macrofauna, such as earthworms which are known to deliver ecosystem good and services (Frampton *et al.*, 2006; Daam *et al.*, 2011; Bertrand *et al.*, 2015). Insecticides alter the eco-physiology of the earthworms (Liang *et al.*, 2007) and there are studies on toxicological effect of carbaryl in different earthworm species such as *Eisenia Andrei*, *P. Excavatus*, *Pheretima Posthuma* and *Metaphire Posthuma* (Lima *et al.*, 2015; Saxena *et al.*, 2014). Few studies have also shown the toxic effect of Imidacloprid, a common neonicotinoid insecticide, on earthworms

(Capowiez *et al.*, 2005, 2006). Majority of work has been carried out on the potential risk of organophosphorus pesticides like fenitrothion, malathion, monocrotophos, phorate in tropical agro-ecosystem using earthworm as test organism (Panda & Sahu, 1999, 2004; Patnaik & Dash, 1990). Much more investigations are needed to study various insecticides and their level of toxicity to non-target soil macro-fauna including various earthworm species.

## World of Earthworms

The earthworms thrive almost all soil types and are known as the indicator of soil health and toxicity including various soil pollutants and pesticides. Lee (1985) categorized earthworms based on their feeding habit as detritivores (feed near the surface on decomposing litter and on dead roots) and geophagous (remain on the subsurface which consumes large quantities of soil). According to Lavelle (1983), geophagous earthworms are further categorized into polyhumic (feed on topsoil and occupy different soil strata), oligohumic (feed on the soil of low organic matter) and mesohumic (feed on humus and soil) and are abundantly found in the tropical regions.

Edward & Bohlen (1992) reported that earthworms are highly susceptible to pesticides such as insecticides, therefore they are considered as a model organism to evaluate the effects of insecticides. There are certain pesticide families that are considered as harmful to earthworms i.e. neonicotinoids, strobilurins, sulfonylureas, triazoles, carbamates and organophosphates (Pelosi *et al.*, 2014). The pesticides affect mortality of earthworms by directly distressing them or by altering their physiology (Sabra & Mehana, 2015). Pesticides have a negative effect on the survival and reproduction of earthworms especially at higher concentration (>25mg/kg). Possible effects of pesticides and insecticides on earthworms in the soil are also depended on earthworm species, type of contaminant and its concentration, soil characteristics etc. (Roriguez-Campos *et al.*, 2014).

The organization for economic cooperation and development (OECD) proposed *Eisenia Fetida* (*Oligochaete*) as a reference earthworm species for toxicity testing because it can easily be cultivated in the laboratory, mature in few weeks and has a high reproductive rate (OECD, 1984, 2004, 2015; ISO, 1993). The different insecticides classes had different toxic effects on *Eisenia Fetida*. Earthworm growth, reproduction (cocoon production, number of hatchlings per cocoon and incubation period) is also influenced by use of pesticide in a dose-dependent manner (Yasmin & D'Souza, 2010).

### Earthworms Morpho groups and their exposure to pesticides

Earthworms are classified into four ecological groups, each group is described by different traits in the soil system (Bouché, 1977; Edwards & Bohlen, 1996) including their exposure to various types of pesticides. **Epigeic** worms are represented by *Lumbricus rubellus*, *Dendrobaena*

*octedra*, *Lumbricus castaneus* and usually found in upper 10–15 cm soil layer and feed on decaying organic matter present in the litter. The species that belong to this group are highly exposed to pesticides while ingesting litter. **Endogeic** worms are represented by *Aporrectodea caliginosa*, *Allolobophora chlorotica* or *Allolobophora icterica* and are of a bigger size ranging from 1 to 20 cm. They feed upon the organic matter which is incorporated and mixed with minerals in the soil where the pesticides have already reached and mixed with soil. **Anecic** worms include *Lumbricus terrestris*, *Aporrectodea longa* and are usually bigger and pigmented. They reflect strong muscles with great burrowing activity and some species reaches to the giant size such as 10 to 110 cm. They feed upon the surface litter mainly during the night and create long sub-vertical burrows (1 to 6 m) and thus ingest more amount of soil and get exposed to pesticides by ingesting contaminated soil. **Compost** worm is represented by *Eisenia fetida* and *Dendrobaena veneta* commonly used in vermicomposting practices. Compost worms are bright red in color and stripy and are commonly called 'tiger worms'. These worms are usually kept in controlled soil pits therefore are less exposed to soil toxicants.

