A STUDY ON THE IMPACT OF PESTICIDES AND HERBICIDES ON THE GROWTH AND REPRODUCTION OF EARTHWORM IN KOTA REGION

A Thesis submitted by

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for the award of

Ph.D. Degree

in the Faculty of Science

to the

UNIVERSITY OF KOTA, KOTA

Supervised by:

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2017

CANDIDATE'S DECLARATION

I hereby declare that the

1. Thesis entitled **"A Study on the impact of Pesticides and Herbicides on the Growth and Reproduction of Earthworm in Kota Region"** submitted by me is an original piece of research work, carried out by me under the supervision of Dr. Anuradha Singh.

2. Matter presented in this thesis has not been submitted by me for the award of any other degree of this or any other University.

Date:

Anamika Khandelwal

<u>SUPERVISOR’S CERTIFICATE</u>

It is certified that the

1. Thesis entitled **“A Study on the impact of Pesticides and Herbicides on the Growth and Reproduction of Earthworm in Kota Region”** submitted by Anamika Khandelwal is an original piece of research work carried out by the candidate under my supervision.

2. Literary presentation is satisfactory and the thesis is a form suitable for publication.

3. Work evinces the capacity of the candidate for critical examination and independent judgment.

4. Candidate has put in at least 200 days of attendance every year.

Date:

Dr. Anuradha Singh
Government College, Kota

Acknowledgements

I feel overwhelmed at this time of submission of my thesis. It was a long journey. During studying for M.Sc. at Rohilkhand University Bareilly in 1994, I aspired to pursue doctoral degree. On account of one or the other reason, I could not do so for the next few years. After my marriage in Jaipur and settling at Kota due to my husband's job in RTU Kota, I started thinking over it again. In the meantime, I passed SLET exam conducted by RPSC Ajmer, which made me eligible for Lecturership in College without having Ph.D. degree. This again extended my dream of pursuing research. Finally, I joined Ph.D. in July 2013. This became possible due to motivation by my mother, Bina Khandelwal and my husband, Dr. Ajay Khunteta. Their constant encouragement and undulating support has made my dream come true. Of course, the unstinting support and co-operation of my daughter, Arunima was very encouraging for me.

I would like to thank my supervisor Dr. Anuradha Singh, Sr. Lecturer in Zoology Department, Government College Kota. Her constant encouragement and keenness towards research made it possible for me. In addition to this, I am thankful to Mr. Irshad (Head, Zoology deptt.), Dr. Prahlad Dube, Smt. Sandhya Singh, Dr. Neeta Jain and all other faculty mambers for their critical and important suggestions from time to time which not only guided me through the research work but also helped me to enrich my research output. I also thank to supporting staff of Zoology Department for their help during this period.

At this moment, I would like to give special thanks to Dr. C. A. M. Van Gestel

(Faculty of Earth and Life Sciences, Vrije Universiteit, Amsterdam, Netherlands) and Dr. Juan Carlos Sanchez Hernandez (University of Castilla-La Mancha, Spain) for their quick response via Email, whenever I contacted them regarding research guidance. I would also like to express my sincere thanks to Dr. C. K. Mandal and Dr. Ch. Satyanarayana of Zoological Survey of India, Kolkata (India) for species identification and Nanta agricultural farm, Kota (Office of Project Director, Crop, Govt. of Raj.) for helping me in soil parameter measurement. Dr. B. S. Meena, Assistant Professor (Agronomy), Agricultural University, Kota provided valuable inputs regarding pesticides use in Kota region and Krishi Vigyan Kendra, Borkhera, Kota provided earthworms *Eisenia fetida* (Lumbricidae) for experimentation. Thanks to their valuable support and guidance.

With tearful eyes, I also remember my father Shrikant Khandelwal, father-in-law Sh. S.N. Khunteta and mother-in-law Smt. Kamla Khunteta. They are no more with me to share this moment of pride. Motivation of my brother Anurag and sister Shalini was also very much valuable to me. All my family members provided me much needed moral and unstinting support during this period.

Finally, I pray God to give me wisdom and strength to return some tiny useful things to the society out of my research work.

Anamika Khandelwal

List of Publications

Journals

1. **Anamika Khandelwal Khunteta** and Anuradha Singh. Avoidance behavior of *Eisenia fetida* to Chlorpyrifos and 2, 4-D Ethyl ester in natural soil from Kota (Rajasthan) in India. *International Journal of Global Science Research*, vol. 3, issue 6, pp. 399–405, Oct. 2016, ISSN: 2348 – 8344 (http://www.ijgsr.com/web/admin/uploads/f2016051533p1.pdf).

2. **Anamika Khandelwal Khunteta** and Anuradha Singh. Study of Diversity of Earthworm Species in Kota, (Rajasthan) India. *Bioglobia*, vol. 3 (2), pp. 25–28, Dec. 2016, ISSN: 2349 – 5626 (http://www.bioglobia.in/Download.html).

3. **Anamika Khandelwal Khunteta** and Anuradha Singh. Individual and Combined Effects of Triazophos and Pendimethalin, Chemicals used in Soybean Crop, on the Growth and Reproduction of *Eisenia fetida* in Kota (India). **(Communicated to Elsevier's Geoderma Regional)**.

Conference

1. **Anamika Khandelwal Khunteta** and Anuradha Singh. Effect of Triazophos and Pendimethalin on Avoidance behavior of *Eisenia fetida* in Natural Soil of Kota (Rajasthan). *Proc. of ESW IV annual national conference on Impact of Global Warming on Environment, Biodiversity and Ecotourism*, Khajuraho, India, 30-31 Jan. 2017 (http://godavariacademy.com/godavari/images/su.pdf).

List of Abbreviations

AERC	:	Agro-Economic Research Centre
a.i.	:	Active ingredient
ARS	:	Agriculture Research Station
ERA	:	Environmental Risk Assessment
FICCI	:	Federation of Indian Chambers of Commerce and Industry
GDP	:	Gross Domestic Product
ha	:	Hectares (area)
ICAR	:	Indian Council of Agricultural Research
IFOAM	:	International Federation for Organic Agriculture Movements
MSL	:	Mean Sea Level
MT	:	Metric Tonnes
NPOF	:	National Project on Promotion of Organic Farming
NPOP	:	National Programme on Organic Production
NR	:	Net avoidance response (%)
NSDP	:	Net State Domestic Product
NSOP	:	National Standards for Organic Production
RAD	:	Recommended Agricultural Dose
TAS	:	Tropical Artificial Soil
Tha	:	Thousand Hectares
TP	:	Transformation Products

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Chapter 1

Introduction

1.1 Indian agriculture at a glance

India traditionally has an agricultural based economy and it is considered to be the backbone of Indian economy. Agricultural sector employs approximately 55% of the total workforce. The total share of agriculture and allied sectors (including agriculture, livestock, forestry and fishery sub sectors) in terms of percentage of gross domestic product (GDP) is 16.96% and 10% of export earnings. For the 12^{th} five year plan (2012-17), a growth target of 4% has been set for the agriculture sector. Total food grain production in the year 2015-16 was 252 million tonnes. As of today, India supports 18% of world's population on 4.2% of world's water resources and 2.3% of global land. India's per capita availability of resources is about 4 to 6 times less as compared to world average. This will decrease further due to increasing demographic pressure and consequent diversion of the land for non-agricultural uses. The present cropping intensity of 136% has registered an increase of only 25% since independence. Further, rain fed dry lands constitute 65% of the total net sown area. India's arable land area of 159.7 million hectares (394.6 million acres) is the second largest in the world, after the United States. Its gross irrigated crop area of 82.6 million hectares (215.6 million acres) is the largest in the world (**Agricultural Situation in India, 2015**). India is among the top three global producers of many crops, which includes wheat, rice, pulses, cotton, peanuts, fruits and vegetables. In addition to growth in total output, agriculture in India has shown an increase in average agricultural output per hectare in last 60 years. India's recent accomplishments in crop yields while being impressive, are still just 30% to 60% of the best crop yields achievable in the farms of developed as well as other developing countries.

Apart from this, we also face many challenges as a second largest populated country in the world. There is an unprecedented degradation of land, ground water resource, and also fall in the rate of growth of total productivity factor. This deceleration needs to be arrested and agricultural productivity has to be doubled to meet growing demands of the population by 2050. The country recorded impressive achievements in agriculture since the onset of green revolution in late sixties. This enabled the country to overcome starvation, achieve self-sufficiency in food, reduce poverty and bring economic transformation in millions of rural families. The situation, however, started turning adverse for the sector around mid-nineties, with slowdown in growth rate of output. Natural resource base of agriculture, like pure water and fertile land, is shrinking and degrading, and is adversely affecting production capacity. However, demand for agriculture is rising rapidly with increase in population and per capita income and growing demand from industry sector. There is, thus, an urgent need to identify severity of problem confronting agriculture sector to restore its vitality and put it back on higher growth trajectory.

In addition to this, challenges associated with agriculture also includes the use of fertilizers and pesticides. It is true that these chemicals enhance the growth but the quest to achieve more has degraded our agriculture land quality severely and has also created numerous health hazards. Organic farming provides a ray of hope in this direction. Organic farming has fed India for centuries and it is a growing sector in India due to public awareness regarding hazards of chemicals on human health.

Organic production offers clean and green production methods without the use of synthetic fertilizers and pesticides and it achieves a premium price in the market place.

1.2 Overview of Rajasthan state

Rajasthan is the largest state of India constituting 10.4% of total geographical area and 5.67% (6.86 crores) of total population of India (Census, 2011). State's rural population is 75% of the total population residing in 7 divisions of its 33 districts, which are further subdivided into 244 tehsils, 249 panchayat sammitees and 9,168 gram panchayats. Physiographically, the state can be divided into four major regions, namely (i) the western desert with barren hills, rocky plains and sandy plains; (ii) the Aravalli hills running south-west to north-east starting from Gujarat and ending in Delhi; (iii) the eastern plains with rich alluvial soils; and (iv) the south-eastern plateau. Mahi, Chambal and Banas are the three major rivers of the state. Rajasthan is endowed with diverse soil and weather conditions comprising of several agro-climatic zones that help the state to adopt a diversified cropping pattern. The state is India's largest producer of mustard, pearl millet (bajra), three spices (coriander, cumin, and fenugreek), cluster beans, and isabgol. It is the second largest producer of maize. The state has a substantial area under vegetable crops. The state has a diverse weather conditions, warm and humid in south-eastern parts to dry and cool in western parts of the state. About 65% population of the state is dependent on agriculture and allied activities for their livelihood. The three major canal irrigations, other than the vast area under arid and dry lands, offer great help for agricultural development of the state. Agriculture in Rajasthan is primarily rain fed, covering country's 13.27% of available land. The diversity in climatic conditions of the state creates potentiality to develop certain belts of horticultural crops. Agriculture and allied sector plays an important role in state's economy. Though its contribution in net state domestic product (NSDP) has fallen from about 35% in 1990-91 to around 20% in 2015-16, agriculture still forms the backbone of the state economy. Around two third of its population is still dependent on agricultural activities for their livelihood. Thus, a higher priority to agriculture will achieve the goals of reducing poverty and malnutrition as well as of inclusive growth. Though agriculture forms the source of livelihood of the majority in the state, it is largely dependent on rainfall. Only 34.5% of the net sown area is irrigated. Since the rainfall amount is very scanty and highly erratic, the expansion of irrigation provisions and efficient water management are major challenging tasks for the policy makers. The structural changes in Rajasthan agriculture have been in favour of growing of oil seeds, pulses and horticultural crops.

1.3 Agriculture scenario in Rajasthan

Agriculture in Rajasthan is primarily rain-fed. As is well known, productivity of crops in the state of Rajasthan varies greatly and largely depends on the behaviour of the rainfall. A well-developed agriculture extension network has been created in the state. The prospect of agriculture in the state largely depend on timely arrival of monsoon. The major crops grown in different parts of Rajasthan are bajra, wheat, jowar, maize, cotton, rapeseed and mustard, groundnut and horticultural crops. As per the cropping pattern in the state, the crop groups such as total cereals (42%), oil seeds (21%), pulses (18%) and fodder crops (15%) are major agriculture produce. Among the cereals, bajra (50.5%), wheat (27.9%), maize (10.5%) and jowar (6.7%) are the major crops; while rapeseed and mustard (45.4%), taramira (21.7%), soybean (14.0%), sesamum (10.0%) and groundnut (6.3%) are the major oil seeds grown in the state. Among total pulses gram (37.5%), moth (33.5%) and moong (22.1%) are the major crops. Agricultural pattern may broadly be subdivided between Rabi and Kharif seasons. Major Rabi produce includes rabi pulses, wheat, barley, gram, mustard, rapeseed and taramira; whereas Kharif crop mainly comprises kharif pulses, bajra, jowar, maize, sesamum, soybean and groundnut (**AERC Report, 2015**). The normal rain fall of the state is 575.10 mm and the total water requirement of major Kharif crops like bajra, maize and jowar is around 450 to 550 mm. It is not only the total amount of rainfall but its distribution which is also important for normal crop production. Sustainability in crop production depends very much on productivity of crops along with the area sown for total production level.

Agriculture sector in Rajasthan has some major challenges such as network of irrigation facilities, efficient water management due to scracity of water, low productivity due to vastly different nature of soil strata. All these become more difficult to attain due to its large geographic area which is also facing challenges associated with adverse climatic condition. Agro forestry programmes for the greening of desert area, agricultural innovations, proper institutional mechanisms and other modern reforms may be used to bring farm prosperity in the state.

1.4 Agro-climatic zones of Rajasthan

The entire country has been delineated into 126 agro-climatic zones by the Indian Council of Agricultural Research (ICAR), out of which the Rajasthan state has been divided into 10 agro-climatic zones as shown in Fig. 1.1 and Table 1.1. These zones have been classified on the basis of agro-climatic parameters like rainfall, temperature regime, topography, soil characteristics, cropping pattern and irrigation availability.

The climate of Rajasthan state has varied contrasts and the presence of Aravallis is the greatest influencing factor. The Aravallis play a significant role here, as on the west of Aravallis the climate is arid having low rainfall, low humidity and extremes of diurnal and annual temperature. To the east of Aravallis the climate is semi-arid to sub-humid having lesser extremes of temperature, higher humidity and rainfall.

The state can broadly be divided into Arid, Semi-arid, Sub-humid, humid and Very humid regions, on the basis of rainfall intensities. Western Rajasthan comes in the arid region with rainfall 10-20 cm. The region is characterized by low and highly variable rainfall years creating inhospitable living condition to both human and livestock population. Arid region covers Jodhpur, Bikaner, part of Ganganagar, Jaisalmer and Barmer. Ganganagar, Hanumangarh, part of Jodhpur region having rainfall of 20-40 cm belongs to Semi-arid region. Sub-humid region belongs to the area having rain fall of 40-60 cm. Alwar, Jaipur, Ajmer, Jalore comes under the Sub-humid region. Humid region with rainfall 60-80 cm comprises the districts of Bharatpur, Dholpur, Sawai Madhopur, Bundi, Kota, Barmer and Rajsamand and the north-eastern parts of Udaipur. Very Humid region with rain fall of 80-150 cm consists of south-east Kota, Baran, Jhalawar, Banswara, south-west Udaipur and adjacent areas of Mt. Abu. In Banswara, Chittaurgarh, Jhalawar, Baran and Kota the rainfall varies between 70 cm to 100 cm. Kota comes under Zone-V which is classified as Humid south Eastern Plain. This zone receives the highest rainfall in the state ranging 70-100 cm. The plain is spread in the south eastern part of the state covering Sawai Madhopur, Karauli, Jhalawar, Baran, Kota and Bundi districts. The landscape is characterized by hill pediments and vast alluvial plain formed by the rivers Chambal, Parbati, Parwan, Kalisindh and their tributaries. River, deep gullies and ravines have been formed in the region due to these rivers. Land is very productive due to the presence of fine textured alluvium deposited by the rivers in this zone. The state witnesses great peculiarities in temperature. Winters are very severe and temperature falls below freezing point at places like Ganganagar; summers are intense and quite severe in region like the western Rajasthan. May is generally the hottest month and generally January records the lowest minimum temperature. Changes in the climate variables like temperature increase can affect the hydrologic cycle and agriculture and allied sectors which exhibit high sensitivity to climate stresses.

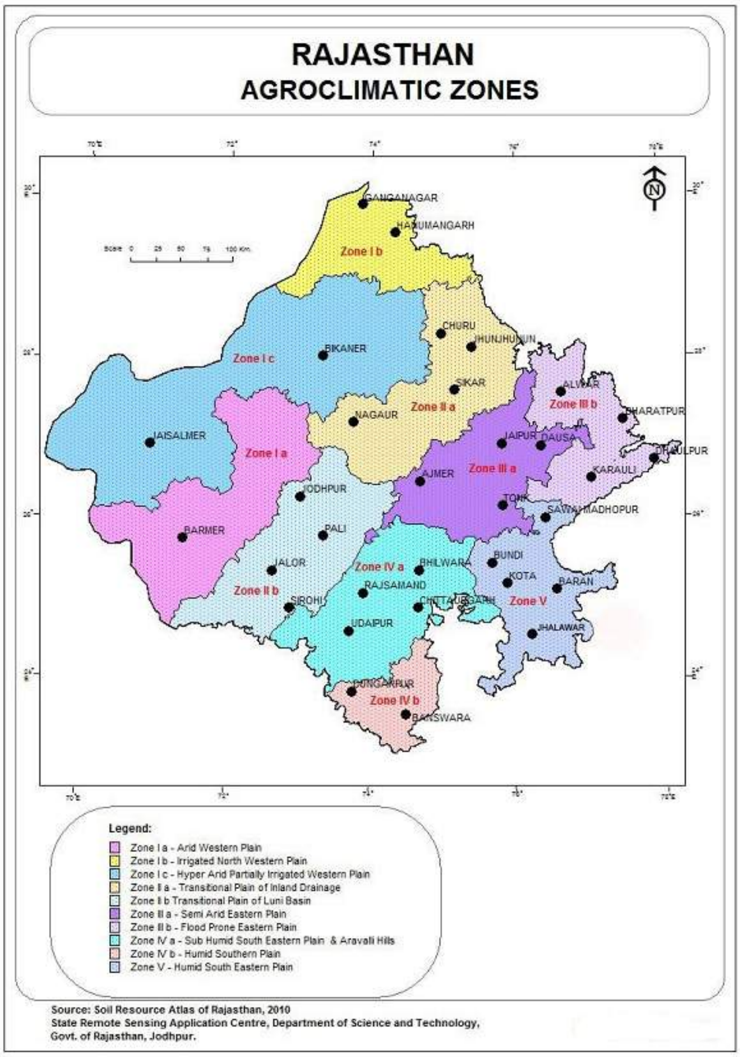

Figure 1.1: Different agro-climatic zones of Rajasthan

Table 1.1: Details of different agro-climatic zones in Rajasthan

Zone	Area	Districts covered	Average rainfall (cm)	Temp. range (^oC)	Major crops		Soil types
					Kharif	Rabi	
IA	Arid western plain	Barmer & part of Jodhpur	20-37	8-40	Pearlmillet, Mothbean, Sesame	Wheat, Mustard, Cumin	Desert soil and sand dunes aeolian soil, coarse sand in texture some places calcareous
IB	Irrigated north western plain	Sriganganagar, Hanumangarh	10-35	4.7-42	Cotton, Clusterbean	Wheat, Mustard, Gram	Alluvial deposits calcareous, high soluble salt & exchangeable sodium
IC	Hyper arid partial irrigated zone	Bikaner, Jaisalmer, Churu	10-35	3-48	Pearlmillet, Mothbean, Clusterbean	Wheat, Mustard, Gram	Desert soil and sand dunes aeolian soil, loamy coarse in texture & calcareous
IIA	Internal drainage dry zone	Nagaur, Sikar, Jhunjhunu, part of Churu	30-50	5.3-39.7	Pearlmillet, Clusterbean, Pulses	Mustard, Gram	Sandy loam, shallow depth red soil in depressions
IIB	Transitional plain of Luni basin	Jalore, Pali, part of Sirohi, Jodhpur	30-50	4.9-38	Pearlmillet, Clusterbean, Sesame	Wheat, Mustard	Red desert soil in Jodhpur, Jalore & Pali sierzems in Pali, Sirohi
IIIA	Semi arid eastern plains	Jaipur, Ajmer, Dausa, Tonk	50-70	8.3-40.6	Pearlmillet, Clusterbean, Sorghum	Wheat, Mustard, Gram	Sierozens, eastern part alluvial, west & north west lithosols, brown soil in foot hills
IIIB	Flood prone eastern plain	Alwar, Dholpur, Bharatpur, Karoli, S. Madhopur	50-70	8.2-40	Pearlmillet, Clusterbean, Groundnut	Wheat, Barley, Mustard, Gram	Alluvial prone to water logging, alluvial calcareous in some places
IVA	Sub-humid southern plains	Bhilwara, Sirohi, Udaipur, Chittorgarh	50-90	8.1-38.6	Maize, Pulses, Sorghum	Wheat, Gram	Soil is lithosols in foot hills & alluvial in plain
IVB	Humid southern plains	Dungarpur, Udaipur, Banswara, Chittorgarh	50-110	7.2-39	Maize, Paddy, Sorghum, Black gram	Wheat, Gram	Predominantly reddish medium texture, well drained calcareous, shallow on hills, deep soil in valleys
V	Humid south eastern plain	Kota, Jhalawar, Bundi, Baran	65-100	10.6-42.6	Sorghum, Soybean	Wheat, Mustard	Black of alluvial origin, clay loam, ground water salinity

1.5 Soil in Rajasthan

Extensive topography of Rajasthan includes rocky terrain, rolling sand dunes, wetlands, barren tracts or land filled with thorny scrubs, river-drained plains, plateaus, ravines and wooded regions. The type of soils in Rajasthan are complex and highly variable, reflecting a variety of different parent material and physiographic land features. The soil in western region is light and coarse textured whereas the soil in eastern parts is heavy and clayey in texture. The soils of the state have been categorized into five specific orders, viz. Aridisols, Alfisols, Entisols, Inceptisols and Vertisols. All the soils have been identified into 22 soil series for particular characteristic and problems. Due to scarcity of surface water in the state, it depends largely on ground resources to a great extent. The ground water exploitation is very high in the eastern as compared to the western region. The annual groundwater recharge is relatively less in the western part of the state, largely owing to very low and erratic nature of the rainfall, absence of surface water sources and high evapotranspiration. The depth of water varies widely throughout the state and varies between 10 m to 25 m and 20 m to 80 m in eastern region to western region, respectively. It has been estimated that over 80% of the state has come under water level depletion zone during the period 1984 to 2015.

The physiography of Kota region comprises vast area formed from the alluvium brought down by Chambal and its tributaries passing through the residual hillocks and gently sloping rocky plateau, which are barren with interspersed veneer of soils. Moderately deep to very deep grayish brown, well drained, fine loamy/coarse loamy either calcareous or non-calcareous soils are dominant feature of the soil scape mainly on the plateau and intervening basin **(NBSS, 1995)**.

1.6 Kota region: An overview on agricultural perspective

Hadoti region, covering Kota, Bundi, Baran and Jhalawar districts, comes under humid south eastern plain (Central Plateau and Hills Region). Regional headquarter Kota is situated at Latitude of $25^o21'$ N, Longitude of $75^o86'$ E and having altitude of 271 MSL.

Land use pattern of Kota district comprises total 521.3 thousand hectares (Tha) of geographical area. Out of this, 269.1 Tha is cultivable area and 125.3 Tha is forest area. This region comprises deep black clayey, deep brown clayey and deep brown loamy soils having percentages of 42, 15 and 11 respectively. Out of the net sown area of 269.1 Tha, 151.8 Tha of area sown more than once. Net irrigated area in the region is 233.9 Tha. Sources of irrigation is canals, tanks and open / bore wells. Rain fed area is 167.1 Tha. Most of the soil is irrigated by canals and wells. Details of Rabi and Kharif crops in the region is as under:

- The Rabi crops are grown in winter season and are seeded in the months of October and November. These crops are harvested in the months of March and April and include barley, wheat, gram, pulses and oil seeds mainly. Rape and mustard are the major oil seeds. Mustard, wheat and coriander are the major crops with productivity of 175.2 kg/ha, 263.2 kg/ha and 40 kg/ha respectively.

- The Kharif crops are summer crops and are sown in months of June and July. Harvesting of these crops takes place in the months of September and October. Principal Kharif crops are bajra, pulses, jowar, maize, soybean and ground nut. Soybean, paddy and maize are the major crops with productivity of 1493 kg/ha, 3452 kg/ha and 1278 kg/ha respectively.

1.7 Pesticides use and trends in India

India, second largest populated country of the world, currently supports nearly 17.84% of the world population having 2.4% land resources and 4% of water resources. To meet the demands of growing population, the country needs to raise its agricultural production to provide food as well as nutrition security. Good emerging trends and solutions for sustainable crop protection through use of time tested agrochemicals, seed treatment, agronomy and bio-technological development have raised the quantity and quality of agricultural production. It is important to note that about 15-25% potential crop production is lost due to insect pests, weeds and diseases. India today imports substantial quantities of pulses and oil seeds on a regular basis and sugar as well as other products. Such imports for longer term can not be afforded by our nation. For ensuring farmer's welfare, self reliance and increasing the agricultural production is the need of the hour. Increasing pest attacks in crops is one of the major challenges in enhancing the output in terms of quality and quantity. At present, per hectare consumption of pesticides in India is amongst the lowest in the world and stands at 0.6 kg/ha against 5-7 kg/ha in the UK and 13 kg/ha in China.

The total number of pests attacking major crops has increased significantly from 1940's. For instance, the number of pests which are harmful for crops such as rice has increased from 10 to 17, whereas for wheat pests increased from 2 to 19. The increased damage to crops from pests and subsequent loss poses a serious threat to food security and further underscores the importance of agrochemicals. Pests, weeds and diseases are causing reduction in global crop output by approximately 25%. In such a scenario, agrochemicals have an increasing role to play in enhancing productivity and crop protection post-harvest. Insecticides and herbicides are the two major chemicals under the class of pesticides. Insecticides are the largest subsegment of agrochemicals with 60% market share, whereas herbicides with 16% market share are the fastest growing segment in India **(FICCI, 2016; Prasad et**

al., 2016). Traditionally, agrochemicals have been manufactured through chemical synthesis but lately biochemical processes are also gaining popularity.

The agrochemicals are broadly classified into different categories as under:

1. *Insecticides*: Insecticides are those agrochemicals which protect the crops either by killing insects or by preventing their attack. They control the population of pest below a desired threshold level. On the basis of their mode of action, further they are classified in two types:

   a. *Contact insecticides*: These kill insects on direct contact. As these chemicals leave no residual activity, they cause minimal damage to environment. Fipronil, carbaryl, pyrethroids (esfenvalerate, bifethrin, cypermethrin, lambdacyhalothin, cyfluthrin, permethrin, deltamethrin, tefluthrin or tralomethrin), pyrethrins and spinosad are some of the examples.

   b. *Systemic insecticides*: These are absorbed by the plant tissues and destroy insects when they feed on the plant. Due to their long term residual activity, they cause damage to environment and human health. Dimethoate, thiamethoxam, imidacloprid, terbufos and dinotefuran are the exmples of such type of agrochemicals.

2. *Fungicides*: Fungicides are the agrochemicals which protect the crops from the attack of fungi. Protectants and eradicates are the two popular types of fungicide. Protectants prevent or inhibit fungal growth, while eradicates kill the pests on application. In this way, fungicides improve productivity, reduce blemishes on crop and improve storage life and quality of harvested crop.

3. *Herbicides*: Herbicides are also known as weedicides. These agrochemicals kill undesirable plants. They can be of two types: selective and non-selective. Selective herbicides kill specific plants, leaving the desired crop unharmed, while non-selective herbicides are used for widespread clearance of ground. Thus, non-selective herbicides are used to control weeds before crop planting.

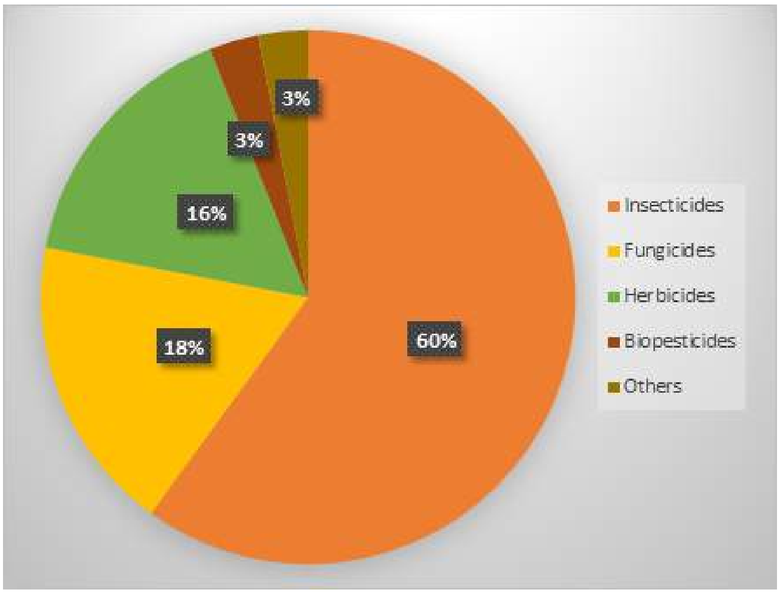

Figure 1.2: Market share of different pesticides in India

4. *Bio-pesticides*: Bio-pesticides are new age crop protection products which are manufactured from natural substances like plants, animals, bacteria and certain minerals. They are eco-friendly, easy to use and require lower dosage amount for the same performance as compared to the chemical based pesticides. Bio-pesticides have huge growth potential due to its non-toxic nature.

5. *Others*: Fumigants and rodenticides are the chemicals which protect the crops from pest attacks during crop storage. Plant growth regulators help in controlling or modifying the plant growth process and are usually used in cotton, rice and fruits.

Though on one side, the use of pesticides in agricultural practices is very much beneficial to increase productivity by protection of crop losses and vector disease control, on the other side, their are numerous hazards associated with pesticides. Some of the important hazards are as under:

- *Direct impact on humans*: Benefit of pesticides includes enhanced economic potential in terms of increased production of food and fiber. On the other hand, disadvantages are in the form of serious health implications to man and his environment. There is now overwhelming evidence that some of these chemicals do pose a potential risk to humans and other life forms and unwanted side effects to the environment.

- *Impact through food commodities*: Pesticide residues in products of plant origin is tested to know the pesticide contamination in the food stuffs. It has been found by a study that 13 pesticides (acephate, carbendazin, chlorothalonil, chlopyriphos, DDT, diazinon, endosulfan, methamidophos, iprodione, metalaxyl, methidathion, thiabendazole, triazophos) were available in five commodities (mandarins, pears, bananas, beans and potatoes). This is just an example to understand the gravity of the situation.

- *Impact on environment*: Pesticides can pollute soil, water, turf, and other vegetation. In addition to killing insects or weeds, pesticides can be toxic to a host of other organisms including birds, fish, beneficial insects, and non-target plants. Insecticides are generally the most acutely toxic class of pesticides, but herbicides can also pose risks to non-target organisms.

- *Surface water contamination*: Pesticides can contaminate surface water also by reaching through runoff from treated plants and soil. Water contamination may cause serious health hazards to human and aquatic organisms.

- *Ground water contamination*: Pesticides also pollute ground water in the long run. This may damage our ground water quality during recharging of ground in rainy season. According to a study, at least 143 different pesticides and 21 transformation products have been found in ground water.

- *Soil contamination*: The pesticides and their transformation products (TP) are retained by soils to different degrees, depending on the interactions between soil and pesticide properties. The most influential soil characteristic is the organic matter content. The larger the organic matter content, the greater the adsorption of pesticides and TP's.

- *Effect on soil fertility (beneficial soil microorganisms)*: Heavy treatment of soil with pesticides can decline the populations of beneficial soil microorganisms also.

1.8 Earthworm: Farmer's friend

Earthworms are terrestrial invertebrates belonging to the Phylum Annelida, Class Chaetopoda, Order Oligochaeta. They have originated about 600 million years ago during the pre-cambrian era **(Piearce et al., 1990)**. Aristotle referred them as "the intestines of earth and the restoring agents of soil fertility" **(Shipley, 1970)**. Earthworms with diverse habitat, improve soil fertility by changing the physical and chemical composition of soil. Many scientists discussed the importance of earthworm in improving the soil fertility **(Darwin, 1881; Edwards et al., 1995; Kale, 1998; Lalitha et al., 2000)**. Earthworms convert organic matter into soil humus by ingesting soil, mixing of different soil components and then produce as surface and sub surface castings **(Jairajpuri, 1993)**. According to **Shuster et al. (2000)**, earthworms play a major role in the decomposition of organic matter through feeding, aeration, fragmentation, turnover and dispersion. Presence of good population of earthworms in soil indicates the presence of a large population of insects, spiders, bacteria, viruses, fungi and other organisms and thus a healthy soil. Due to this, earthworms are biological indicators of soil quality **(Lachnicht and Hendrix, 2001)**.

1.8.1 Ecological strategies of earthworms

Bouche (1977) and **Lee (1985)** categorised earthworms into three main ecological groups. This classification is done on the basis of soil horizons in which the earthworms were commonly found i.e., litter, topsoil and sub soil. Classified three major groups are the epigeics, anecics and endogeics. They may be defined as under :

- *Epigeic*: Species of earthworms which live on the soil surface are called Epigeic. They are litter feeders, e.g. *Allolobophora parva* and *Eisenia fetida*.
- *Anecic*: Earthworms which live on topsoil are called Anecic and feed on the leaf litter mixed with the soil. They predominantly form vertical burrows in the soil, e.g. *Lampito mauritii*.
- *Endogeic*: These species of earthworms consume more soil than epigeic or anecic species, deriving their nourishment from humus. They preferably form horizontal burrows in soil, e.g. *Metaphire posthuma*.

1.8.2 Factors affecting distribution of earthworms

Physical and chemical characters of the soil, such as pH, moisture, organic matter, temperature and soil texture affect the distribution of earthworms in soil **(Edwards and Bohlen, 1996)**.

1. As earthworms are sensitive to the hydrogen ion concentration, their distribution and abundance are very much affected by pH and factors that are related to pH. **(Staaf, 1987; Chalasani et al., 1998)**. Soils, having neutral pH, are usually preferred by most species of earthworms.
2. Soil moisture plays an important role in the survival of earthworms, as their bodies have 75-90% of water by weight **(Grant, 1955)**. Soil moisture affects

the number and biomass of earthworms **(Wood, 1974)**. They can even survive in adverse moisture conditions, either by moving to a region with more moisture **(Valle et al., 1997)** or by means of aestivation **(Baker et al., 1992)**.

3. The distribution of earthworms is greatly influenced by the distribution of organic matter. **Doube et al., 1997** reported that there is a strong positive correlation between the organic matter content of the soil and earthworm numbers and biomass. Earthworms are also recognised as soil managers because they bring about physical, chemical and biological changes in the soil due to their activities.

1.8.3 Importance of earthworms

- *As a growth enhancer of plants*: Earthworms prepare the ground in an excellent manner for the growth of plants **(Darwin, 1881)**. Many workers demonstrated that earthworms have beneficial effects on soil as these effects increase plant growth as well as yield of crops **(Decaens et al., 1999; Lalitha et al., 2000)**. Earthworms release auxins and cytokinins which are beneficial substances for plant growth **(Krishnamoorthy and Vajranabhaiah, 1986)**.

- *In organic farming* : Burning of organic wastes originated from various sources like domestic, agriculture and industrial has caused serious environmental hazards and economic problems. This problem can be overcome by earthworms as they can process city refuse, household garbage, sewage and waste from paper, food and wood industries. This composting process decreases the time of stabilization of the waste and converts into an efficient bio-product, i.e., vermicompost. Vermiculture and vermicompost associated with other biological inputs have been found to be economically very productive. This way it offers a solution to recycle and reuse organic agro wastes in ecofriendly manner. By organic farming, we can eliminate the use of chemicals in the form of fertilizers/pesticides, convert waste into useful compost. This improves soil, plant, animal and human health and creates an ecofriendly, sustainable and economical bio-system models **(Ansari and Ismail, 2001, 2001b)**.

- *Vermicomposting*: Vermicomposting is a process through which earthworms and microorganisms biologically degrade and stabilize the organic waste into vermicompost. The earthworms stimulate microbial activity, fragment the organic waste and induce rate of mineralization **(Kale et al., 1982)**. All these processes convert waste into humus-like substances with finer structure. By adding vermicompost with clay soil, soil loosening takes place. This finally improves the passage for the entry of air and hence improves soil porosity **(Lavelle and Spain, 2001)**. Vermicast improves water holding capacity of the soil, as mucus associated with the cast is hydroscopic in nature. This also prevents water logging of soil due to its better absorbing capacity. Nutrients, beneficial for plants, are released slowly and steadily from the organic carbon present in vermicompost. Epigeic earthworms like *Perionyx excavatus, Eisenia fetida, Lumbricus rubellus* and *Eudrilus eugeniae* are used for vermicomposting but the local species like *Perionyx excavatus* has proved efficient composting earthworms in tropical or subtropical conditions **(Kale, 1998)**. In this way, vermicompost is able to improve the physical, chemical and biological properties of soil. It promotes growth of plants and has been found to have a favourable influence on all crops.

- *As a biomarker*: A biomarker is defined as a "biochemical, cellular, physiological or behavioural variations that can be measured in tissue or body fluid samples, or at the level of whole organisms, to provide evidence of exposure and/or effects from one or more contaminants" **(Depledge, 1994)**. The effects of contaminants occur more rapidly at lower levels, e.g. biochemical, cellular, physiological in comparison to higher levels (e.g., ecological effects). This helps to indicate a more sensitive early warning of toxicological effects within earthworm populations. Earthworm biomarkers represent useful tools in soil monitoring and assessment as an early warning of adverse ecological effects **(Sanchez-Hernandez, 2006; Rodriguez-Castellanos & Sanchez-Hernandez, 2007)**. As indicated by **Sanchez-Hernandez (2006)**, four types of approaches can be performed in soil pollution monitoring : 1) biomarker analysis on native earthworm populations; 2) use of transplanted organisms in in situ exposure bioassays; 3) exposure of a selected earthworm population to the environmental medium (soil) in laboratory standardized conditions; 4) simulated field studies.

Earthworm as biomarkers have become relevant for assessing the effects of contaminants on soil organisms. Biomarker approach is used for soil pollution monitoring. It is a relatively recent approach as compared to aquatic environment monitoring. To develop this methodology to perfection, it is to be further evaluated. First, it is necessary to identify and characterise appropriate earthworm species for quick assessment of soil pollution. Second, earthworm biomarkers studies have been mostly conducted for heavy metals. It is necessary to study the exposure for a wider range of chemicals related to soil pollution. Third, there is a need to increase the knowledge of biological responses of earthworms to pollutants for the purpose of standardization. This will help in detection of the pollutant induced stress syndrome in soil organisms. Cytological, biochemical and transcriptomic parameters are also used to assess the effect of contaminants by examining the effect on physiological fluids, such as coelomic fluids and blood of earthworms. In this regard, granulocyte morphometric alteration has been recently demonstrated as a suitable general biomarker of contaminant's effect. Finally, earthworm biomarkers have been scarcely investigated under field conditions.

1.9 Organic farming

The concept of organic farming includes the efficient use of local resources, soil fertility management, recycling of nutrient to the possible extent, control of pests and diseases by natural products. It can be a promising option for sustainable agricultural development. Associated benefits of this are: (i) It provides greater yield stability, particularly in tropical ecosystems, (ii) By using improved and the adapted technology, higher yields and incomes are attainable in traditional farming systems, (iii) It improves soil fertility which leads to long-term sustainability of farming systems, (iv) It reduces farmer's dependence on external inputs, (v) By using organic farming, restoration of degraded or abandoned land is possible and (vi) It strengthens the self-confidence as well as autonomy of farmers.

By organic farming, it is possible to produce safe and healthy food and fiber with minimum or no adverse effects on the environment and resources. Organic cultivation follows different management approaches depending on climates, locations and cropping systems.

Over the years, it has been scientifically proven that organic farming promises environmental preservation, protection of variety and species, protection of soil, purity of water and reduces the impact of agriculture on the atmosphere. Today's awareness regarding use of organically grown food and fiber has attracted the attention of agricultural policy planners, promoters and producers all over the world. Efforts were made by International Federation for Organic Agriculture Movements (IFOAM) for promotion of organic farming has ensured the growing acceptance of organic agricultural systems world over. Various countries, including India are working seriously in this direction to develop and implement quality assurance systems to promote organic agriculture.

In India, ministry of commerce launched the "National Programme on Organic Production" (NPOP) defining the National Standards for Organic Production (NSOP) and the procedure for accreditation and certification in 2000. India now has 30 accredited certification agencies for facilitating the certification to growers. For area expansion and technology transfer, ministry of agriculture launched a National project on Promotion of Organic Farming (NPOF). To augment the research needs ICAR launched a Network Project on Organic Farming (NPOF-ICAR) under Project Directorate of Farming System Research with 13 collaborating centers across the country. As on March 2014, India has brought 4.72 million hectares area under organic certification process, which includes 0.6 million hectares of cultivated agricultural land and 4.12 million hectares of wild harvest collection area in forests. Since 2004, many states embraced organic farming and drafted policies. So far 11 states, namely Andhra Pradesh, Karnataka, Kerala, Uttarakhand, Maharashtra, Madhya Pradesh, Tamil Nadu, Himachal Pradesh, Sikkim, Nagaland and Mizoram have drafted the organic agriculture promotion policies. Sikkim became the first state in the country by converting the entire state into organic.

Chapter 2

Review of Literature

Agrochemicals play a major role in fulfilling the ever growing demand of food by increasing the productivity and protect the crops by controlling pests and weeds. While their excessive use has resulted in increased yield, they have also resulted in serious health complications to man and environment. Pesticides pollute water, soil, and quality of other vegetation. These chemicals are found to be useful not only in killing insects and weeds, but are toxic to host of other organisms which are beneficial to crop and our ecosystem. Birds, fish, beneficial insects, and non-target plants comes in this category. Insecticides are generally the most acutely toxic class of pesticides, but the non-target organisms can be at a greater risk due to herbicides also.