### Effect of insecticides on earthworms

Solaimalai *et al.* (2004) investigated effect of various pesticides and their sub-lethal effect on earthworms and demonstrated that the sub-lethal effects cause rupturing of cuticle, oozing out of coelomic fluid, swelling, paling of body and softening of body tissues. Other studies include the cellular autolysis (Luo *et al.*, 1999), damage to male reproductive system (Sorour & Larnik, 2001), swelling (Bharathi & Subbarao, 1984) and coiling of tail (Espinoza-Navarro & Bustos-Obregon, 2004). The higher and the lower dose of insecticides cause physiological damage (cellular dysfunction and protein catabolism) to earthworms (Schreck *et al.*, 2008).

Temperature also plays an important role in degree of pesticide toxicity. Bindsbol *et al.* (2009) investigated effects of freezing temperatures on toxicities of abamectin and carbendazim. De Silva *et al.* (2009) investigated influence of temperature and soil type on the toxicities of chlorpyrifos and carbofuran. Lima *et al.* (2015) investigated effects of carbaryl under low and high temperatures and Garcia *et al.* (2008) assessed effects of three pesticides on the avoidance behavior under temperate and tropical conditions. These investigations showed that change in temperature may influence the pesticide toxicity, but the results obtained from these studies were not definite and substantiated by any other studies.

There are many studies on neurotoxicity caused by various insecticides namely neonicotinoid imidacloprid, oxadiazine indoxacarb, pyrethroids alpha-cypermethrin and lambda-cyhalothrin and the combination of organophosphate chlorpyrifos and pyrethroid cypermethrin. All these insecticides primarily affect nervous system – neonicotinoids interfere with the transmission of stimuli in the nervous system causing irreversible blockage of acetylcholine receptors, oxadiazines act as voltage-gated

sodium channel blockers, pyrethroids cause excitation of the sodium and potassium channels of neurons and the delay of closing of the channels during the phase of depolarization and organophosphates inhibit the action of enzyme acetylcholinesterase (AChE) leading to accumulation of acetylcholine, excessive stimulation of the cholinergic receptors and disruption of neural activity (Stenersen 2004; Casida, 2009; Ribera *et al.*, 2001; Gracia *et al.*, 2011; Nasr & Badawy, 2015). Jeyanthi *et al.* (2016) reported that Carbaryl at higher concentration (50 kg/ha) decreases protein content and antioxidant enzymes glutathione-S-transferase (GST). The antibiotics, carbamates and organophosphates induced intermediate toxicity response to earthworms. Wang *et al.*, (2012) reported that the neonicotinoids are the most toxic to *Eisenia Foetida* among the six chemical classes followed by pyrethroids, while IGRs exhibited the lowest toxicity. Organophosphates are not very toxic to earthworms. Considering the high efficacy of neonicotinoids against target organisms, environmental managers should carefully evaluate the use of them in integrated pest management (IPM) programs to avoid serious damage to earthworms.

#### Impact of insecticides on earthworm growth and reproduction

Various reproductive parameters such as maturation, cocoon production, viability, hatching and sperms production were studied with reference to the genotoxicity when exposed to different types of insecticides and other chemical classes (Espinoza-Navaroo & Bustos, 2004; Govindarajan & Prabakaran, 2014). Pawar & Ahmad (2013) reported that the effect of Chlorpyrifos which is an organophosphate insecticide with the exposure period of 7, 14, 21, 28, and 35 days, the dose concentration of 0.1 and 0.2 showed less effect on growth with the exposure period of 7 and 14 days, but effected earthworms growth when exposed more than 14 days.