Earthworms play a pivotal role in farming through use of natural resources. Earthworms are capable to recycle the organic waste and convert it into useful organic manure which is very useful for agricultural application. Organic farming helps to provide many advantages like providing the alternative to fertilizers/pesticides, recycle and regenerate waste into useful manure, improve soil quality and thus beneficial to plant, animal and human health.

Researchers studied the potential of earthworms for soil quality management and enhancing its fertility. Following sections present the various key contributions.

These research works were a motivation to explore further in this direction.

2.1 Study of avoidance behaviour in earthworms

Lukkari et al. (2005) assessed the toxicity of copper and zinc with standardised earthworm acute toxicity and reproduction tests .They used avoidance test to find out whether the earthworm *Aporrectodea tuberculata* avoids soils simultaneously contaminated with Cu and Zn, and whether earlier exposure to metal-polluted soil affects its avoidance response and also to compare the sensitivity of the earthworm avoidance test to the standardised acute toxicity and reproduction tests. They observed that *A. tuberculata* clearly avoided lower soil metal concentrations than those that induced responses in the acute toxicity and reproduction tests. They found that the standard species in the earthworm tests, *Eisenia fetida*, appeared to be more tolerant to metals and it seemed to regulate the tissue metal concentrations more strictly than *A. tuberculata* and concluded that the earthworms living in the metal contaminated area were either better adapted or acclimatized to live in metal contaminated soil.

Zhou et al. (2007) assessed toxicity of chlorpyrifos-contaminated soil on earthworm with three different earthworm test methods. The test methods covered all important ecological relevant endpoints (acute, chronic, and behavioural). They found that at concentration of 78.91 mg/kg, chlorpyrifos caused significant toxic effects in all test methods, but at lower test concentrations, only significant chronic toxic effects could be observed. Results showed that chlorpyrifos had adverse effect on growth and fecundity in earthworm exposed to 5 mg/kg chlorpyrifos after eight weeks. The avoidance response test, however, showed significant repellent effects at concentration of 40 mg/kg chlorpyrifos. They concluded that for chlorpyrifos, concentration affecting avoidance response was far greater than growth and fecundity, it seemed likely that earthworms were not able to escape from pesticide-contaminated soil into the clean soil in field and hence were exposed continuously to elevated concentrations of pesticides.

Garcia et al. (2008) evaluated the avoidance behaviour of earthworms *Eisenia fetida* for three pesticides (benomyl, carbendazim, lambda-cyhalothrin) in laboratory tests performed under temperate and tropical conditions. The results showed that this test gives reproducible and reliable results. Toxicity values (NOEC, EC 50) are lower than those determined in 14 day-acute mortality tests and are approximately in the same range such as those found in 56 day-chronic reproduction tests with the same earthworm species, which were performed in parallel. Therefore, the use of the earthworm avoidance tests is recommended as a screening tool for the risk assessment of pesticides.

De Silva et al. (2009) studied comparative sensitivity of *Eisenia andrei* and *Perionyx excavatus* in earthworm avoidance tests in the tropics, using chlorpyrifos and carbofuran in artificial and natural soil. They exposed earthworms to concentrations of 1-900 (chlorpyrifos) and 1-32 (carbofuran) mg a.i./kg dry soil in a two-chamber system under tropical conditions $(26 \pm 2^{o}C, 48h)$. They found no significant difference in the control tests comparing the two soils used; suggesting soil type did not affect the distribution of the worms. Their results suggest a higher sensitivity of *E. andrei*, with EC 50 for the effect on avoidance behaviour for both pesticides being a factor of 2-3 lowers than for *P. excavatus*. They concluded that earthworm avoidance tests with local species should therefore be used with caution when applied as a tool for pesticide risk assessment in the tropics. Endpoints generated through avoidance tests in their study are shown to be less sensitive than reproduction and more sensitive than survival. They also concluded that *Eisenia andrei* was more sensitive in avoidance test with two pesticides than *P. excavatus* under tropical conditions.

Marques et al. (2009) analysed the toxicity of formulated herbicides and their active ingredients on natural soils by using earthworm avoidance behaviour. For this, they used two herbicide active ingredients (a.i.) sulcotrione and penoxsulam and their respective commercial formulations–Mikado and Viper and earthworm species was *Eisenia andrei*. Both herbicides induced avoidance behaviour on *E. andrei*, stronger effects were denoted by penoxsulam and its respective formulated product, Viper. Overall, avoidance tests provided a sensitive, valuable and feasible response either to compare the habitat function of different standard and agricultural natural soils or to test the effect of herbicides.

De Sousa and De Andrea (2011) studied the avoidance behaviour of *Eisenia andrei* in three different agricultural soils treated with cypermethrin. Their experimental result showed that after 48 h, there was no mortality, but the avoidance was clear because all earthworms were found in the untreated section of each type of soil ($p < 0.05$). They concluded that the different soil characteristics, cypermethrin concentrations and formulation did not influenced the avoidance behaviour of the earthworms.

Santos and Forrer (2011) observed avoidance behaviour of *Eisenia fetida* to carbofuran, chlorpyrifos, mancozeb and metamidophos in natural soils from the highlands of Colombia. Their observations showed that for the carbofuran and chlorpyrifos 100% avoidance was not reached and no significant avoidance behaviour trend was found for mancozeb and methamidophos. However, for the case of carbofuran and methamidophos, differences of more than double in avoidance were obtained.

Farrukh and Ali (2011) examined the effect of endosulfan on growth, reproduction and avoidance behaviour of earthworm *Eisenia fetida*. Their result showed that growth was significantly affected at all three concentrations of pesticide used, whereas earthworm of control group showed normal increase in growth. Reproduction seemed to be deliberately affected and avoidance test of 48 hours was also found to be a sensitive parameter in evaluation of the toxic chemical.

Farruk and Ali (2011b) observed the effects of dichlorovos organophosphate on growth, reproduction, and avoidance behaviour of earthworm *Eisenia fetida*. Their observation showed that all three doses of dichlorovos decreased the weight of earthworms, whereas reproduction and avoidance behaviour which are sensitive parameters were found to be significantly affected.

Santos et al. (2012) assessed the effect of three commercial formulations containing the insecticides chlorpyrifos and endosulfan and the herbicide glyphosate to non-target soil organisms. For this, they collected soil after spraying and dilution series were prepared with untreated soil to determine the impact of the pesticides on the avoidance behaviour and reproduction of the earthworm *Eisenia andrei*. Result showed that a significant avoidance was observed at the recommended field dose in case of endosulfan by earthworms (60%). In addition, both insecticides endosulfan and chlorpyrifos affected the number of juveniles produced by the earthworms (EC 50 were below the recommended field dose). They concluded that glyphosate did not seem to affect earthworms in the recommended field dose.

Alves et al. (2013) assessed the impact of the insecticides imidacloprid, fipronil, thiametoxam, fungicides captan and carboxin plus thiram on the survival, reproduction, and behaviour of *Eisenia andrei* (Oligochaeta). All these chemicals are used in the chemical treatment of crop seeds. Results showed that with the exception of imidacloprid, none of the pesticides tested caused mortality in *E. andrei* in artificial soils.

Morcillo et al. (2013) quantified the avoidance response of *Lumbricus terrestris* in chlorpyrifos-spiked soils, depending on the pesticide concentration and exposure duration. The effects of different chlorpyrifos concentrations were examined in a standardised test (two-chamber system) with 0.6, 3 and 15 mg/kg chlorpyrifos. A modification of the test involved a pre-exposure step (24, 48 or 72 hrs) in soils spiked with 15 mg/kg. In both protocols, earthworms were unable to avoid the contaminated soils. They concluded that the avoidance behaviour test for organophosphorus-contaminated soils could be supported by specific biomarkers to facilitate a better understanding of pesticide exposure and toxicity during this test.

Bucha et al. (2013) studied the toxicity of three pesticides, carbendazim, carbofuran and glyphosate to *Pontoscolex corethrurus* (Muller, 1857) and *Eisenia andrei* (Bouche, 1972) by using avoidance and mortality tests. They found that concentrations applied in the field of these two pesticides have toxic effects on both species and glyphosate showed no toxic effects for either species even at the highest concentration tested (47 mg a.i./kg), although earthworm species displayed avoidance behaviour at this concentration. They observed that the sensitivity of *P. corethrurus* appears to be similar to the standard species for the pesticides evaluated reinforcing the notion that *E. andrei* is a good test species.

Acute and sub-acute effects of enrofloxacin on the earthworm species *Eisenia fetida* in an artificial soil substrate were examined by **Li et al. (2015)**. The results showed that the LC 50 of enrofloxacin to earthworms was 11.01 gm/kg at day 14 , which was far higher than the environmentally relevant concentration of at day 14 residues. They observed that the reduction in the growth and reproduction of earthworms was a dose dependent effect. Earthworms exposed to > 0.25 gm/kg of enrofloxacin displayed avoidance response while the soil only spiked with > 2 gm/kg of enrofloxacin showed decreased habitat function. The respiration of earthworms was inhibited when exposed to 2.0 gm/kg of enrofloxacin for more than 14 days or 1.0 gm/kg of enrofloxacin for more than 28 days. They concluded that enrofloxacin could cause acute and sub-acute toxicity to earthworms (*E. fetida*) at relatively high concentration.

2.2 Study on the impact of chemicals on the growth and reproduction of different earthworm species

Various researchers reported the effect of pesticides on growth and reproduction on different species of earthworms. In this section, detailed discussion is done on species other than *Eisenia fetida*.

Choo and Baker (1998) studied the influence of four commonly used pesticides on the survival, growth, and reproduction of the earthworm *Aporrectodea trapezoides* (Lumbricidae) and found that endosulfan significantly reduced the weight of juvenile *Aporrectodea trapezoides* within 5 weeks when applied to soil at normal application rate in both the field and laboratory condition while fenamiphos did so at normal application rate in the field only. Both fenamiphos and methiocarb reduced earthworm weight in the laboratory when applied at 10 times normal rate. They also found that cocoon production in *A. trapezoides* was inhibited by endosulfan and fenamiphos at normal application rates and methiocarb at 10 times normal rate.

Panda and Sahu (1999) investigated the decline and recovery of the growth and reproduction of an earthworm *Drawida willsi*, for different doses of malathion to soil. Study found that sharp decline in the growth of *D. willsi* (57% in 2.2 and 80% in 4.4 mg malathion per kg soil) was observed after 15 days. Study indicated decline in growth and reproduction of *D. willsi* following application of a normal or a double agricultural dose of malathion. However, the worms were able to resume normal growth and reproduction after an interval of 105 days.

Maboeta et al. (1999) studied the effects of sublethal concentrations of lead nitrate on growth and reproduction of earthworm *Perionyx excavatus* by exposing worms in an organic substrate to lead nitrate-contaminated food over a period of 76 days. Their results showed that growth was affected negatively by the presence of lead while maturation rate and cocoon production was not affected. Lead accumulation was of the same order of magnitude as for other previously studied species.

Panda et al. (1999b) studied the accumulation of zinc and its effects on the growth, reproduction and life cycle of *Drawida willsi* (Oligochaeta), a dominant earthworm in Indian crop fields. *D. willsi* did not revealed any significant changes in their mass at any of the concentrations of Zn (50, 200 and 400 mg/kg) compared to in untreated soils. They found that concentrations in the exposed earthworms were significantly increased, but they were able to regulate their body content of Zn within a range of 116–125 mg/kg (dry wt) in 200–400 mg/kg Zn-treated soil. Reproduction was significantly reduced when the Zn concentration in soil exceeded 200 mg/kg. The drop in reproduction at elevated concentrations of Zn apparently resulted in a delay in completion of the life cycle and a decline in the total population.

Booth et al. (2000) examined the effect of two organophosphates, chlorpyrifos and diazinon in the earthworm *Aporrectodea caliginosa* and observed a reduction in growth rate in all pesticide-treated worms.

Panda and Sahu (2000) assessed the recovery of population, biomass and reproduction of the earthworm *Drawida willsi* following the application of two recommended agricultural doses of malathion (2.2 mg/kg single dose and 4.4 mg/kg double dose) in a rice field agroecosystem for a period of 105 days. Observations showed that the average worm population and biomass were declined 12% and 21%, respectively, in plots treated with single and double doses of malathion, compared with control. The peak of worm population was observed after 60 days in control and this peak value was shifted by 15 days (i.e. observed after 75 days) in malathion-treated plots indicating delay in growth and maturation of the worms following the application of malathion. Inhibition in the total number of cocoons produced, and thus in the rate of reproduction, was also observed in malathion-treated plots. One-way analysis of variance showed significant differences in the population size and biomass of *D. willsi* up to 75 days, in rate of reproduction up to 90 days, and no difference thereafter. They concluded that under field conditions, *D. willsi* worms took about 75–90 days after application of malathion to resume normal population, biomass and reproduction, and hence they suggested that a second application of malathion in single and double agricultural doses should be avoided before this time.

Panda and Sahu (2004) investigated the recovery of acetylcholine esterase (AChE) activity of *Drawida willsi* (Oligochaeta) by application of two recommended agricultural (single and double) doses of butachlor (1.1 and 2.2 mg a.i./kg dry soil), malathion (2.2 and 4.4 mg a.i./kg dry soil) and carbofuran (1.1 and 2.2 mg a.i./kg dry soil) to the soil under laboratory conditions. A sharp decline in the AChE activity of *D. willsi* was observed up to 9 and 12 days following treatment of carbofuran and malathion in both single and double doses, respectively, whereas very little inhibition was noticed in case of butachlor. They found that *D. willsi* worms took 45 and 75 days to resume normal AChE activity after exposure to both single and double doses of malathion and carbofuran, respectively. They strongly suggested that the time gap between the first and second application of malathion, irrespective of single and double dosage, should be at least 90 days, whereas it should be at least 105 days for carbofuran. Butachlor was found to be very toxic, suppressing growth, sexual maturation and cocoon production of *D. willsi* at both single and double doses. Researchers suggested that application of organochlorine pesticides like butachlor should be avoided as far as possible to ensure maintenance of good soil health.

Capowiez et al. (2005) tested lethal and sublethal effects of imidacloprid on two earthworm species (*Aporrectodea nocturna* and *Allolobophora icterica*), which is the major component of many widely used insecticides and is relatively persistent in soils. Their result was consistent with previous findings obtained with other earthworm species and natural soils, i.e. significant decreases in weight.

De Silva et al. (2009b) investigated the influence of temperature and soil type on the toxicity of three pesticides to *Eisenia andrei*. They compared the toxicity of chlorpyrifos, carbofuran and carbendazim to the earthworm *Eisenia andrei* at two different temperatures reflecting temperate and tropical conditions. The toxicity of the three pesticides in both conditions decreased in the order carbendazim $>$ carbofuran $>$ chlorpyrifos. For chlorpyrifos and carbofuran, but not for carbendazim, survival was more sensitive at the higher temperature, probably due to increased earthworm activity. Sub-lethal effects (reproduction and growth) however, varied inconsistently with temperature and soil types. They concluded that toxicity of pesticides in tropics may not be predicted from data generated under temperate conditions, even within the same species.

De Silva et al. (2010) assessed the toxicity of chlorpyrifos, carbofuran, mancozeb and their formulations on survival, growth and reproduction of the tropical earthworm *Perionyx excavates* in standard artificial soil. They found that toxicity of the three chemicals decreased in the order carbofuran > chlorpyrifos > mancozeb. In general, formulations were more toxic than the active ingredients, but differences in LC 50 and ECx values were significant only in two cases and notmore than a factor of 2.0. This could mainly be due to masking of the effects of additives in the soil. Comparison with available survival data revealed that *P. excavatus* is more sensitive.

De Silva et al. (2010b) found that chlorpyrifos causes decreased organic matter decomposition by suppressing earthworm and termite communities in tropical soil. In their study, litterbag and earthworm field tests were performed simultaneously at the same tropical field site sprayed with chlorpyrifos. Their experimental results showed that the recommended dose of chlorpyrifos (0.6 kg a.i./ha) and two higher doses (4.4, 8.8 kg a.i./ha) significantly decreased litter decomposition during the first 3 months after application, which could be explained from lower earthworm and termite abundances during this period. They observed species-specific effects of chlorpyrifos on organism abundance and biomass, with termites being mostly affected followed by the earthworm *Perionyx excavatus*; the earthworm *Megascolex* species was least affected.

Capowiez et al. (2010) examined earthworm cast production as a new behavioural biomarker for toxicity testing. They proposed a new and relatively simple ecotoxicity test based on the estimation of cast production by *Lumbricus terrestris* under laboratory conditions. Cast production was found to be linearly correlated to earthworm biomass and to be greatly influenced by soil water content. Their experimental result showed that azinphos-methyl had no effect on cast production at all the concentrations tested. Significant decreases were observed at the normal application rate for other pesticides with (imidacloprid, carbaryl, methomyl) or without (ethyl-parathion and chlorpyrifos-ethyl) a clear concentration effect response. Cast production is straight forward and rapidly measured and ecologically meaningful. They thus believed it to be of great use as an endpoint in ecotoxicity testing.

Dittbrenner et al. (2010) studied the physiological and behavioural effects of imidacloprid on two ecologically relevant earthworm species (*Lumbricus terrestris* and *Aporrectodea caliginosa*) commonly found in different agricultural soils. In laboratory experiments they assessed sub-lethal effects (body mass change and cast production) of imidacloprid. They observed a significant loss of body mass after seven days in both species exposed to imidacloprid concentrations as low as 0.66 mg/kg dry soil. Cast production increased by 26.2% in *A. caliginosa* and by 28.1% in *L. terrestris* at the lowest imidacloprid concentration tested (0.2 mg/kg dry soil), but significantly decreased at higher concentrations (equal to and above 0.66 mg/kg dry soil) in both earthworm species after the 7 days exposure experiment. They concluded that the biomarkers used in this study, body mass change and changes in cast production, may be of ecological relevance and have shown high sensitivity for imidacloprid exposure of earthworms. The measurement of changes in cast production should be considered for inclusion in current standard tests.

Lister et al. (2011) studied a combined approach using mixture toxicity testing, toxicokinetic studies and modelling to address the link between joint toxicity and internal concentration. The study was conducted in Lumbricid earthworms (*Lumbricus rubellus*) with a binary mixture of a metal (nickel) and an organophosphate insecticide (chlorpyrifos).

Dasgupta et al. (2012) studied the effects of carbaryl, chlorpyrifos and endosulfan on growth, reproduction and respiration of tropical epigeic earthworm, *Perionyx* *excavatus* (Perrier) under laboratory conditions. The experimental results showed significant reduction in biomass, production and hatching of cocoon and production of juveniles of the worms exposed to these insecticides. Endosulfan was found most dangerous among the three insecticides followed by carbaryl and chlorpyrifos. Chlorpyrifos produced no change in respiration of the worms except at the highest dose, while the worms showed an increase in evolution of CO_2 at all doses of carbaryl and endosulfan. Based on the recommended agricultural dose of each insecticide, they concluded that application of endosulfan and carbaryl was potentially dangerous to earthworms.

Bhattacharya and Sahu (2013) studied the effect of imidacloprid on mortality of earthworm *Drawida willsi*, under ideal laboratory conditions to rice field soil. They found that the 96 hrs LC 50 value for with their 95% confidence limit of juvenile, immature and adult earthworm was 4.43, 7.96 and 12.45 mg a.i. imidacloprid /kg dry soil respectively. Although the recommended dose of imidacloprid was lower the 96 h LC 50 values of *D. willsi* for imidacloprid, but still it could affect the soil biota by altering its vital rates and metabolism.

Suthar (2014) examined the toxicological impact of pesticide methyl parathion on growth and reproduction performance in tropical earthworms: *Metaphire posthuma* (endogeic), *Lampito mauritii* (anecic) and *Allolobophora parva* (epigeic). They applied a total of three concentrations (a.i. g/kg dry test soil), 1.00, 1.125 and 2.25 of methyl parathion in test substrate over 60 days under laboratory conditions and observed that methyl parathion caused significant mortality in all tested species and the individual live weight loss was 27–37% in *L. mauritii*, 36–57.1% in *M. posthuma* and 1.2–11.0% in *A. parva* in different test concentrations. The pesticide-exposed worms produced less cocoons than control, but in *L. mauritii*, an unusual reproduction (hormesis) was recorded. They suggested that the methyl parathion had species-specific toxicity against tropical earthworms.

Murugan et al. (2014) assessed the stress of the monocrotophos (pesticide) and glyphosate (herbicide) on *Eudrilus eugeniae*. They found that the biomass of the earthworm was decreased on using monocrotophos and observed that most earthworms showed their preference to be of neutral to slightly acidic when incorporated into glyphosate, monocrotophos soil. They also observed that among the herbicides and pesticides, the concentration of 0.44 ± 0.02 mg/kg of glyphosate accumulated in the different regions of earthworms which were analysed during the study.

Sanchez-Hernandez et al. (2014) examined the integrated biomarker analysis of chlorpyrifos metabolism and toxicity in the earthworm *Aporrectodea caliginosa*. For this, they measured acetylcholinesterase (AChE), carboxylesterase (CbE), cytochrome P450-dependent monooxygenase (CYP450), and glutathione S-transferase (GST) activities in the body wall of earthworms.

Annapoorani (2014) studied the toxic effect of aluminium on reproduction and survival of *Eudrilus eugeniae* (Kinberg) by exposing in moist leaf litter with aluminium. The morphological changes observed during the exposure of animals in laboratory conditions. The reproduction and survival rate were higher in the control than Al treatments. The activity of Al concentrations was found to influence the size of the population and cocoon production and abnormal bulging of clitellar region and over all emancipation of the size of the body.

Hattab et al. (2015) examined the stress response of earthworms (*Eisenia andrei*) to exposure to a commonly used herbicide, 2,4 dichloro-phenoxy-aceticacid (2,4-D). They exposed earthworms to three sublethal concentration of 2,4-D (3.5, 7, and 14 mg/kg) for 7 and 14 days. Results showed that exposure to 7 and 14 mg/kg of 2,4-D significantly reduced both worm body weight and lysosomal membrane stability. The latter is a sensitive stress biomarker in coelomocytes.

Singh and Singh (2015) exposed *Eutyphoeus waltoni* to different concentrations of 2,4-D (200, 300, 400, 450 mg/kg) in feed material (i.e., buffalo dung, wheat straw and gram bran) and different concentrations of 2,4-D (150, 300, 350, 400 mg/kg) in different types of soil (i.e., loamy soil, clay soil and sandy soil) from 24 h up to

240 h. Result indicated that the toxic effect of herbicide 2,4-D on the earthworm *Eutyphoeus waltoni* was both time and dose dependent. Maximum toxicity was observed in the sandy soil, whereas minimum in the feed material of buffalo dung with gram bran.

The toxicity of some selected widely used agricultural pesticides on earthworm *lumbricus terrestris* were studied by **Yuguda et al. (2015)**, using two different types of bioassay, a 48 hours contact filter paper bioassay test and a 14 days soil bioassay test. They also tested some selected heavy metals in the soil before and after the experiment to determine the level of their presence in the selected pesticides. The result of 48 hours contact filer paper test showed that pyrethroid, neonicotinoids and organophosphates to be highly toxic to earthworm (*lumbricus terrestris*) with LC 50 values range from 0.000 ml–0.001 ml. Herbicides and fungicides were relatively low toxic with LC 50 values ranges from 0.002 ml for butachlor and 0.006 ml for mancozeb respectively. Their result for 14 days soil bioassay test revealed different pattern of toxicity. Insecticides, pyrethroid and neonicotinoids, still showed high level of toxicity under soil condition. Herbicides and fungicides do not pose a serious threat to earthworm's survival under soil condition. They concluded that insecticides pyrethroid and neonicotinoids were highly toxic to earthworms in the soil based on agricultural recommended dose/rate of application.

Govindarajan and Prabaharan (2015) determined the impact of monocrotophos on the cocoon production of the earthworm *Eudrilus eugeniae* in laboratory controlled soil. They observed that cocoon production was more sensitive than other parameters such as growth, mortality, etc. They concluded that food played a major role in reproduction potential (cocoon production).

Ahmed (2015) examined the influence of four pesticides (cyren, ridomil, triplen and mamba) on *Lumbricus terrestris* earthworm for 4 weeks in the laboratory. He observed that there were loss of weight in all cultures, signs and symptoms of toxicity like, coiling, body swollen, sluggish movement and discharge of coelomic fluid.

They concluded that among the four pesticides tested cyren was most toxic to earthworm *Lumbricus terristris* causing sever mortality, while triplen and mamba were moderately toxic and ridomil was the least toxic pesticide.

Gaupp-Berghausen et al. (2015) evaluated the effect of glyphosate-based herbicide on *Lumbricus terrestris* and *Aporrectodea caliginosa*. They observed that the surface casting activity of vertically burrowing earthworms (*L. terrestris*) almost ceased three weeks after herbicide application, while the activity of soil dwelling earthworms (*A. caliginosa*) was not affected. Reproduction of the eathworms was reduced by 56% within three months after herbicide application. They found that herbicide application led to increased soil concentrations of nitrate by 1592% and phosphate by 127%, pointing to potential risks for nutrient leaching into streams, lakes, or groundwater aquifers.

Jeyanthi et al. (2016) studied the effect of pesticide (carbaryl) and metal (lead) on the biochemical responses in three earthworm species, *Eudrilus eugeniae, Perionyx ceylanensis and Perionyx excavates*. Their result showed that protein content was slightly increased in *E. eugeniae, P. ceylanensis* when exposed to the lower concentration of Pb (75 mg/kg). But decreased protein content was observed in *P. excavatus* at the higher concentration of carbaryl (50 mg/kg), and with exposure to 300 mg/kg Pb in *P. ceylanensis*. They observed that due to exposure to carbaryl and Pb the levels of antioxidant enzymes glutathione-S-transferase, glutathione peroxidase, reduced glutathione, superoxide dismutase and catalase were changed. But other antioxidant enzymes showed little variation at the time of exposure. They suggested that both carbaryl and Pb induced the production of reactive oxygen species by causing oxidative damage to cells.

Singh and Singh (2016) analyzed the lethal effect of different concentrations of herbicide butachlor (2-chloro 2,6 diethyl N, butoxymethyl acetanilide) on the earthworm *Eutyphoeus waltoni* in different combination of feed materials i.e. buffalo dung, wheat straw and gram bran and soils under the laboratory conditions. Their result showed that maximum toxicity was observed in the sandy soil whereas, minimum in combination of buffalo dung with gram bran.

Dominguez et al. (2016) studied the toxic effect of aminomethyl phosphonic acid (AMPA) on the earthworm *Eisenia andrei*. Aminomethyl phosphonic acid is one of glyphosate's main metabolites which have been classified as persistent in soils, raising concern regarding the widespread use of glyphosate in agriculture and forestry. They found that field-relevant concentrations of AMPA had no significant effects on mortality in acute or chronic assays. Except at the highest concentration tested, a significant biomass loss was observed compared to controls in the chronic assay. Their experimental results showed that the number of juveniles and cocoons increased with higher concentrations of AMPA applied, but their mean weights decreased. This mass loss indicates higher sensitivity of juveniles than adults to AMPA. They suggested that earthworms coming from parents grown in contaminated soils may have reduced growth, limiting their beneficial roles in key soil ecosystem functions.

Pelosi et al. (2016) examined the sublethal effects of epoxiconazole on the earthworm *Aporrectodea icterica*. In their study, they used different doses of a commercial formulation of epoxiconazole, in a natural soil to evaluate their effect on earthworm mortality, uptake, weight gain, enzymatic activities (catalase and glutathione-S-transferase), and energy resources (lipids and glycogens). Weight gains were 28, 19, and 13% of the initial weight after 28 days of exposure in the control and D1 and D10 (1 and 10 times the recommended dose) treatments, respectively. They found that no difference was observed for catalase activity between the three treatments, at 7, 14, or 28 days. The glutathion-S-transferase (GST) activity was two times as high in D1 as in D0 at 14 days. At 28 days, glycogen concentration was lower in D10 than in the D1 treatment. This study highlighted moderate sublethal effects of the commercial formulation for earthworms. Considering that these effects were observed on a species found in cultivated fields, even at recommended rates, muchmore attention should be paid to this pesticide.

2.3 Study on the impact of chemicals on the growth and reproduction of earthworm *Eisenia fetida* together with other species

Mahanthswamy and Patil (2003) studied on the toxicity of different pesticides on four different species of earthworms *Eudrillus eugeniae* (Kinberg), *Eisenia foetida* (Savigny), *Perionyx excavatus* (Stepenson), *Lampito mauritii*. Pesticides were applied by incorporation method to food material (mixture of soil, dried leaves and farmyard manure). They observed that there was a variation in the toxicity of different pesticides to different species of earthworms. Endosulfan 35 EC caused highest mortality to all the species of earthworm. It caused cent percent mortality to *L. mauritii* which was significantly higher than the remaining treatments. Dicofol caused higher mortality next to endosulfan in all the species of earthworms except in *E. fetida*. Carbendazim 50 WP caused maximum mortality to *P. excavatus* (40%) and the lowest mortality to *L. mauritii* (10%). Trifluralin 48 EC caused maximum mortality to *E. eugeniae* (52.50%) and lowest mortality to *L. mauritii* (22.5%).

Tripathi and Bhardwaj (2004) compared the composting potential, biomass growth and biology of a non-native (*Eisenia fetida*) and an endemic (*Lampito mauritii*) species of earthworm in the semiarid environment of Jodhpur district of Rajasthan in India. They reared earthworm in a mixed bedding material comprising of biogas slurry, cowdung, wheat straw, leaflitter, sawdust and kitchen waste. They observed that percentage of organic carbon of the culture bedding material declined upto 105 days with *E. fetida* and 120 days with *L. mauritii* and percentage of nitrogen, phosphorous and potassium increased as a function of the vermicomposting period. In contrast, C/N and C/P ratios decreased day by day. They found that both species were effective for decomposition and mineralization of mixed bedding in the semi-arid environment. They assessed biomass growth and reproductive rates of *E. fetida* and *L. mauritii* under controlled laboratory conditions using mixed bedding. Experimental results showed that cocoon production was higher for *E. fetida* than *L. mauritii* and the net reproductive rate was 9 per month in the case of *E. fetida* and 1 per month for *L. mauritii*. Fertilized eggs of *E. fetida* and *L. mauritii* developed into adults within 4 and 5.25 months, respectively. Their observations indicated that the *E. fetida* may be a more efficient breeder than *L. mauritii* in the desert region of Rajasthan.

The impact of organophosphate pesticides in orchards on earthworms in the Western Cape, South Africa was observed by **Reinecke and Reinecke (2007)**. Worm densities were very low in orchards (22 per m^2) compared to adjacent uncultivated fields (152 per m^2) at a distance from the orchards. The possible effect of organophosphate pesticides on the earthworms was investigated. Background soil concentrations of chlorpyrifos prior to the start of the spraying season were low (0.2–2.7 mg/kg) but persistent for up to 6 months after the last spraying event, and the pesticide was, as a result of rainfall, transported to nontarget areas by runoff. Background concentrations of azinphos methyl were higher than those of chlorpyrifos (1.6–9.8 mg/kg) but not detectable 2 weeks after the spraying event. Azinphos methyl was mostly transported by wind (spray drift) to adjacent areas. A microcosm study indicated the effects of chlorpyrifos on earthworms as determined by measuring biomass change and cholinesterase inhibition. It was concluded that earthworms were affected detrimentally by the pesticides due to chronic (chlorpyrifos) and intermittent (azinphos methyl) exposure.

Reinecke and Reinecke (2007b) also worked on biomarker response and biomass change of earthworms exposed to chlorpyrifos in microcosms. For this microcosms were filled with soil from the same areas and earthworms of the species *A. caliginosa* were introduced. They treated microcosms with a series of concentrations of chlorpyrifos in the laboratory under controlled conditions. These concentrations were chosen to fall within the background ranges found in the soils. They determined biomass of the worms regularly for a period of 5 weeks and worms in a state of estivation were noted. Earthworms were removed from the microcosms for biomarker tests: for cholinesterase (ChE) inhibition assays every week and for a neutral red retention determination 2 weeks after the exposures started. They noted that most prominent biomass loss was in earthworms exposed to the highest pesticide concentration of 8.0 mg/kg. Estivation was higher among earthworms exposed to higher exposure concentrations. Inhibition of ChE increased with higher exposure concentrations and with time but there was no clear dose–related response. They established a clear dose-related response with exposure concentration for the neutral red retention assay. A correlation between ChE inhibition and biomass change existed directly after the second application of chlorpyrifos.

Tu et al. (2011) examined the effect of fungicides and insecticides on earthworm behaviour in controlled environments and on the dynamics of earthworm community in the field by using single application of insecticides sevin (carbaryl) and merit (imidacloprid) at the manufacturer's suggested dose. They observed that these insecticides significantly inhibited earthworm feeding activity for at least three weeks without leading to any earthworm death. While fungicides did not show significant toxicity to earthworms when applied only once, but their toxicities increased as application frequency increased. T-methyl and sevin significantly reduced the abundance and biomass of earthworms in the field, when applying six consecutive weeks. Their suppressive effects lasting for at least 6 weeks after the chemical application was terminated. They suggested that the surface activities of earthworms in turfgrass systems may be managed through moderate application of pesticides at peak periods of earthworm activities.

Dittbrenner et al. (2011) compared the sensitivity of *Eisenia fetida* to *Aporrectodea caliginosa* and *Lumbricus terrestris* after imidacloprid exposure. Results showed significant changes in body mass in *E. fetida* and *A. caliginosa* occurred after exposure to imidacloprid concentrations as low as 0.2 (7 days) and 0.66 mg/kg dry weight (14 days), significant body mass changes in *L. terrestris* observed to 2 and 4 mg/kg dry weight, for 7 and 14 days of exposure, respectively. They found that significant cellular changes already occurred after 24 h exposure to the lowest test concentrations in all species by histopathological examinations, but the degree of detrimental effects as well as species-specific differences were dependent on the monitor tissue. They concluded that *E. fetida* seemed to be more sensitive than *L. terrestris* concerning cellular alterations.

Saxena et al. (2014) performed experiment to establish the use of *Metaphire posthuma* as a sensitive test model for ecotoxicological studies. They carried out acute toxicity testing of carbaryl, carbofuran, cypermethrin and fenvalerate on *Eisenia fetida* and *Metaphire posthuma* by using contact filter paper toxicity and soil toxicity bioassays. They found among the tested chemicals, carbofuran was the most toxic to both the earthworm species. On comparing the toxicity data of these chemicals for both the earthworm species, they found *M. posthuma* was to be more sensitive than *E. fetida*. Based on the acute toxicity data, the order of toxicity of insecticides in both the test procedures was carbofuran > cypermethrin > carbaryl > fenvalerate for *M. posthuma* where as for *E. fetida* it was carbofuran > carbaryl > fenvalerate > cypermethrin. They observed some morphological changes in the organisms exposed to these chemicals which were more pronounced in *M. posthuma* at lower concentrations than *E. fetida* in both the test procedures. They concluded that the use of *M. posthuma* for ecotoxicity studies, being a more sensitive and reliable model than *E. fetida*.

2.4 Study on the impact of chemicals on the growth and reproduction of earthworm *Eisenia fetida*

Helling et al. (2000) studied effects of the fungicide copper oxychloride on the growth and reproduction of *Eisenia fetida* in the experiment lasting for 8 weeks. They selected following life-history parameters: earthworm growth in consecutive weeks, survival rate, maturation time, cocoon production, reproduction success, total number of hatchlings produced, and incubation time for their study. Earthworm growth and cocoon production were significantly reduced at copper oxychloride exposure concentrations of 8.92 mg/kg and higher. Reproduction success in the 8.92 mg/kg Cu substrate was highest. From an exposure concentration of 15.92 mg/kg Cu substrate and higher, there was a considerable impact of copper oxychloride on reproduction. They concluded that from a reduced reproduction success, a reduced mean and maximum number of hatchlings per cocoon, and a longer incubation time, indicating a strong effect of low copper oxychloride concentrations on this earthworm species.

Vermeulen et al. (2001) determined the acute and sublethal effects of the fungicide mancozeb on the earthworm *Eisenia fetida* in laboratory experiments. They selected following life-history parameters for measurement: growth in consecutive weeks over a 6-week period, survival rate, maturation time, cocoon production, hatching success of cocoons, number of hatchlings per cocoon, and incubation time of cocoons over another 4 weeks. Their results indicated that mancozeb had no significant detrimental effect on either growth or reproduction of *E. fetida* at the recommended dose (8 mg/kg) or at an estimated environmental concentration (44 mg/kg). The endings do not support the hypothesis that avoidance response to mancozeb could serve as an indication of toxicity.

Toxic effects of chlorpyrifos on morphology and acetylcholinesterase activity in the earthworm, *Eisenia fetida* was studied by **Rao et al. (2003)**. They carried out a

48-h contact test for acute toxicity of chlorpyrifos in the earthworm, *E. fetida*, as described by OECD guideline 207. The LC 50 of chlorpyrifos was 0.063 mg per m^2. Scanning electron microscopic studies revealed the morphological abnormalities in the worms. The study demonstrated a dose- and time-dependent exposure of chlorpyrifos through skin results, morphological abnormalities, and inhibition of AChE in the earthworm, *E. fetida*.

Xiao et al. (2004) studied single and joint effects of acetochlor and urea on earthworm *Eisenia foetida* populations in phaiozem. They found that acetochlor had an enhanced toxicity from low concentration to high concentration. The mortality of earthworms after a 6-day exposure was changed from 0 to 86.7%, and the weight change rate ranged from 7.86 to 30.43%, when the concentration of acetochlor was increased from 164 to 730 mg/kg. They observed that urea expressed its positive and beneficial effects on earthworms when its concentration was lower than 500 mg/kg, but strongly toxic when the concentration of urea was higher than 1000 mg/kg. The mortality of earthworms exposed to urea reached 100% when its concentration was more than 1500 mg/kg. When the concentration of urea was lower than 500 mg/kg, there were antagonistic effects between the two agrochemicals on earthworms; when the concentration of urea was higher than 500 mg/kg, joint toxic effects of acetochlor and excessive urea on earthworms were synergic. In any case, excessive urea application is very harmful to the health of soil ecosystems.

Maboeta et al. (2004) studied the relationship between lysosomal biomarker and organismal responses in an acute toxicity test with *Eisenia foetida* (Oligochaeta) exposed to the fungicide copper oxychloride. Their aim was to establish whether a lysosomal subcellular response, measured as neutral red retention times, could be linked to the LC 50 and biomass changes. They found that changes in coelomocyte membrane stability manifested earlier than effects on biomass and it may have predictive value and may contribute information during acute toxicity tests, which could be of greater ecological relevance than mortality data alone.

Effects of perchlorate on earthworm (*Eisenia fetida*) survival and reproductive success was examined by **Landrum et al. (2006)** using three types of tests: dermal contact (filter paper), sand, and artificial soil. All studies utilized a range of perchlorate concentrations in order to simulate levels that are likely to occur in the environment under different scenarios (typical soil levels vs. spill levels). Cocoon production was highest in the control group, although overall cocoon production appeared to be low. In contrast to the acute toxicity tests, perchlorate did affect earthworm reproduction at environmentally relevant soil concentrations. In addition, preliminary data suggest that cocoons produced under perchlorate contamination did not hatch as well as cocoons produced in control soil despite incubation of both sets of cocoons in clean soil or sand.

Xiao et al. (2006) showed the fate of herbicide acetochlor and its toxicity to *Eisenia fetida* under laboratory conditions. They studied the effect of the herbicide on growth, reproduction, glutathione-S-transferases (GST) activity and cellulase activity of earthworms by taking 5 concentrations (5, 10, 20, 40 and 80 mg/kg soil). They found that low concentrations had not significant effect on growth of *E. fetida* except after 15 and 30 days of exposure. They observed that when concentration was more than 20 mg/kg, growth rates and numbers of juveniles per cocoon decreased significantly compared to the control in all treatments. However, cellulase activity decreased significantly in all treatments. They concluded that growth, numbers of juveniles per cocoon and cellulase activity can be regarded as sensitive parameters to evaluate the toxicity of acetochlor on earthworms.

Yasmin and D'Souza (2007) investigated the effects of three different pesticides (carbendazim, dimethoate, and glyphosate) and their mixture on the growth and reproduction of the earthworm species, *Eisenia fetida*. Their results showed that the pesticide treatment had a marked negative impact on the growth and reproduction of earthworms. Carbendazim and dimethoate were found to cause greater harm to the selected earthworm species than glyphosate.

Shi et al. (2007) conducted laboratory tests to compare the effects of various concentrations of lindane and deltamethrin on mortality, growth, and cellulase activity in earthworms (*Eisenia fetida*) cultured in artificial soil during typical acute (14 d) and subchronic (42 d) exposure periods. They observed that the toxicity order for earthworm mortality from the 14-day exposure was lindane $>$ deltamethrin, with median lethal concentrations (LC 50) of 162.1 and 432.9 mg/kg, respectively. Earthworms exposed to deltamethrin showed dose-dependent toxic effects on growth and cellulose activity only from the acute exposures, whereas lindane's effects on these activities were seen correlated with both the acute and subchronic doses.

Rombke et al. (2007) investigated the effects of the fungicide benomyl on earthworms in laboratory tests under tropical and temperate conditions. They studied whether the effects of the fungicide benomyl (chosen as a model substance) differ between tropical and temperate regions and whether data generated under temperate conditions can be used for the environmental risk assessment (ERA) in tropical regions. The effect of benomyl on earthworms was evaluated in acute and chronic laboratory tests modified for tropical conditions. These tests were performed at two temperatures $(20^oC$ and $28^oC)$ and with two strains (temperate and tropical) of the compost worm *Eisenia fetida*. The fungicide was spiked in two natural and two artificial soils. The results from the laboratory tests and a literature review showed that the effects of benomyl were, on average, lower under tropical conditions (LC 50: 450–630 mg a.i./kg; EC 50: 0.8–12.9 mg a.i./kg) than under temperate conditions (LC 50: 61–67 mg a.i./kg; EC 50: 1.0–1.6 mg a.i./kg) by a maximum factor of 10.3 (acute tests) and 12.9 (chronic tests). They concluded that this result might be caused by an increased degradation of benomyl, and/or its first metabolite carbendazim, at higher temperatures, but a different sensitivity of the two worm strains cannot be ruled out. Despite the lower toxicity under tropical conditions and assuming comparable application rates, a preliminary assessment confirms the risk of benomyl to soil invertebrates under both conditions.