Booth & O'Halloran (2001) found significant reduction in growth of *A. Caliginosa* by exposure to two organophosphates, diazinon and chlorpyrifos, at 60 and 28 kg/ha dose. Rajshree *et al.* (2014) also found that Methyl parathion and phorate are very toxic to earthworms and showed progressive symptoms of toxicity such as coiling, curling and excessive mucous secretion with sluggish movements, swelling of the clitellum, degenerative changes in nervous system and loss of pigmentation which is elicited by organophosphorus insecticide.

Malathion, the organophosphate, showed a significant reduction in body weight and negative impact on the male reproductive organs that alter the cell proliferation and affect the DNA structure of spermatogonia of earthworms (Espinoza-Navarro, 2004). Sperm count is also a sensitive marker (Mosleh *et al.*, 2003; Venter & Reinecke 1985). Malathion could affect the sperm count, but in addition, its metabolites could affect the sperm quality (Espinoza-Navarro, 2004). Mosleh *et al.* (2003) assumed that the weight loss may indicate a reduced food intake, by which earthworms regulate intake of pesticides and leads to growth inhibition.

Mosleh *et al.* (2003) investigated that the toxicity of aldicarb, cypermethrin, profenofos, chlorfluzuron, atrazine, endosulfan and metalaxyl in the earthworms *Aporrectodea caliginosa* and *Lumbricus terrestris* causes a reduction in growth rate. Zhou *et al.* (2007) assessed and found that chlorpyrifos had an adverse effect on growth in earthworm exposed to 5 kg/ha chlorpyrifos after eight weeks. Some studies have shown that the growth of earthworms appeared to be more severely affected at juvenile stage than the adult stage.

Chlorpyrifos exposure had a significant effect on reproduction in earthworm as it shows the effect on fecundity when exposed to 5 kg/ha after eight weeks (Zohu *et al.*, 2006) According to Zohu *et al.*, 2008 reproduction of earthworm appeared to be more severely affected by cypermethrin at juvenile stage than at adult stage. Application of 20 kg/ha cypermethrin caused significant toxic effects in the reproduction of worms. Apart from the above mentioned facts there are many more effects and responses that have been studied by various researchers (Table 6).

#### Effect of insecticides on earthworm gut bacteria and cast production

In soil, earthworms explicate soil property and regulate biochemistry of terrestrial soil. The cast of earthworms contribute significantly to cyclic processes carried out in soil ecosystem by supplying nutrient to the plant roots and maintain pedological characteristics of the soil. The earthworms are voracious feeders and the nutrient-rich organic matter along with the soil flows through earthworms gut. The gut of earthworms is a straight tube bioreactor and maintains stable temperature by the regulatory mechanism (Karthikeyan *et al.*, 2004). The gut of earthworm is known as ideal habitat for many agriculturally important microbes (Wolter & Scheu, 1999) and mostly derives its energy and nutrient from gut-specific microbiota rather than from microbiota present in ingested soil (Sampredo *et al.*, 2006). Shi *et al.* (2007) examined that earthworm exposed to deltamethrin for 14 days exposure showed dose-dependent toxic effect on growth and cellulose activity. A decrease in cast production was found in *L. Terrestris* when exposed to methomyl, carbaryl, and imidacloprid respectively for 7 days (Capoweiz *et al.*, 2010).

#### Conclusions

The study highlights the use of pesticides in agriculture system results in many ecological problems. There is clear evidence that the population of earthworm and other non-target soil biota are influenced by pesticides and fertilizers use and the impact is wide-ranging and causing the unwanted shift in the community. Initially, pesticides were used for the benefit to human life by an increase in agricultural productivity and by controlling infectious diseases but their adverse effect on human health and environment were ignored. Multifarious and tremendous uses of pesticides are causing harm to the environment and its components. Some of the adverse effects emerged