Reddy and Rao (2008) evaluated acute toxicity, morphological alterations and histological effects of an organophosphorus insecticide, profenofos (PFF) to earthworm, *Eisenia foetida* by direct contact through a filter paper. They observed that earthworms had 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. They assessed neurotoxic potentiality of PFF by measuring acetylcholinesterase (AChE).

Rai and Bansiwal (2009) studied the impact of sublethal doses of an organophosphate pesticide- malathion on growth and reproduction of earthworm *Eisenia foetida*. They analyzed the effects of malathion exposure for 35 days duration and recovery for 35 days on growth and reproductive parameters of earthworm *Eisenia foetida*. They found that the worms of control group gained more weight and produced more numbers of cocoons and hatchlings in compare to malathion exposed worms. Their study revealed that reduction in growth and cocoons productions were due to the toxicity of malathion. It causes concentration dependent changes in growth and reproduction.

Sarojini et al. (2009) studied effect of lignite fly ash on the growth and reproduction of earthworm *Eisenia fetida*. Supply of nutrients from fly ash with biosolids may enhance their agricultural use. They assessed the growth and reproduction of *Eisenia fetida* during vermicomposting of fly ash with cowdung and pressmud in four different proportions and one control i.e., cow dung and pressmud alone. The growth, cocoon and hatchlings production were observed at the interval of 15 days over a period of 60 days. The maximum worm growth and reproduction was observed in bedding material alone.

Owojori et al. (2009) investgated the combined stress effects of salinity and copper on the earthworm *Eisenia fetida*. They exposed *Eisenia fetida* in OECD artificial soil spiked with a range of sub-lethal concentrations of NaCl and Cu singly and as mixtures. Mortality, weight change, and internal copper concentrations were assessed in worms while Cu concentrations in the soil (total, di-ethylene-triamine-penta acetic acid and $CaCl^2$ extractable) were also determined. Their result showed no worm mortality during this study in both individual and joint toxicity tests and increased NaCl and Cu significantly affected the weight change and cocoon production, as individual substances. In combination, the contaminants had mostly additive effects on these worm parameters. They concluded that the effects of salinity, resulting from increased NaCl, on the toxicity of copper to these earthworms were mainly additive but also depended on the concentrations of both substances.

Correia and Moreira (2010) conducted laboratory tests to compare the effects of various concentrations of glyphosate and 2,4-D on earthworms (*Eisenia foetida*) cultured in argissol during 56 days of incubation. The effects on earthworm growth, survival, and reproduction rates were verified for different exposure times. They reported that there was no mortality in glyphosate treated soil samples, but showed gradual and significant reduction in mean weight (50%) at all test concentrations. For 2,4-D, 100% mortality was observed in soil treated with 500 and 1,000 mg/kg. At 14 days, 30%-40% mortality levels were observed in all other concentrations. They found no cocoons or juveniles in soil treated with either herbicide. They concluded that glyphosate and 2,4-D demonstrated severe effects on the development and reproduction of *Eisenia foetida* in laboratory tests in the range of test concentrations.

Gobi and Gunasekaran (2010) explored the effect of butachlor on the life history parameters (biomass, clitellum development, and cocoon production) and the histological changes in the earthworm *Eisenia fetida* over 60 days. They took the dried cow dung contaminated with 0.2575 mg/kg, 0.5150 mg/kg, and 2.5750 mg/kg of butachlor based on the LC 50 value, and a control was maintained. They found mean earthworm biomass decreased with increasing herbicide concentration. Similarly, cocoon production was also reduced by the increasing herbicide concentration. All earthworms in the exposed group were found to have glandular cell enlargement and to be vacuolated.

Zhu et al. (2010) examined the bioaccumulation of penta-BDE (DE-71) in earthworms (*Eisenia fetida*) and the induced toxicities of penta-BDE (DE-71) on the growth and reproduction of earthworms. They observed that all the major congeners in DE-71 could be bioaccumulated in earthworms and the concentration found in earthworms correlated to the spiked concentration in soil. DE-71 might inhibit the growth and reproduction of cocoons and juveniles of earthworms. The toxicities were dose dependent and increased with exposure time.

Zhou et al. (2011) assessed individual and combined toxic effects of cypermethrin and chlorpyrifos on earthworm *Eisenia fetida andrei* for different responses (acute, chronic, behavioural). Their results showed that the toxicity of the mixture of cypermethrin and chlorpyrifos was significantly higher than either of these pesticides individually, especially on the earthworms chronic responses. They found that at a concentration of 5 mg/kg, the mixture caused significant reductions on the growth and reproduction rates of earthworms, but did not cause any significant effect when the individual was tested. They concluded that the increase in toxicity of the pesticide mixture means that the use of toxicity data obtained exclusively from single-pesticide experiments may underestimate the ecological risk of pesticides that actually present in the field.

Dasgupta et al. (2011) evaluated ecotoxicological risks of agricultural application of six insecticides to *Eisenia fetida* by acute toxicity tests under laboratory condition following OECD guidelines. They found that the organochlorine insecticide endosulfan (LC 50: 0.002 mg/kg) and the carbamate insecticides aldicarb (LC 50: 9.42 mg/kg) and carbaryl (LC 50: 4.81 mg/kg) were ecologically most dangerous because LC 50 values of these insecticides were lower than the respective recommended agricultural dose (RAD). Although *E. fetida* was found highly susceptible to the pyrethroid insecticide cypermethrin (LC 50: 0.054 mg/kg), the value was higher than its RAD. The organophosphate insecticides chlorpyrifos (LC 50: 28.58 mg/kg), and monocrotophos (LC 50: 39.75 mg/kg) were found less toxic and ecologically safe because the LC 50 values were much higher than their respective RAD.

Daam et al. (2011) compared the sensitivity of soil invertebrates to pesticides with that of *Eisenia fetida*. They used the relative tolerance approach to enable comparing toxicity thresholds obtained from the US-EPA ECOTOX database, for main pesticidal types of action (insecticides, fungicides, herbicides, and other) and terrestrial taxonomic groups separately. Their analysis reported lower and higher sensitivity of collembolans to fungicides and insecticides, respectively as previously reported. They found that arachnids and isopods were more sensitive to insecticides, and nematodes to fungicides, as compared to *E. fetida*.

The effects of the insecticide lambda-cyhalothrin on the earthworm *Eisenia fetida* under experimental conditions of tropical and temperate regions were studied by **Garcia et al. (2011)**. They performed their experiment with two strains (temperate and tropical) of the compost worm *Eisenia fetida* and acute and chronic laboratory tests modified for tropical conditions, i.e at selected temperatures ((20^oC and (28^oC). The insecticide was spiked in two natural soils, in OECD artificial soil and a newly developed tropical artificial soil. They observed that the effects of lambda-cyhalothrin did rarely vary in the same soil at tropical and temperate temperatures. In tests with tropical soils and high temperature, effect values differed by up to a factor of ten.

Wang et al. (2012) conducted a 48-h filter paper contact test to investigate comparative toxicity of 45 pesticides, including insecticides, acaricides, fungicides, and herbicides, toward the epigeic earthworm *Eisenia fetida*. Results indicated that clothianidin, fenpyroximate, and pyridaben were supertoxic to *E. fetida*, followed by carbaryl, pyridaphenthion, azoxystrobin, cyproconazole, and picoxystrobin, while the other pesticides ranged from being relatively nontoxic to very toxic to the worms.

When experiment conducted in artificial soil for 14 days, clothianidin and picoxystrobin showed the highest intrinsic toxicity against *E. fetida*, followed by fenpyroximate. However, the herbicides fluoroglycofen, paraquat, and pyraflufen-ethyl exhibited the lowest toxicities. In contrast, the other pesticides exhibited relatively low toxicities.

Wu et al. (2012) examined biomarker responses of earthworms, *Eisenia fetida*, exposured to phenanthrene and pyrene both singly and combined in microcosms. For this, they considered following biomarker responses; growth inhibition, enzyme activity, malondialdehyde content, sperm count, neutral-red retention time (NRRT) and annetocin and translationally controlled tumor protein gene transcriptions. Their two-way ANOVA analysis showed that the combination of these two compounds decreased growth, superoxide dismutase activities, NRRT and sperm count synergistically, but increased the catalase activities and malondialdehyde content. A clear dose-related response with exposure concentration was established for the NRRT. Their results demonstrated that earthworms were under physiological stress at field dose of 0.5 (Phe) + 100 (Pyr) mg/kg soils. They stated that phenanthrene and pyrene synergistically decreased sperm count and NRRT, but antagonistically caused changes in antioxidant enzyme activities to disrupt the detoxification functions and inhibit earthworm growth.

Neaman et al. (2012) explored the effects of lime and compost on earthworm (*Eisenia fetida*) reproduction in copper and arsenic contaminated soils from the Puchuncavi Valley, Chile to determine the effectiveness of lime and compost for in situ immobilization of trace elements in the soils. Researchers concluded that compost treatment was effective in improving the quality of soils of Puchuncavi Valley, increasing earthworm reproduction.

Pal and Patidar (2013) studied the effect of insecticide malathion on cocoon production in earthworm *Eisenia foetida*. They observed that the group of earthworms that had been exposed to the high concentration of malathion, matured slowly and cocoon production in the resulting adults was reduced to 55.55% and 43.75% compared to controls exposed for 15 days and 30 days respectively.

Chen et al. (2014) assessed the acute toxicity of butachlor, imidacloprid and chlorpyrifos with different modes of action on earthworm, *Eisenia fetida*. They also compared ecotoxicities of these pesticides for earthworm *E. fetida* separately and in combination in artificial soil and contact filter paper tests. They found that imidacloprid was the most toxic for *E. fetida* with LC 50 (lethal concentration 50) values three orders magnitude lower than that of butachlor and chlorpyrifos in both tests. They compared toxicity of the mixtures to that predicted by the concentration addition (CA) model and found the observed toxicities of all binary mixtures were less than additive. They observed that the combined effects of the pesticides in contact filter paper tests were not consistent with the results in artificial soil toxicity tests, which may be associated with the interaction of soil salts with the pesticides.

Chen et al. (2014b) also studied combined toxicity of butachlor, atrazine and k-cyhalothrin on the earthworm *Eisenia fetida* by combination index (CI)-isobologram method in artificial soil and filter paper tests. The order of toxicity for the individual pesticides was ranked as atrazine $>$ k-cyhalothrin $>$ butachlor in both tests. They found that synergism was observed in majority of the mixtures except for the combination of butachlor plus k-cyhalothrin for most cases in artificial soil test. This particular combination displayed opposite interaction in filter paper test. They compared CI method with the classical models of concentration addition (CA) and independent action (IA) and found that CI method could accurately predict the combined toxicity and can serve as a useful tool in ecotoxicological risk assessment.

Jovana et al. (2014) assessd the toxic effects of three pesticides on the earthworm *Eisenia fetida* (Savigny 1826) to artificial soil supplemented with different concentrations of the examined pesticides based on the recommended agricultural doses (RAD) under laboratory conditions. Their commercial formulations were: galition G-5 (insecticide: a.i. malathion and fenitrothion), terbis (herbicide: a.i. terbuthylazine), and gardene (limacide: a.i. metaldehyde). They chose mortality, biomass, and growth inhibition as toxic endpoints. No death was recorded at the lowest concentration (1/4 RAD) of the insecticide or the limacide after 7 and 14-day exposures, nor was it recorded at the highest concentration (4-RAD) of the insecticide or the limacide after 7-day exposure. They found triazine herbicide terbis the most toxic and ecologically dangerous to *E. fetida*, because it LC 50 value (1.26 mg per kg) was very close to the respective RAD and the growth inhibition in all concentrations was significantly positive. Although *E. fetida* was found susceptible to the galition, due to the significant positive growth inhibition at the highest concentration, the value of LC 50 was higher than its RAD. On the other hand, gardene was found ecologically safe because the LC 50 value was higher than its RAD and weight was not significantly changed.

Garcia-Torres et al. (2014) studied adult mortality, biomass, fecundity and viability of cocoons in *Eisenia fetida* in response to glyphosate exposure in soil. They found 71% mortality for *E. fetida* at the highest concentration of glyphosate (50,000 mg/kg), by day 7 of exposure. Although biomass of *E. fetida*, between the control and 5,000 mg/kg dose at day 14, was not affected and only showed a significant weight loss after 7 days of exposure to 50,000 mg/kg. They observed adverse effects upon adult fecundity and cocoon viability at glyphosate concentrations of 5,000 mg/kg and above.

Santadino et al. (2014) observed sublethal effects of the widely used herbicide glyphosate, on the earthworm (*Eisenia fetida*). They found that the control population had a positive growth rate, both glyphosate treatments showed negative growth rates.

Xu et al. (2014) studied the toxicity and bioaccumulation of ethofumesate enantiomers in earthworm *Eisenia fetida*. They found a slight difference in toxicity to earthworm between two enantiomers and indicated that the acute toxicity of ethofumesate enantiomers was enantioselective. Earthworm can uptake ethofumesate but the bioaccumulation curve did not reach the steady state. In the elimination experiment, the concentrations of ethofumesate in earthworm declined following a first-order decay model with a short half life of 1.8 day. The bioaccumulation and elimination of ethofumesate in earthworm were both nonenantioselective.

Wang et al. (2015a) examined the combined toxicities of five insecticides (chlorpyrifos, avermectin, imidacloprid, cyhalothrin, and phoxim), two herbicides (atrazine and butachlor), and a heavy metal (cadmium) using the acute toxicity test on the earthworm, *Eisenia fetida*. They observed that imidacloprid exhibited the highest acute toxicity toward the earthworm *Eisenia fetida* with a concentration of 2.75 mg/kg being lethal for 50% of the organisms. They also studied toxicological interactions of these chemicals internary mixtures using the combination- index (CI) equation method. They observed that twenty-one ternary mixtures exhibited various interactive effects, in which 11 combinations showed synergistic effects, four led to dual synergistic / additive behaviours, one exhibited an additive effect, and five showed increasing antagonism within the entire range of effects. The CI method was compared with the classical models of concentration addition and independent action, and it was found that the CI method could accurately predict combined toxicity of the chemicals studied. The predicted synergism in the majority of the mixtures, especially at low-effect levels, might have implications in the real terrestrial environment.

Wang et al. (2015b) applied the combination index(CI)-isobologram method which allows computerized quantitation of synergism, additive effect and antagonism to determine the nature of toxicological interactions of two pesticides λ-cyhalothrin, imidacloprid, and heavy metal cadmium towards earthworm *Eisenia fetida*. They found that, in an artificial soil test, λ-cyhalothrin and Cd combination was slightly synergistic at low effect levels which turned into a slight antagonism above effect level values of 0.6, while the binary mixtures containing imidacloprid exhibited antagonism. The presence of imidacloprid in the ternary mixture also resulted in anantagonistic effect to the earthworms. This behaviour became more antagonistic in the ternary mixture in filter paper tests.

Schnug et al. (2015) studied the effects of the insecticide esfenvalerate, the fungicide picoxystrobin and the bactericide triclosan, applied individually and as a mixture, on an earthworm (*Eisenia fetida*) community in the field. They observed that effects on juvenile proportions were less pronounced and more variable than effects on abundance. In general, effects were species-specific and chemical-specific as well as temporal variations distinct. The mixture affected abundance and juvenile proportions, but the latter only at high mixture concentrations.

Wang et al. (2015c) investigated the effects of five neonicotinoid insecticides on reproduction, cellulase activity and the tissues of *Eisenia fetida*. The LC 50 of imidacloprid, acetamiprid, nitenpyram, clothianidin and thiacloprid was 3.05, 2.69, 4.34, 0.93 and 2.68 mg/kg, respectively. These insecticides seriously affect the reproduction of *E. fetida*, reducing the fecundity by 84.0%, 39.5%, 54.3%, 45.7% and 39.5% at the sub-lethal concentrations of 2.0, 1.5, 0.80, 2.0 and 1.5 mg/kg, respectively. **Wang et al. (2015d)** also studied the toxic effect of a neonicotinoid insecticide guadipyr, in earthworm *Eisenia fetida*.

Yang et al. (2015) studied toxicological interactions of two pesticides chlorpyrifos and atrazine and a heavy metal cadmium toward earthworm *Eisenia fetida* by artificial soil and filter paper acute toxicity tests. Their result showed that the binary mixture of chlorpyrifos and atrazine was antagonistic toward *E. fetida*. The combination of atrazine and Cd exhibited a slight degree of synergism throughout the exposure range, while chlorpyrifos plus Cd combination led to dual antagonistic/ synergistic behaviour. The nature of binary combinations in filter paper displayed opposite interaction to that in the artificial soil test, and the toxicity of ternary mixtures was not significantly synergistic than their binaries.

Shi et al. (2016) studied the survival, growth, activity of the biotransformation system phase II enzyme glutathione-S-transferase (GST) and the oxidative defense enzyme catalase (CAT) of earthworms (*Eisenia fetida*) exposed to the contaminated soils from a former DDT plant and reference soils, and compared with the corresponding indicators in simulated soil-earthworm system, unpolluted natural soils with spiked-in DDT series. Their results showed that mortality, growth inhibition rates, GST and CST activities of earthworms exposed to the contaminated soils were significantly higher than that in reference soils.

In addition to this, numerous review papers have been published in this area of work. **Frampton et al. (2006)** reviewed and analysed effects of pesticides on soil invertebrates in laboratory studies using species sensitivity distributions. **Kale and Karmegam (2010)** presented the role of earthworms in tropics with emphasis on Indian ecosystems. **Bhadauria and Saxena (2010)** studied role of earthworms in soil fertility maintenance through the production of biogenic structures. Basic research tools for earthworm has been compiled by **Butt and Grigoropoulou (2010)**. **Yasmin and D'Souza (2010)** presented a review on effects of pesticides on growth and reproduction of earthworms. **Weyers and Spokas (2011)** studied the impact of biochar on earthworm populations. **Pelosi et al. (2013)** published a review on pesticides and earthworms.

Chapter 3

Materials and Methods

Earthworms are pivotal to achieve organic farming. They modify soil organic matter both chemically and physically, mix leaf litter with the soil, facilitate the formation and stabilization of soil aggregates and improve soil porosity. They are ideal test organisms for soil risk assessment due to their high biomass in soil and frequently observed sensitivity to relatively low concentrations of environmental toxicants.

3.1 Test soil

The natural soil was collected from an abandoned area in Kota (India), where no prior agricultural activity was reported and was an area with no known history of pesticides use. The physicochemical characterization of soil was provided by Nanta agricultural farm (Office of Project Director, Crop) in the Kota district.

3.2 Test organisms

The earthworms *Eisenia fetida* (Lumbricidae), were obtained from Krishi Vigyan Kend-ra, Borkhera, Kota. The animals were bred in cattle manure as food at temperature ranging $20 - 30°C$. For all tests, only adult worms with clitellum with a fresh weight between 200 to 300 mg were used. All earthworms were fed according to demand, usually once a week, with finely ground cattle manure free of any chemical contamination. In addition, mass cultures of *E. fetida* were established where the cultures were kept in room temperature at a light cycle of 16 hrs /8 hrs. Finely ground cow dung, free of any chemical contamination, was used to feed the earthworms with an interval of 7 to 10 days. Acclimatization of selected worms was done in the selected soil 24 hrs before the onset of the experiment.

3.3 Test chemicals

Based on the data provided by the Agriculture Research Station (ARS), Umed Ganj, Kota and by interaction with local farmers as well as shopkeepers, four chemicals were selected to study their effect on avoidance, growth and reproduction of earthworms. The objective of the research work was to observe the effect of pesticides used for one Rabi and Kharif season crop on earthworms. Wheat and soybean have been selected as a Rabi and Kharif crop respectively for this study as these two are main crops of the Kota region.

Chemicals used in Wheat crop:

- Chlorpyrifos (O,O-diethyl-O-3.5.6-trichloro-2-pyridyl phosphorothioate) is an organophosphate insecticide. Chlorpyrifos was tested as Radar 20 EC (100 ml, Isagro (Asia) Agrochemical Pvt Ltd, Mumbai). It is used to kill insect pests by disrupting their nervous system. Chlorpyrifos has an advantage over other products in that it is effective against a wide range of plant-eating insect pests.

- 2, 4-D Ethyl ester 38% EC (based on 86% w/w 2,4 dichloro-phenoxy-acetic acid) is a selective herbicide effective against broad leaves weeds in sorgham, maize, wheat, aquatic weeds etc. It was tested as Cut Off 38 (250 ml, Crystal Crop Protection Pvt Ltd). It is a selective, systematic, post emergent herbicide used mainly for control of annual/perennial broad leaved weeds in millets, cereals, wheat, maize, rice, sugarcane and aquatic weeds etc. in accordance with climatic conditions and as per advisory given by agriculture department.

Chemicals used in Soybean crop:

- Triazophos (O, O-diethyl O-(1- Phenyl -1 H -1,2,4-triazol -3 yl) phosphorothioate) is an organophosphates and it is a acetylcholinesterase (AChE) class of inhibitor. Triazophos was tested as Trizocel 40% EC (250 ml, Excel Crop Care Ltd). It is a broad-spectrum insecticide which acts upon insects through contact and stomach action. It penetrates deeply into plant tissues. It is effective for control of stem borer, leaf folder, jassids, green leaf hopper, aphids and bollworms in soybean.
- Pendimethalin (3, 4-Dimethyl-2,6-dinitro-*N*-pentan-3-yl-aniline) is an herbicide of the dinitroaniline class. Pendimethalin was tested as Panida 30 EC (250 ml, Rallis India Ltd). It is used to control annual grasses and certain broadleaf weeds by inhibiting their cell division as well as cell elongation in wheat, barley, corn, soybeans, rice, potato, legumes, fruits, vegetables, nuts and other ornamental plants.

3.4 Identification of earthworm species in Kota

In the present work, earthworm species were collected from different areas around Kota city and their species identified by renowned scientific organization of Government of India.

Earthworms were collected from six different sites on the outskirt areas of Kota city as shown by blue and red rectangles in Figure 3.1. For collecting the earthworms, a pit was dug (four in each area at different places) by ploughing the area (Figure 3.2). Earthworms were collected by hand picking and then transferred to jars and preserved

Figure 3.1: Kota region map showing search areas of earthworm

Figure 3.2: Digging of pit to search earthworms in Kota region

in formaldehyde (formalin). Identification of the earthworms was done at Zoological Survey of India, Kolkata (India). Soil samples from these areas were also collected and their physico-chemical parameters were obtained by sample test at Nanta agricultural farm (Office of Project Director, Crop) in the Kota.

3.5 Study of avoidance behaviour of *Eisenia fetida*

The earthworm avoidance test was developed in USA **(Yeardley et al., 1996)** and since then several studies have been conducted to observe the effects on earthworms for various classes of chemicals.

Avoidance test is an easy and quick to perform. It is known to be sensitive towards a wide range of chemicals. The principle of this test is that the earthworms are simultaneously exposed to the soil sample spiked with the pesticide, and to the control soil. The location of the earthworms is determined after the test period of two days **(ISO, 2008)**. The tendency of a species to avoid a certain study soil in favour of the control soil (free of contaminants) is used as avoidance test to control soil quality and the effects of certain chemicals on the behaviour of earthworm species **(ISO, 2011)**. These tests are based on the fact that chemicals in soil are in different factions depending on the contamination level and soil type and can be absorbed by earthworms. Earthworms can detect a wide range of contaminants due to their chemoreceptors on their anterior segments and sensory tubercles located on the surface body **(Reinecke et al., 2002)**.

The avoidance assays with the earthworms were made based on the ISO guideline 17512-2 (ISO, 2011) and has been performed by two compartment method in a container. Each plastic container (15.5 cm height and diameter 13 cm) was divided into two equal sections with a plastic card (Figure 3.3), one-half of the container received 250 gm (dry weight) of control soil and the other half 250 gm (dry weight) of soil contaminated with the pesticide (Figure 3.4). All combinations of contaminated/ uncontaminated soil were tested, each one with four replicates. After placing the soils into each container, the card divider was removed and 10 worms were placed on the middle line (Figure 3.5). Afterwards, each container was covered with a transparent lid perforated in order to allow aeration. The organisms were incubated at $(20 \pm 2^{o}C)$ with a photoperiod of 16 hrs : 8 hrs (light : dark) for 2 days. After the test period, the divider was put back to separate the control and test soils, and the number of worms in both sections were counted.

The chosen eight concentrations tested were 0.316, 1, 3.162, 10, 31.62, 100, 316.22 and 1000 mg a.i./kg dry soil for all chemicals. For each replicate, the avoidance response is calculated using

$$NR = \frac{(C - T) * 100}{N} \tag{3.1}$$

where, NR = net avoidance response (%), C = number of worms in control soil, T = number of worms in pesticide-amended soil, and N = total number of worms exposed.

Figure 3.3: Creating partition for avoidance test in earthworm

Figure 3.4: Top view of beaker after elimination of partition

Figure 3.5: Placing of earthworm *Eisenia fetida* in the gap

3.6 Study on the impact of chemicals on the growth and reproduction of *Eisenia fetida*

The chosen agrochemicals for the present study were: Chlorpyrifos, 2,4-D Ethyl ester, Triazophos and Pendimethalin. The concentrations used in our experimentation were chosen based on recommended dose of these chemicals for Wheat and Soybean crops in Kota, Rajasthan (India). These doses are 1.2 mg/kg for Chiorpyrifos, 0.75 mg/kg for 2,4-DE, 0.5 mg/kg for Triazophos and 1.5 mg/kg for Pendimethalin. Doses are as per the recommendation of Department of Agriculture, Govt. of Rajasthan, Kota division office. Dose calculations are as per the following:

- **Chiorpyrifos dose calculation:**

    1. Recommended dose is 0.8 kg/hectare.
    2. Assuming that the chemical would disperse into the top 5 cm soil (as per literature), then volume of 1 hectare soil may be calculated as V = 9997.36 x 104 x 5 cm^3 (1 hectare = 9997.36 x 104 cm^2).
    3. Test soil density calculation: 1 kg of dry powdered soil contained 750 ml of volume in a container, so density d of test soil is = 1000/750 = 1.33 gm/cm^3.
    4. Now weight of soil in 1 hectare area (for 5 cm soil depth) is V.d = 9997.36 x 104 x 5 cm^3 x 1.33 gm/cm^3 = 6664824440 gm.
    5. Recommended dose calculation in mg/kg, for dry soil, can now be obtained by using dose of 0.8 kg/hectare. This comes out to be 1.2 mg/kg.
    6. Five chosen concentrations are 1.2, 3.79, 12, 37.9 and 120 mg/kg dry soil weight.

- **2,4-DE dose calculation:**

    1. Recommended dose is 0.5 kg/hectare.

2. Same soil is being used in all sets of experimentation, so soil density under test and weight of 1 hectare soil are same as mentioned earlier.
3. Recommended dose calculation in mg/kg, for dry soil, can now be obtained by using dose of 0.5 kg/hectare. This comes out to 0.75 mg/kg.
4. Five chosen concentrations are 0.75, 2.37, 7.5, 23.7 and 75 mg/kg dry soil weight.

- **Triazophos dose calculation:**

1. Recommended dose 800 ml/hectare (v/w), now converted to (w/w) as per formulation gives 0.320 kg/hectare.
2. Same soil is being used in all sets of experimentation, so soil density under test and weight of 1 hectare soil are same as mentioned earlier.
3. Recommended dose calculation in mg/kg, for dry soil, can now be obtained by using dose of 0.320 kg/hectare. This comes out to be 0.5 mg/kg.
4. Five chosen concentrations are 0.158, 0.50, 1.58, 5, and 15.8 mg/kg dry soil weight.

- **Pendimethalin dose calculation:**

1. Recommended dose is 1 kg/hectare.
2. Same soil is being used in all sets of experimentation, so soil density under test and weight of 1 hectare soil are same as mentioned earlier.
3. Recommended dose calculation in mg/kg, for dry soil, can now be obtained by using dose of 1 kg/hectare. This comes out to be 1.5 mg/kg.
4. Five chosen concentrations are 0.474, 1.50, 4.74, 15, and 47.4 mg/kg dry soil weight.

In our experimentation, five different concentrations were used with a multiplicative factor of 3.167 times. This led to the scenario where every alternative concentration is either 10 times or factored by 10. In our experimentation, one lower concentration of Triazophosis and Pendimethalin is chosen from the recommended dose. This has been done due to observed strong avoidance behaviour of earthworm at 10 mg/kg in Triazophos and 31.6 mg/kg in Pendimethalin in our earlier experimentation. Strong avoidance behaviour at certain concentration levels indicated regarding avoiding very high dose of chemicals in our experimentation. In addition to this, study has been conducted for observing the combined effect of Chiorpyrifos and 2,4-DE as well as Triazophos and Pendimethalin . Combination values of five doses taken in our experimentation were taken as per Tables 3.1 and 3.2.

3.6.1 Earthworm growth and reproduction test

Five sets of different concentrations were prepared to evaluate the growth of earthworms (in four weeks) and number of juveniles (after eight weeks) to test the efficacy of pesticides. For experimentation, raw cow dung was procured from dairy farm and sun dried (Figures 3.6, 3.7 and 3.8). Test soil and cow dung powder were taken in the ratio of 2:1. Dry weight of this mixture was 500 gms for each sample. For experimentation, transparent plastic containers of 1 litre capacity were taken. The soil was artificially contaminated by adding Chiorpyrifos, 2,4-DE and their combination. In another set, Triazophos, Pendimethalin and their combination by adding chosen concentrations of pesticides as stated earlier. In addition to this, one set of experiment was conducted by taking control soil (no contamination). Earthworms were sorted out from the culture and acclimatized for 24 hrs in test soil. After which they were washed with fresh water and left over the blotting paper for some time before measuring their weight on electronic balance. Ten earthworms having weight between 2 to 3 gms were selected (Figure 3.9) and left over on the soil surface of each container. They immediately buried themselves into the soil. Close watch was kept on moisture content of the soil by checking it weekly and maintaining it at 50% by adding water, as required. Quantity of the water to be added was decided by measuring the difference in the weight of the container as compared to weight at the time of sampling. For growth rate observation, experiment lasted for 28 days. Adult earthworms were removed from the chemically treated soil as well as from control soil after 28 days and their weight measured to observe the impact on the biomass growth rate. Biomass growth rate is calculated by dividing average weight of earthworms after 28 days of exposure with average weight of earthworms at the begining of incubation.

For the study of effect on reproduction, cocoons were left in the soil for four additional weeks. After the completion of the period, young worms present in the soil were counted (Figures 3.10, 3.11 and 3.12). This count divided by ten provided the parameter of number of juveniles produced per earthworm.

Table 3.1: Combined concentration values of Chiorpyrifos and 2,4-DE alongwith their labels

1	2	3	4	5
Chiorpyrifos 1.2 mg/kg + 2,4-DE 0.75 mg/kg	Chiorpyrifos 3.79 mg/kg + 2,4-DE 2.37 mg/kg	Chiorpyrifos 12 mg/kg + 2,4-DE 7.5 mg/kg	Chiorpyrifos 37.9 mg/kg + 2,4-DE 23.7 mg/kg	Chiorpyrifos 120 mg/kg + 2,4-DE 75 mg/kg

Table 3.2: Combined concentration values of Triazophos and Pendimethalin alongwith their labels

1	2	3	4	5
Triazophos 0.158 mg/kg + Pendimethalin 0.474 mg/kg	Triazophos 0.5 mg/kg + Pendimethalin 1.5 mg/kg	Triazophos 1.58 mg/kg + Pendimethalin 4.74 mg/kg	Triazophos 5 mg/kg + Pendimethalin 15 mg/kg	Triazophos 15.8 mg/kg + Pendimethalin 47.4 mg/kg

Figure 3.6: Sun drying of raw cow dung

Figure 3.7: Preparing manure in stages

Figure 3.8: Final form of manure for use

Figure 3.9: Weighing of earthworms by electronic machine

Figure 3.10: Counting of juveniles

Figure 3.11: Juveniles after 56 days of exposure to chemicals (view 1)

Figure 3.12: Juveniles after 56 days of exposure to chemicals (view 2)

Chapter 4

Results

4.1 Identification of earthworm species in Kota

Survey was conducted in six different areas of Kota for the presence of earthworms. Of these, three areas showed presence of earthworms. These areas were Bundi road, Rangpur road and Baran road areas (shown as red rectangle in Figure 3.1). Three other areas Rawatbhata road, Jhawalwar road and Abhera areas (shown as blue rectangle) were predominantly marked by the absence of earthworm species. A total number of three species of earthworms were identified belonging to two families (Lumbricidae and Megascolecidae). The species identified under family Lumbricidae is *Eisenia fetida* (Savigny, 1826). The number of species identified under family Megascolecidae are *Lampito mauritii* (Kingberg, 1866) and *Perionyx*. All three species are vermicomposting species. *Eisenia fetida*, as shown in Figure 4.1, was found to be most abundant in Bundi road, Rangpur road and Baran road areas around Kota city. *Lampito mauritii* (Figure 4.2) was found at Bundi road and Rangpur road area and *Perionyx*, as shown in Figure 4.3, was found only at Rangpur road area. Earthworm identification report as received (by Email) from Zoological Survey of India (Kolkata) is enclosed in Appendix.

Figure 4.1: *Eisenia fetida*

Figure 4.2: *Lampito mauritii*

Figure 4.3: *Perionyx excavatus*

Sample soil collected from survey areas contained the pH range 7.89 − 8.10, conductivity in the range 0.320 − 0.475 m Mhos/cm. and organic carbon content between 0.42 − 0.60 %. Earthworm populations depend on both physical and chemical properties of the soil; such as moisture, temperature, pH, salts, texture and aeration as well as available food, and the ability of the species to reproduce and disperse. These soil samples are represented as samples 1 to 3 in soil parameter test report as received from Nanta agricultural farm (Office of Project Director, Crop) and enclosed in Appendix.

4.2 Avoidance behaviour of *Eisenia fetida*

In the present study, it was found that there were no dead or missing worms in the test for all the different concentrations of four chemicals used in experiments, except at the highest concentration of Chlorpyrifos and Pendimethalin i.e. 1000 mg a.i./kg of dry soil, where mortality was 100%. Therefore, this concentration was excluded for the statistical analysis of avoidance behaviour.

When exposed to Chlorpyrifos, *E. fetida* showed a higher avoidance with higher concentrations. Avoidance was seen 40% at very low concentration of 0.316 mg/kg. 55% avoidance ($p < 0.05$) was observed at 1 mg/kg. 75% avoidance was observed at 3.16 mg/kg concentration and 60% avoidance was observed at 10 mg/kg. Earthworm showed more than 90% avoidance behaviour at highest three concentrations (31.6, 100 and 316 mg/kg). Avoidance (%) of *Eisenia fetida* as well as mean and standard deviation (SD) for different concentrations of Chlorpyrifos for four samples are given in Tables 4.1 and 4.2 respectively. Graphical representation of the results of this experiment is shown in Figure 4.4. Experiment exhibited that avoidance behaviour increased with higher concentration of Chlorpyrifos. The only variation was observed at 10 mg/kg concentration, where the avoidance found to be little lower than previous concentration (3.16 mg/kg).

For 2,4-DE chemical, the initial avoidance decreased with increase in concentration value upto 10 mg/kg and thereafter avoidance behaviour showed higher values with higher concentration of the chemical. Avoidance was around 60% at 0.316 mg/kg and 1 mg/kg. Avoidance was seen 20% at 3.16 mg/kg. Minimum avoidance of 5% was shown at 10 mg/kg. 25% avoidance was reflected at the concentration of 31.6 mg/kg. The result of the experiment showed gradual increase for the remaining higher three concentrations and varied between 55% to 90% progressively. Avoidance (%) of *Eisenia fetida* as well as mean and standard deviation (SD) for different concentrations of 2,4-DE for four samples are given in Tables 4.3 and 4.4 respectively. Graphical representation of the results of this experiment is shown in Figure 4.5.

For Triazophos, earthworm showed strong attraction behaviour 85% at 0.316 mg/kg of dry soil. Avoidance was minimum 10% at 1 mg/kg concentration. Avoidance was 70% at 3.16 mg/kg. 85% avoidance was shown at 10 mg/kg. Avoidance was 95% at concentrations of 100 mg/kg and 316 mg/kg. 100% avoidance was observed in earthworms at the highest concentration of 1000 mg/kg. Avoidance (%) of *Eisenia fetida* as well as mean and standard deviation (SD) for different concentrations of Triazophos for four samples are given in Tables 4.5 and 4.6 respectively. Graphical representation of the results of this experiment is shown in Figure 4.6.

When exposed to Pendimethalin, *E. fetida* showed attraction response of 15% and 10% at the very low concentrations of 0.316 mg/kg and 1 mg/kg respectively. 30% and 50% avoidance was observed in earthworms at concentration values of 3.16 mg/kg and 10 mg/kg respectively. Earthworm showed 70% avoidance behaviour at concentrations of 31.6 and 100 mg/kg. 80% avoidance was showed at concentration 316 mg/kg of dry soil. Avoidance (%) of *Eisenia fetida* as well as mean and standard deviation (SD) for different concentrations of Pendimethalin for four samples are given in Tables 4.7 and 4.8 respectively. Graphical representation of the results of this experiment is shown in Figure 4.7.

Table 4.1: Avoidance (%) of *Eisenia fetida* for different concentrations of Chlorpyrifos

Chemical name	Replica no.	0.316 mg/kg	1 mg/kg	3.16 mg/kg	10 mg/kg	31.6 mg/kg	100 mg/kg	316 mg/kg	1000 mg/kg
Chlor-pyrifos	Sample 1	40	60	80	60	100	100	100	died
	Sample 2	40	60	80	80	80	100	100	died
	Sample 3	60	60	60	40	80	100	80	died
	Sample 4	20	40	80	60	100	80	100	died

Table 4.2: Mean and standard deviation (SD) of avoidance of *Eisenia fetida* for different concentrations of Chlorpyrifos

Chemical name	Statistical parameter	0.316 mg/kg	1 mg/kg	3.16 mg/kg	10 mg/kg	31.6 mg/kg	100 mg/kg	316 mg/kg	1000 mg/kg
Chlor-pyrifos	Mean	40	55	75	60	90	95	95	–
	SD	16.33	10	10	16.33	11.55	10	10	–

Table 4.3: Avoidance (%) of *Eisenia fetida* for different concentrations of 2,4-DE

Chemical name	Replica no.	0.316 mg/kg	1 mg/kg	3.16 mg/kg	10 mg/kg	31.6 mg/kg	100 mg/kg	316 mg/kg	1000 mg/kg
2,4-DE	Sample 1	60	60	20	00	20	60	60	80
	Sample 2	60	60	00	20	40	40	80	100
	Sample 3	80	40	40	00	20	60	60	80
	Sample 4	40	60	20	00	20	60	60	100

Table 4.4: Mean and standard deviation (SD) of avoidance of *Eisenia fetida* for different concentrations of 2,4-DE

Chemical name	Statistical parameter	0.316 mg/kg	1 mg/kg	3.16 mg/kg	10 mg/kg	31.6 mg/kg	100 mg/kg	316 mg/kg	1000 mg/kg
2,4-DE	Mean	60	55	20	5	25	55	65	90
	SD	16.33	10	16.33	10	10	10	10	11.55

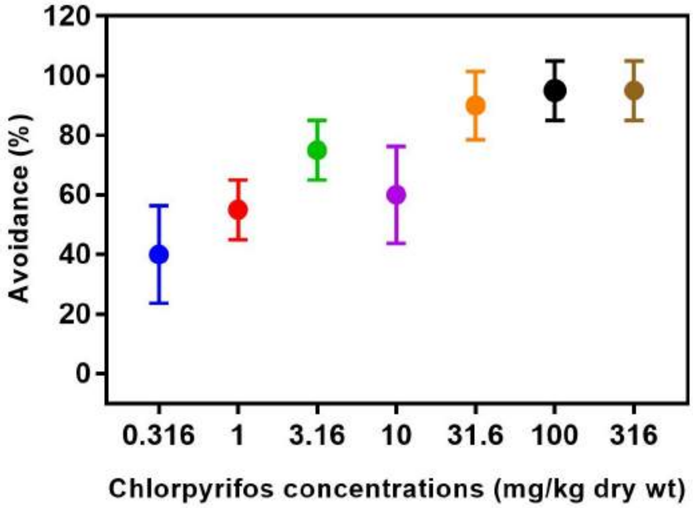

Figure 4.4: Avoidance behaviour of *E. fetida* in Chlorpyrifos with varying concentrations

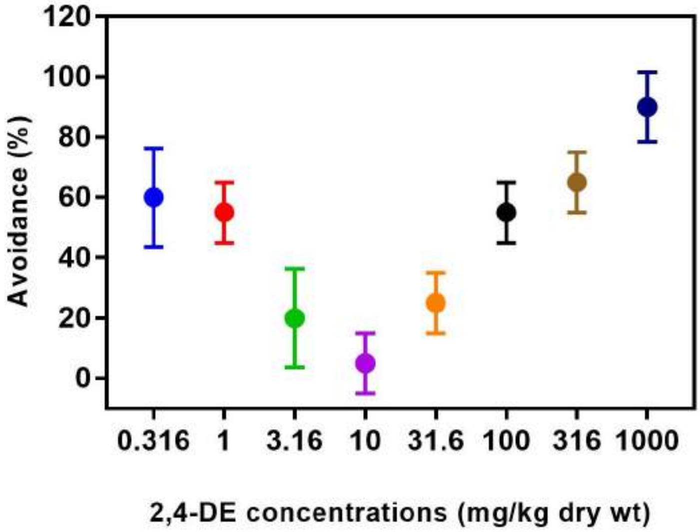

Figure 4.5: Avoidance behaviour of *E. fetida* in 2,4-DE with varying concentrations

Table 4.5: Avoidance (%) of *Eisenia fetida* for different concentrations of Triazophos