**Table 6.** Response of various insecticides on earthworm species at different concentrations

Insecticide	Concentration of Insecticide/exposure	Species	Responses	References
Aldrin, DDE, parathion and carbaryl	LD <sub>50</sub> value 45 µg/g	<i>Lumbricus terrestris</i>	With drawl responses and discoloration of the skin	Cathey, 1982
Endrin	LD <sub>50</sub> value 45 µg/g	<i>Lumbricus terrestris</i>	With drawl responses and discoloration of the skin	Cathey, 1982
DDE	LD <sub>50</sub> value 46 µg/g	<i>Lumbricus terrestris</i>	With drawl responses and discoloration of the skin	Cathey, 1982
Parathion	LD <sub>50</sub> value 34 µg/g	<i>Lumbricus terrestris</i>	With drawl responses and discoloration of the skin	Cathey, 1982
Carbaryl	LD <sub>50</sub> value 28 µg/g	<i>Lumbricus terrestris</i>	With drawl responses and discoloration of the skin	Cathey, 1982
Chlorpyrifos	LC <sub>50</sub> value 0.063 mg/cm <sup>2</sup>	<i>Eisenia foetida</i>	Inhibition of acetylcholinesterase activity, Behavioral and morphological abnormalities	Rao <i>et al.</i> , 2003
Malathion	LD <sub>50</sub> value 880 mg/kg soil	<i>Eisenia foetida</i>	Decreased the spermatocytic viability in spermatheca, altering the cell proliferation and modifying the DNA structure of spermatogonia.	Espinoza-Navarro & Bustos-Obregon, 2004
Carbaryl		<i>Metaphire posthuma</i>	Sperm head abnormalities	Gupta & Saxena, 2003
Dieldrin	LC <sub>50</sub> value 100 mg/kg	<i>Eisenia foetida</i> (Juveniles)	Clitellum development retarded, Influencing reproduction. Growth was retarded even at the agricultural dose of 5 kg/ha	Venter & Reinecke, 1985
Imidacloprid	LC <sub>50</sub> value 25.53 mg/kg	<i>Eisenia andrei</i>	Retarded development, reduced fertility, and teratogenic effects reveal qualitative and quantitative changes in earthworm population, mortality does not occur	Alves <i>et al.</i> , 2013
Dimethoate	LC <sub>50</sub> value 28 mg/kg d.w.	<i>Eisenia foetida</i>	Significantly reducing earthworm weight and showing an avoidance response at soil concentrations	Rico <i>et al.</i> , 2016
Profenofos	LC <sub>50</sub> value 4.56 and 3.55 µg/cm <sup>2</sup>	<i>Eisenia foetida</i>	Body ruptures, bloody lesions, and internal excessive formation of glandular cell mass and disintegration of circular and longitudinal muscles, which failed to regulate the internal coelomic pressure, leading to fragmentation in earthworms	Reddy & Rao, 2008
Dichlorvos	LC <sub>50</sub> value 76 mg/kg d.w	<i>Eisenia foetida</i>	The weight of earthworm decreases. Reproduction and avoidance behavior significantly affected.	Farrukh & Ali, 2011
Cypermethrin	LC <sub>50</sub> value 0.008 mg/kg	<i>Perionyx excavatus</i>	Order of toxicity – cypermethrin > endosulfan > carbaryl > chlorpyrifos > aldicarb > monocrotophos	Gupta <i>et al.</i> , 2010
Endosulfan	LC <sub>50</sub> value 0.03 mg/kg	<i>Perionyx excavatus</i>	Order of toxicity – cypermethrin > endosulfan > carbaryl > chlorpyrifos > aldicarb > monocrotophos	Gupta <i>et al.</i> , 2010
Carbaryl	LC <sub>50</sub> value 6.07 mg/kg	<i>Perionyx excavatus</i>	Order of toxicity – cypermethrin > endosulfan > carbaryl > chlorpyrifos > aldicarb > monocrotophos	Gupta <i>et al.</i> , 2010
Chlorpyrifos	LC <sub>50</sub> value 7.3 mg/kg	<i>Perionyx excavatus</i>	Order of toxicity – cypermethrin > endosulfan > carbaryl > chlorpyrifos > aldicarb > monocrotophos	Gupta <i>et al.</i> , 2010
Aldicarb	LC <sub>50</sub> value 10.63 mg/kg	<i>Perionyx excavatus</i>	Order of toxicity – cypermethrin > endosulfan > carbaryl > chlorpyrifos > aldicarb > monocrotophos	Gupta <i>et al.</i> , 2010
Monocrotophos	LC <sub>50</sub> Value 13.04 mg/kg	<i>Perionyx excavatus</i>	Order of toxicity – cypermethrin > endosulfan > carbaryl > chlorpyrifos > aldicarb > monocrotophos	Gupta <i>et al.</i> , 2010
Chlorpyrifos	LC <sub>50</sub> value 0.5 mg/kg	<i>Eisenia foetida</i>	Effects on growth and weight of earthworms	Pawar & Shahzad, 2013
Lambda-cyhalothrin, Cypermethrin, Didcot, Termicot	LC <sub>50</sub> ranging from 0.000 ml–0.002 ml	<i>Lumbricus terrestris</i>	Presents the highest number of mortality in all concentration	Yuguda <i>et al.</i> , 2015
Methyl Parathion and Phorate	Conc 0.05g/500 g of soil and Methyl parathion 0.12g/500g	<i>Eurilus eugeniae</i>	Coiling, curling and excessive mucous secretion with sluggish movements, Swelling of the clitellum, Extrusion of coelomic fluids resulting in bloody lesions. Earthworms also showed degenerative changes in the anterior part of the nervous system. The disappearance of metameric segmentations and loss of pigmentations.	Rajashree <i>et al.</i> , 2014
Dimethoate	LC <sub>50</sub> value 300 mg/kg	<i>Eisenia foetida</i>	The decrease in cocoon production and cocoon viability	Pal & Patidar, 2013
Carbofuran	LC <sub>50</sub> value 23.5 and 9.3 mg/kg	<i>Eisenia Andrei</i> and <i>Pontoscolex corethrurus</i>	After 7 days biomass reduction was observed only with <i>E. andrei</i> and after 14 days a biomass of both the species reduced significantly	Buch <i>et al.</i> , 2013
Chlorpyrifos (pure)	LC <sub>50</sub> value 80 mg/kg soil	<i>Eisenia Fetida</i>	Adverse impact on growth and reproduction	Zhou <i>et al.</i> , 2007
Parathion	LC <sub>50</sub> value 1478 mg/kg soil	<i>Eisenia foetida</i>	Adverse effect on cocoon production, cocoon viability and hatching success rate.	Bustos-Obreg & Goicochea, 2002