Chemical name	Replica no.	0.316 mg/kg	1 mg/kg	3.16 mg/kg	10 mg/kg	31.6 mg/kg	100 mg/kg	316 mg/kg	1000 mg/kg
Tria-zophos	Sample 1	-80	00	80	100	100	100	100	100
	Sample 2	-80	20	60	80	100	80	100	100
	Sample 3	-80	20	60	80	80	100	100	100
	Sample 4	-100	00	60	80	80	80	80	100

Table 4.6: Mean and standard deviation (SD) of avoidance of *Eisenia fetida* for different concentrations of Triazophos

Chemical name	Statistical parameter	0.316 mg/kg	1 mg/kg	3.16 mg/kg	10 mg/kg	31.6 mg/kg	100 mg/kg	316 mg/kg	1000 mg/kg
Tria-zophos	Mean	-85	10	65	85	90	90	95	100
	SD	10	11.55	10	10	11.55	11.55	10	00

Table 4.7: Avoidance (%) of *Eisenia fetida* for different concentrations of Pendimethalin

Chemical name	Replica no.	0.316 mg/kg	1 mg/kg	3.16 mg/kg	10 mg/kg	31.6 mg/kg	100 mg/kg	316 mg/kg	1000 mg/kg
Pendi-methalin	Sample 1	-20	00	40	40	60	60	80	died
	Sample 2	-20	-20	20	60	60	80	80	died
	Sample 3	00	-20	20	40	80	60	60	died
	Sample 4	-20	00	40	60	80	80	80	died

Table 4.8: Mean and standard deviation (SD) of avoidance of *Eisenia fetida* for different concentrations of Pendimethalin

Chemical name	Statistical parameter	0.316 mg/kg	1 mg/kg	3.16 mg/kg	10 mg/kg	31.6 mg/kg	100 mg/kg	316 mg/kg	1000 mg/kg
Pendi-methalin	Mean	-15	-10	30	50	70	70	75	–
	SD	10	11.55	11.55	11.55	11.55	11.55	10	–

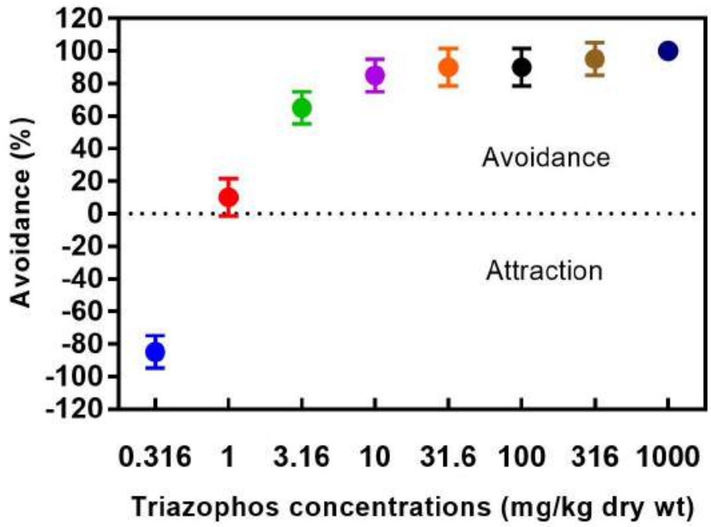

Figure 4.6: Avoidance behaviour of *E. fetida* in Triazophos with varying concentrations

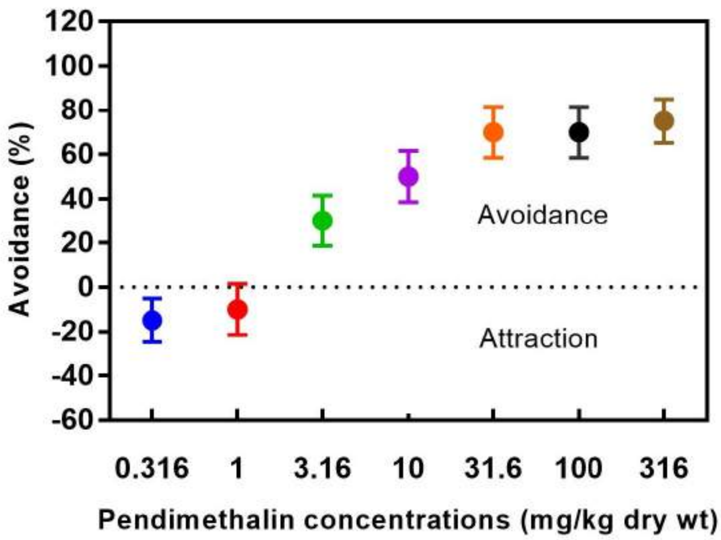

Figure 4.7: Avoidance behaviour of *E. fetida* in Pendimethalin with varying concentrations

4.3 Impact of chemicals on the growth and reproduction of *Eisenia fetida*

The natural soil for the purpose of experimentation was collected from an abandoned area in Kota with no history of agricultural activity and hence no known history of pesticides use. Sample soil of our experiments contained the pH 8.33, conductivity 0.291 m Mhos/cm and organic carbon content is 0.03%. Soil color was brown. This natural soil is represented as sample 4 in soil parameter test report as received from Nanta agricultural farm (Office of Project Director, Crop) and enclosed in Appendix.

4.3.1 Effect of Chlorpyrifos and 2,4-DE on growth and reproduction of *Eisenia fetida*

- **Effect on growth**

  Chlorpyrifos caused decline in growth rates of the treated earthworms compared to the growth rates of the control earthworms. After 28 days of exposure, growth rate of the control earthworms was found $138\% \pm 19.1\%$ while growth reduced to $122.9\% \pm 9.8\%$ in treated soil with concentration of 1.2 mg/kg. Growth rates were $80.4\% \pm 6.47\%$, $64.38\% \pm 3.19\%$, $51.18\% \pm 8.78\%$ and $13.8\% \pm 7.35\%$ at the other concentration values of 3.79 mg/kg, 12 mg/kg, 37.9 mg/kg and 120 mg/kg respectively. The results of five samples for five different concentrations of Chlorpyrifos on biomass growth of *Eisenia fetida* together with biomass growth in control soil are shown in Table 4.9. Graphical results of effect on growth of *Eisenia fetida* due to Chlorpyrifos is shown in Figure 4.8.

  2,4 DE produced a sharp decline in the growth rates as compared to control. This is found to be $89.54\% \pm 3.39\%$, $78.5\% \pm 7.83\%$ and $78.66\% \pm 14.12\%$

for concentration values of 0.75 mg/kg, 2.37 mg/kg and 7.5 mg/kg respectively. Similarly, growth rates were $44.32\% \pm 5.47\%$ and $26.36\% \pm 1.91\%$ for concentration values of 23.7 mg/kg and 75 mg/kg respectively. Table 4.10 shows the result of five samples for five different concentrations of 2,4-DE on biomass growth of *Eisenia fetida* together with biomass growth in control soil. Graphical results of effect on growth of *Eisenia fetida* due 2,4-DE is shown in Figure 4.9.

Combined effects of these two chemicals have also been studied in our experiments and their combined concentrations are reflected in Table 3.1. Growth rate for combination no. 1 was found $80\%\pm4.73\%$, while for combination no. 2, 3, 4 and 5 growth rates were $62.4\%\pm3.12\%, 43.6\%\pm2.99\%, 33.4\%\pm3.35\%$ and $10.2\% \pm 1.84\%$ respectively. Results are shown in Table 4.11. Graphical results of effect on growth of *Eisenia fetida* due to Chlorpyrifos plus 2,4-DE chemicals are shown in Figure 4.10.

- **Effect on reproduction**

The effect of Chlorpyrifos on reproduction was observed and found to be drastically reduced as compared to control soil. After 56 days of exposure, number of juveniles produced per earthworm in control soil was found 22.82 ± 2.72. In treated soil, number of juveniles reduced to 13.2 ± 0.69 with concentration of 1.2 mg/kg Chlorpyrifos. Number of juveniles produced per earthworm were $6.52 \pm 1.022, 4.7 \pm 0.412, 3.86 \pm 0.919$ and 1.08 ± 0.104 at the other concentration values of 3.79 mg/kg, 12 mg/kg, 37.9 mg/kg and 120 mg/kg respectively. The results of five samples for five different concentrations of Chlorpyrifos on reproduction of *Eisenia fetida* together with reproduction in control soil are shown in Table 4.12. Graphical results of effect on reproduction of *Eisenia fetida* due to Chlorpyrifos is shown in Figure 4.11.

2,4-DE also caused decline in number of juveniles produced per earthworm.

This is found to be $8.96 \pm 1.08, 11.41 \pm 1.71, 8.82 \pm 1.38, 7.96 \pm 2.13$ and 6.22 ± 0.222 for concentration values of 0.75 mg/kg, 2.37 mg/kg, 7.5 mg/kg, 23.7 mg/kg and 75 mg/kg respectively. Table 4.13 show the results of five samples for five different concentrations of 2,4-DE on reproduction of *Eisenia fetida* together with reproduction in control soil. Graphical results of effect on reproduction of *Eisenia fetida* due to 2,4-DE is shown in Figure 4.12.

Combined effects of Chlorpyrifos and 2,4-DE on reproduction have also been studied in our experiments. Number of juveniles produced per earthworm in combination no. 1 was found 8.76 ± 0.845, while for combination no. 2, 3, 4 and 5, number of juveniles produced per earthworm were $7.7 \pm 0.978, 5.04 \pm 0.347, 3.9 \pm 0.362$ and 1.9 ± 0.439 respectively. Results show that combined concentrations caused severe negative impact on growth and reproduction. Results are shown in Table 4.14. Graphical results of effect on reproduction of *Eisenia fetida* due to Chlorpyrifos plus 2,4-DE chemicals are shown in Figure 4.13.

The test chemicals, Chlorpyrifos and 2,4-DE not only caused a decline in the growth rate and number of juveniles reduced, but at higher concentrations they also caused swelling on the earthworms body surface. The effect of Chlorpyrifos is shown in Figure 4.14 and the effect of 2,4-DE is shown in Figure 4.15. The cocoons of the earthworm and the emergence of the earthworms from the cocoon are depicted in Figure 4.16 and Figure 4.17 respectively. Figures 4.18 and 4.19 show the earthworm juveniles.

Table 4.9: Effect of different concentrations of Chlorpyrifos on biomass growth of *Eisenia fetida*

Chemical name and concentration	Replica no.	Biomass growth (%)	Mean growth (AvG)	Standard deviation (SD)	Std. error of mean (SE)	Lower and upper 95% CI mean
Control soil	Sample 1	120	138.8	15.42	6.895	119.7
	Sample 2	157				and
	Sample 3	152				157.9
	Sample 4	130				
	Sample 5	135				
Chlorpyrifos	Sample 1	112.9	122.9	7.877	3.523	113.1
1.2	Sample 2	126.5				and
mg/kg	Sample 3	134				132.7
	Sample 4	120				
	Sample 5	121				
Chlorpyrifos	Sample 1	74.6	80.4	6.063	2.711	72.87
3.79	Sample 2	85.5				and
mg/kg	Sample 3	87.9				87.93
	Sample 4	75				
	Sample 5	79				
Chlorpyrifos	Sample 1	64.8	64.38	2.566	1.147	61.19
12	Sample 2	68				and
mg/kg	Sample 3	61.1				67.57
	Sample 4	63				
	Sample 5	65				
Chlorpyrifos	Sample 1	39	51.18	7.072	3.163	42.4
37.9	Sample 2	57				and
mg/kg	Sample 3	54.9				59.96
	Sample 4	53				
	Sample 5	52				
Chlorpyrifos	Sample 1	5.7	13.8	5.92	2.647	6.449
120	Sample 2	18.3				and
mg/kg	Sample 3	20				21.15
	Sample 4	15				
	Sample 5	10				

Table 4.10: Effect of different concentrations of 2,4-DE on biomass growth of *Eisenia fetida*

Chemical name and concentration	Replica no.	Biomass growth (%)	Mean growth (AvG)	Standard deviation (SD)	Std. error of mean (SE)	Lower and upper 95% CI mean
Control soil	Sample 1	120	138.8	15.42	6.895	119.7
	Sample 2	157				and
	Sample 3	152				157.9
	Sample 4	130				
	Sample 5	135				
2,4-DE	Sample 1	93.5	89.54	2.734	1.223	86.15
0.75	Sample 2	90.3				and
mg/kg	Sample 3	86.9				92.93
	Sample 4	87				
	Sample 5	90				
2,4-DE	Sample 1	68	78.5	6.305	2.82	70.67
2.37	Sample 2	79				and
mg/kg	Sample 3	80.5				86.33
	Sample 4	85				
	Sample 5	80				
2,4-DE	Sample 1	98.9	78.66	11.37	5.085	64.54
7.5	Sample 2	75.2				and
mg/kg	Sample 3	72.2				92.78
	Sample 4	74				
	Sample 5	73				
2,4-DE	Sample 1	38.1	44.32	4.375	1.956	38.89
23.7	Sample 2	47.5				and
mg/kg	Sample 3	49				49.75
	Sample 4	45				
	Sample 5	42				
2,4-DE	Sample 1	25	26.36	1.533	0.6856	24.46
75	Sample 2	25.1				and
mg/kg	Sample 3	28.7				28.27
	Sample 4	26				
	Sample 5	27				

Table 4.11: Effect of different combined concentrations of Chlorpyrifos and 2,4-DE on biomass growth of *Eisenia fetida*

Chemical name and concentration	Replica no.	Biomass growth (%)	Mean growth (AvG)	Standard deviation (SD)	Std. error of mean (SE)	Lower and upper 95% CI mean
Control soil	Sample 1	120	138.8	15.42	6.895	119.7
	Sample 2	157				and
	Sample 3	152				157.9
	Sample 4	130				
	Sample 5	135				
Chlorpyrifos	Sample 1	85	80	3.808	1.703	75.27
1.2 mg/kg	Sample 2	80				and
+	Sample 3	75				84.73
2,4-DE	Sample 4	82				
0.75 mg/kg	Sample 5	78				
Chlorpyrifos	Sample 1	65	62.4	2.51	1.122	59.28
3.79 mg/kg	Sample 2	60				and
+	Sample 3	62				65.52
2,4-DE	Sample 4	60				
2.37 mg/kg	Sample 5	65				
Chlorpyrifos	Sample 1	45	43.6	2.408	1.077	40.61
12 mg/kg	Sample 2	42				and
+	Sample 3	47				46.59
2,4-DE	Sample 4	41				
7.5 mg/kg	Sample 5	43				
Chlorpyrifos	Sample 1	35	33.4	2.702	1.208	30.05
37.9 mg/kg	Sample 2	30				and
+	Sample 3	32				36.75
2,4-DE	Sample 4	37				
23.7 mg/kg	Sample 5	33				
Chlorpyrifos	Sample 1	10	10.2	1.483	0.6633	8.358
120 mg/kg	Sample 2	8				and
+	Sample 3	12				12.04
2,4-DE	Sample 4	11				
75 mg/kg	Sample 5	10				

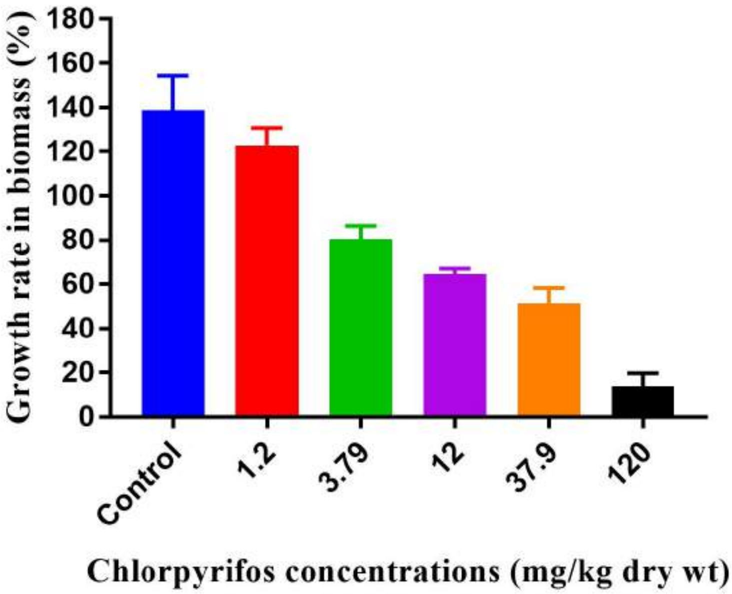

Figure 4.8: Growth rate of *E. fetida* for different concentrations of Chlorpyrifos

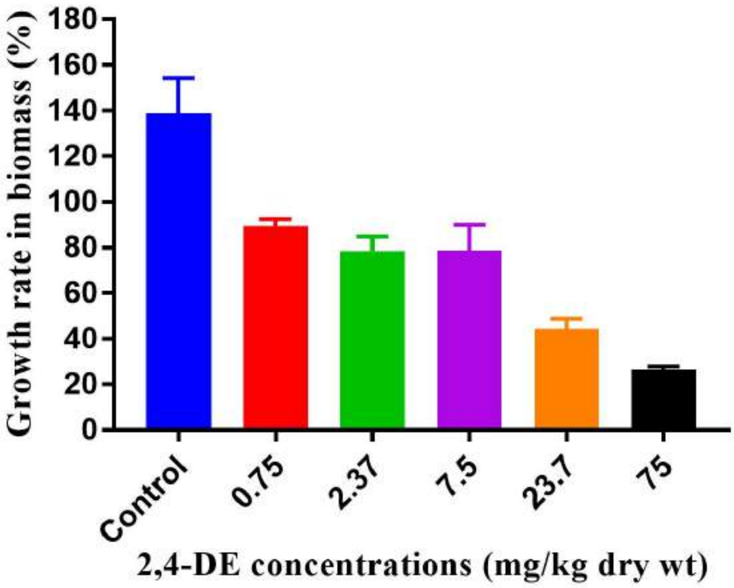

Figure 4.9: Growth rate of *E. fetida* for different concentrations of 2,4-DE

Table 4.12: Effect of different concentrations of Chlorpyrifos on reproduction of *Eisenia fetida*

Chemical name and concentration	Replica no.	No. of juveniles/ earthworm	Mean no. of juv. (AvJ)	Standard deviation (SD)	Std. error of mean (SE)	Lower and upper 95% CI mean
Control soil	Sample 1	22.5	22.82	2.995	1.34	20.10
	Sample 2	25				and
	Sample 3	18.4				25.54
	Sample 4	19				
	Sample 5	24.2				
Chlorpyrifos	Sample 1	12.6	13.2	0.5523	0.247	12.51
1.2	Sample 2	12.9				and
mg/kg	Sample 3	13				13.89
	Sample 4	14				
	Sample 5	13.5				
Chlorpyrifos	Sample 1	5.5	6.52	0.8228	0.368	5.498
3.79	Sample 2	7.6				and
mg/kg	Sample 3	6.5				7.542
	Sample 4	7				
	Sample 5	6				
Chlorpyrifos	Sample 1	4.3	4.7	0.3317	0.1483	4.288
12	Sample 2	4.8				and
mg/kg	Sample 3	4.4				5.112
	Sample 4	5				
	Sample 5	5				
Chlorpyrifos	Sample 1	3.8	3.86	0.7403	0.3311	2.941
37.9	Sample 2	5				and
mg/kg	Sample 3	4				4.779
	Sample 4	3				
	Sample 5	3.5				
Chlorpyrifos	Sample 1	1.1	1.08	0.0836	0.0374	0.976
120	Sample 2	1				and
mg/kg	Sample 3	1.2				1.184
	Sample 4	1				
	Sample 5	1.1				

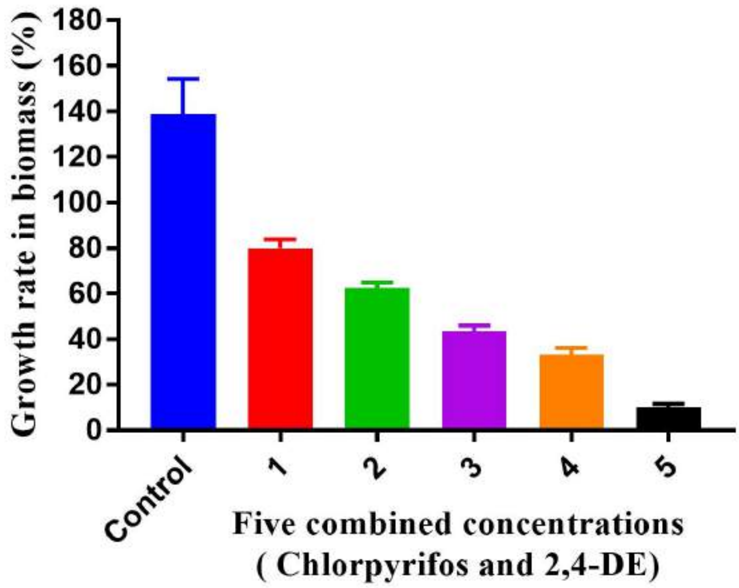

Figure 4.10: Growth rate of *E. fetida* for different combined concentration labels of Chlorpyrifos and 2,4-DE