Table 6. Continued ...

Insecticide	Concentration of Insecticide/exposure	Species	Responses	References
Imidacloprid	LC <sub>50</sub> value 0.77 mg/kg dry soil	<i>Eisenia foetida</i>	Adult survival decreased significantly	Silva <i>et al.</i> , 2017
Thiacloprid	LC <sub>50</sub> value 7.1 mg/kg dry soil	<i>Eisenia foetida</i>	Acting on sub-lethal endpoints leading to a reduction in the number of offspring.	Silva <i>et al.</i> , 2017
Cycloxaprid	LC <sub>50</sub> of 10.21 mg/kg dry soil	<i>Eisenia fetida</i>	It induced tissue damage to the epidermis, gut, and neurochord at sublethal doses and also induce oxidative stress	Suzhen <i>et al.</i> , 2018

in the form of an increase in resistant pest population, decline in beneficial soil microorganisms, predators, pollinators and earthworms. Earthworm which is one of the important soil fauna is extremely at the edge of the exposure to pesticides. Such sensitivity of earthworms to pesticides, especially to the major class of pesticides i.e. the insecticides, is well documented in the present review. The toxicity of the insecticides to earthworms varies with the category of chemicals affecting the earthworm life cycle parameters. The persistent nature of pesticides has impacted our ecosystem too that have entered into various food chains and into the higher trophic levels such as that of humans and other large mammals. In order to reduce the effect of pesticides there should be input of sufficient organic manures instead of chemical fertilizers with minimal disturbances in soil and can be adapted for optimum activity of earthworms in the soil for healthy and fertile soil. A little effort has been made to provide a comprehensive review of the toxicity level of insecticide to one of the non-target taxa i.e. earthworm. Therefore farmers must be educated regarding the beneficial role of earthworms because of its importance and to reduce or minimize the use of pesticide to provide the threshold to the environment and biodiversity.

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