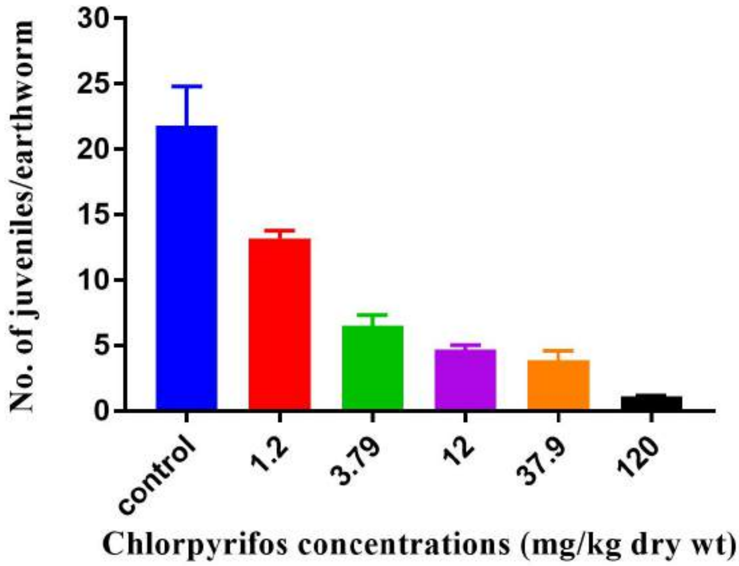

Figure 4.11: No. of juveniles produced per earthworm (*E. fetida*) for different concentrations of Chlorpyrifos

Table 4.13: Effect of different concentrations of 2,4-DE on reproduction of *Eisenia fetida*

Chemical name and concentration	Replica no.	No. of juveniles/ earthworm	Mean no. of juv. (AvJ)	Standard deviation (SD)	Std. error of mean (SE)	Lower and upper 95% CI mean
Control soil	Sample 1	22.5	22.82	2.995	1.34	20.10 and 25.54
	Sample 2	25				
	Sample 3	18.4				
	Sample 4	19				
	Sample 5	24.2				
2,4-DE 0.75 mg/kg	Sample 1	8.4	8.96	0.8735	0.3906	7.875 and 10.04
	Sample 2	7.8				
	Sample 3	9.1				
	Sample 4	9.5				
	Sample 5	10				
2,4-DE 2.37 mg/kg	Sample 1	11.9	11.14	1.374	0.6145	9.434 and 12.85
	Sample 2	12				
	Sample 3	8.8				
	Sample 4	11				
	Sample 5	12				
2,4-DE 7.5 mg/kg	Sample 1	7.1	8.82	1.112	0.4974	7.439 and 10.2
	Sample 2	10				
	Sample 3	8.5				
	Sample 4	9.5				
	Sample 5	9				
2,4-DE 23.7 mg/kg	Sample 1	6	7.96	1.718	0.7685	5.826 and 10.09
	Sample 2	8.9				
	Sample 3	10.4				
	Sample 4	7				
	Sample 5	7.5				
2,4-DE 75 mg/kg	Sample 1	6.5	6.22	0.1789	0.08	5.998 and 6.442
	Sample 2	6.2				
	Sample 3	6.2				
	Sample 4	6				
	Sample 5	6.2				

Table 4.14: Effect of different combined concentrations of Chlorpyrifos and 2,4-DE on reproduction of *Eisenia fetida*

Chemical name and concentration	Replica no.	No. of juveniles/ earthworm	Mean no. of juv. (AvJ)	Standard deviation (SD)	Std. error of mean (SE)	Lower and upper 95% CI mean
Control soil	Sample 1	22.5	22.82	2.995	1.34	20.10
	Sample 2	25				and
	Sample 3	18.4				25.54
	Sample 4	19				
	Sample 5	24.2				
Chlorpyrifos	Sample 1	9.8	8.76	0.6804	0.3043	7.915
1.2 mg/kg	Sample 2	9				and
+	Sample 3	8.5				9.605
2,4-DE	Sample 4	8				
0.75 mg/kg	Sample 5	8.5				
Chlorpyrifos	Sample 1	9	7.7	0.7874	0.3521	6.722
3.79 mg/kg	Sample 2	7.5				and
+	Sample 3	7.8				8.678
2,4-DE	Sample 4	7				
2.37 mg/kg	Sample 5	7.2				
Chlorpyrifos	Sample 1	4.8	5.04	0.2793	0.1249	4.693
12 mg/kg	Sample 2	5.3				and
+	Sample 3	5.3				5.387
2,4-DE	Sample 4	5.1				
7.5 mg/kg	Sample 5	4.7				
Chlorpyrifos	Sample 1	4	3.9	0.2915	0.1304	3.538
37.9 mg/kg	Sample 2	4.1				and
+	Sample 3	4.2				4.262
2,4-DE	Sample 4	3.7				
23.7 mg/kg	Sample 5	3.5				
Chlorpyrifos	Sample 1	2.4	1.9	0.3536	0.1581	1.461
120 mg/kg	Sample 2	2.1				and
+	Sample 3	1.5				2.339
2,4-DE	Sample 4	1.7				
75 mg/kg	Sample 5	1.8				

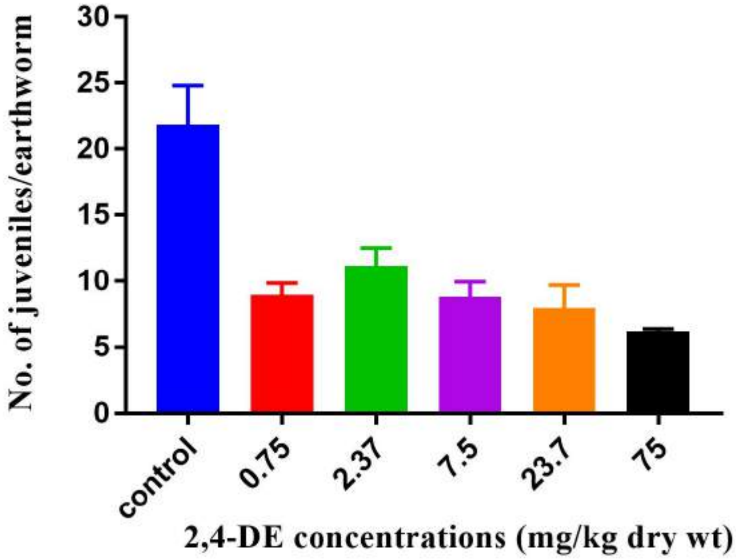

Figure 4.12: No. of juveniles produced per earthworm (*E. fetida*) for different concentrations of 2,4-DE

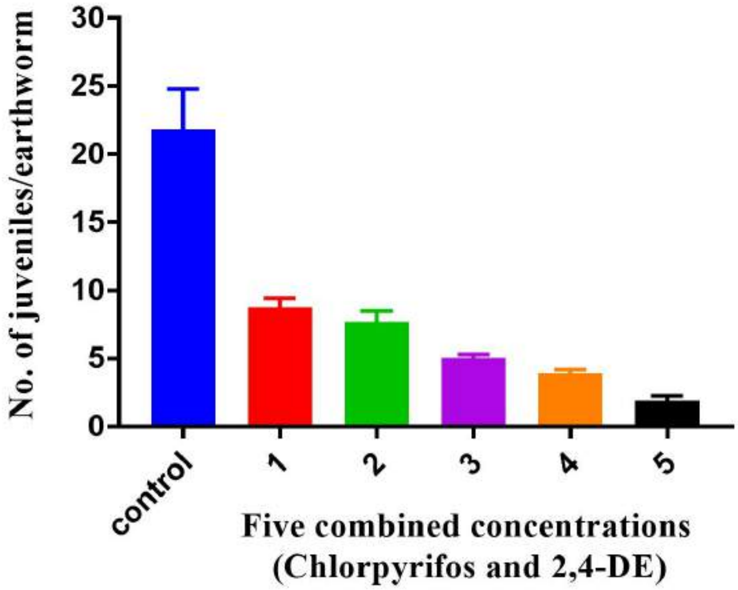

Figure 4.13: No. of juveniles produced per earthworm (*E. fetida*) for different combined concentration labels of Chlorpyrifos and 2,4-DE

Figure 4.14: Earthworm swelling and shrinking due to the effect of Chlorpyrifos

Figure 4.15: Earthworm swelling due to the effect of 2,4-DE

Figure 4.16: Earthworm cocoons

Figure 4.17: Earthworm cocoons showing emergence of juvenile (view 1)

Figure 4.18: Earthworm juveniles (view 1)

Figure 4.19: Earthworm juveniles (view 2)

4.3.2 Statistical analysis of effect of Chlorpyrifos and 2,4-DE

The statistical data of growth rate under influence of Chlorpyrifos, 2,4-DE and their combined concentrations are presented in Table 4.15. Data shows that growth rate is not significant in Chlorpyrifos for 95% confidence interval (CI) of mean. Whereas for 2,4-DE and combined concentrations, results are significant for 95% CI of mean. Correlation value for these chemicals lies in the range of -0.995 to -0.8579. This ascertains the adverse effect on growth rate.

The statistical data showing the number of juveniles produced per earthworm under influence of Chlorpyrifos, 2,4-DE and their combined concentrations is given in Table 4.16. Results show that effect on number of juveniles produced per earthworm under influence of Chlorpyrifos and 2,4-DE is not significant with 95% CI of mean. Whereas for combined concentrations, result is significant with 95% CI of mean. Correlation values between number of juveniles produced per earthworm and concentration lies in the range of -0.9920 to -0.7240. This ascertains the adverse effect on number of juveniles produced per earthworm due to the effect of chemicals.

Most of the P (two-tailed) values are closer to zero as reflected in Tables 4.15 and 4.16. This result ascertains negative effect of chemicals over important life history parameters of earthworm. While $R_{squared}$ values are nearer to one. This shows that data is closer to the fitted regression line around its mean.

The combined plot showing correlation between growth rate of earthworms and number of juveniles produced per earthworm for different concentrations of Chlorpyrifos, 2,4-DE and their combined concentrations are shown in Figures 4.20, 4.21 and 4.22.

Table 4.15: Statistical analysis of biomass growth in *Eisenia fetida* due to Chiorpyrifos and 2,4-DE

Chemical name	Correlation Concentration v/s Biomass growth (%)	95% CI	R squared	P (two-tailed)	Significant (alpha=0.05) Confidence 95%
Chlorpyrifos	-0.8579	-0.9905 to 0.1001	0.7361	0.0629	No
2,4-DE	-0.9205	-0.9948 to -0.2035	0.8473	0.0266	Yes
Chlorpyrifos and 2,4-DE	-0.995	-0.9997 to -0.9233	0.9901	0.0004	Yes

Table 4.16: Statistical analysis of reproduction in *Eisenia fetida* due to Chlorpyrifos and 2,4-DE

Chemical name	Correlation Concentration v/s Juvenile/earthworm	95% CI	R squared	P (two-tailed)	Significant (alpha=0.05) Confidence 95%
Chlorpyrifos	-0.7240	-0.9802 to 0.4382	0.5241	0.1667	No
2,4-DE	-0.8488	-0.9898 to 0.1333	0.7204	0.0690	No
Chlorpyrifos and 2,4-DE	-0.992	-0.9995 to -0.8795	0.9841	0.0009	Yes

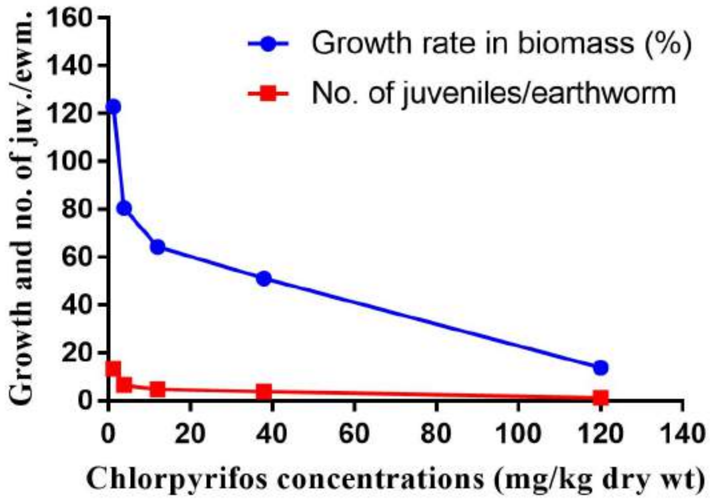

Figure 4.20: Correlation between growth rate and no. of juveniles produced per earthworm for different concentrations of Chlorpyrifos

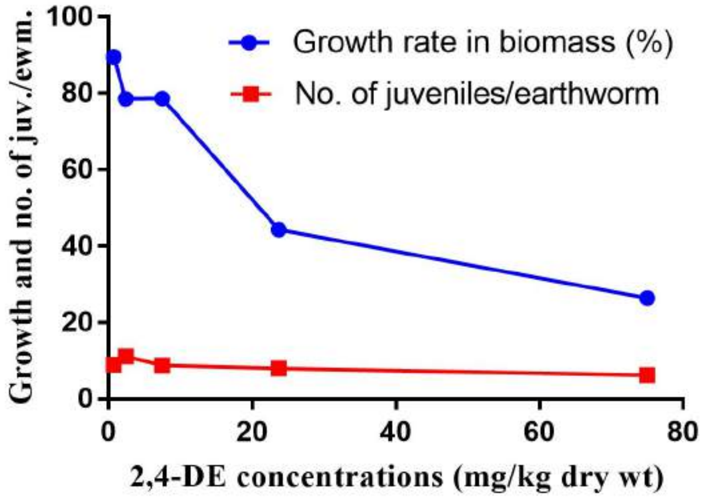

Figure 4.21: Correlation between growth rate and no. of juveniles produced per earthworm for different concentrations of 2,4-DE

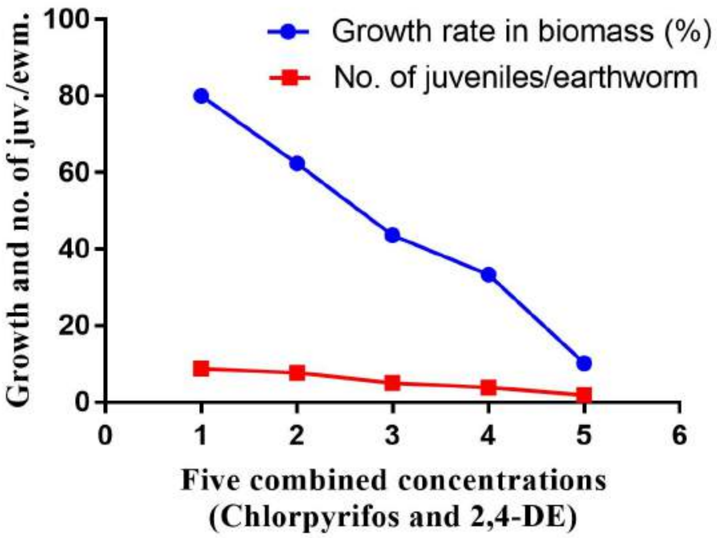

Figure 4.22: Correlation between growth rate and no. of juveniles produced per earthworm for different combined concentration labels of Chlorpyrifos and 2,4-DE

4.3.3 Effect of Triazophos and Pendimethalin on growth and reproduction of *Eisenia fetida*

- **Effect on growth**

Triazophos caused decline in growth rates of the treated earthworms compared to the growth rates of the control earthworms. After 28 days of exposure, growth rate of the control earthworms was found $153\% \pm 20.9\%$ while in treated soil with concentration of 0.158 mg/kg growth reduced to $97.94\% \pm 4.04\%$. At the other concentration values of 0.50 mg/kg, 1.58 mg/kg, 5 mg/kg and 15.8 mg/kg, growth rates were $93.6\% \pm 12.6\%, 69.6\% \pm 4.53\%, 40\% \pm 4.73\%$ and $13.6\% \pm 7.58\%$ respectively. The results of five samples for five different concentrations of Triazophos on biomass growth of *Eisenia fetida* together with biomass growth in control soil are shown in Table 4.17. Graphical results of the effect on growth of *Eisenia fetida* exposed to Triazophos is shown in Figure 4.23.

Pendimethalin also produced a sharp decline in the growth rates. The growth rate at concentration values of 0.474 mg/kg, 1.5 mg/kg, 4.74 mg/kg, 15 mg/kg and 47.4 mg/kg is found to be $38.88\% \pm 11.45\%, 25.48\% \pm 2.29\%, 20.54\% \pm 4.70\%, 19.68\% \pm 6.35\%$ and $10.2\% \pm 4.99\%$ respectively. Triazophos caused lesser decline in growth rate as compared to Pendimethalin. The result of five samples for five different concentrations of Pendimethalin on growth of *Eisenia fetida* together with growth in control soil are shown in Table 4.18. Graphical results of the effect on growth of *Eisenia fetida* due to Pendimethalin is shown in Figure 4.24.

Combined effects of these two chemicals have also been studied in our experiments and their combined concentrations are given in Table 3.2. Growth rate for combination no. 1 was found $106.3\% \pm 4.1\%$, while for combination no. 2, 3, 4 and 5 growth rates were $69.96\% \pm 4.97\%, 57\% \pm 4.56\%, 40\% \pm 4.73\%$

and $11\% \pm 1.96\%$ respectively. Tabular results are shown in Table 4.19. Graphical results of effect on growth of *Eisenia fetida* due to Triazophos plus Pendimethalin chemicals are shown in Figure 4.25.

- **Effect on reproduction**

The effect on reproduction has been found to be drastically reduced as compared to control soil. After 56 days of exposure, number of juveniles produced per earthworm in control soil was found 20.2 ± 3.21, while in soil treated with concentration of 0.158 mg/kg Triazophos, number of juveniles reduced to 8.68 ± 1.41. At the other concentration values of 0.50 mg/kg, 1.58 mg/kg, 5 mg/kg and 15.8 mg/kg, number of juveniles produced per earthworm were $7.5 \pm 0.47, 5.9 \pm 0.8, 4.04 \pm 0.45$ and 2.1 ± 0.53 respectively. The results for five different concentrations (five samples) of Triazophos on reproduction of *Eisenia fetida* together with reproduction in control soil are shown in Table 4.20. Graphical results of effect on reproduction of *Eisenia fetida* due to Triazophos is shown in Figure 4.26.

Pendimethalin also caused decline in number of juveniles produced per earthworm. For concentration values of 0.474 mg/kg, 1.5 mg/kg, 4.74 mg/kg, 15 mg/kg and 47.4 mg/kg, this is found to be $5.84 \pm 0.69, 4.1 \pm 0.36, 3.28 \pm 0.87, 2.82 \pm 1.23$ and 1.38 ± 0.78 respectively. Triazophos caused lesser decline in juvenile numbers as compared to Pendimethalin. The results for five different concentrations (five samples) of Pendimethalin on reproduction of *Eisenia fetida* together with reproduction in control soil is shown in Table 4.21. Graphical results of effect on reproduction of *Eisenia fetida* due to Pendimethalin is shown in Figure 4.27.

Combined effect of Triazophos and Pendimethalin on reproduction have also been studied in our experiments. Number of juveniles produced per earthworm in combination no. 1 was found 7.26 ± 2.11, while for combination no. 2, 3, 4 and 5, number of juveniles produced per earthworm were $6.2 \pm 0.71, 3.32 \pm 0.62, 2.5 \pm 0.34$ and 1.7 ± 0.5 respectively. Results show that combined concentrations caused severe negative impact on growth and reproduction. Result of the combined effects of these two chemicals on reproduction are shown in Table 4.22. Graphical results of effect on reproduction of *Eisenia fetida* due to Triazophos plus Pendimethalin chemicals are shown in Figure 4.28.

Figure 4.29 shows swelling on the body surface of earthworm when exposed to higher concentration of Triazophos and Figure 4.30 shows lesions on earthworm body due to the effect of higher concentration of Pendimethalin. Figure 4.31 shows emergence of juvenile. Figures 4.32 and 4.33 show juveniles of earthworms during counting process done after 56 days.

Table 4.17: Effect of different concentrations of Triazophos on biomass growth of *Eisenia fetida*

Chemical name and concentration	Replica no.	Biomass growth (%)	Mean growth (AvG)	Standard deviation (SD)	Std. error of mean (SE)	Lower and upper 95% CI mean
Control soil	Sample 1	161	153.3	16.81	7.52	132.4
	Sample 2	130.9				and
	Sample 3	140				174.2
	Sample 4	166.6				
	Sample 5	168				
Triazophos	Sample 1	101	97.94	3.251	1.454	93.9
0.158	Sample 2	100.7				and
mg/kg	Sample 3	99				102
	Sample 4	95				
	Sample 5	94				
Triazophos	Sample 1	93	93.6	10.14	4.534	81.01
0.5	Sample 2	111				and
mg/kg	Sample 3	90				106.2
	Sample 4	85				
	Sample 5	89				
Triazophos	Sample 1	65	69.6	3.647	1.631	65.07
1.58	Sample 2	68				and
mg/kg	Sample 3	70				74.13
	Sample 4	75				
	Sample 5	70				
Triazophos	Sample 1	45	40	3.808	1.703	35.27
5	Sample 2	42				and
mg/kg	Sample 3	40				44.73
	Sample 4	38				
	Sample 5	35				
Triazophos	Sample 1	10	13.6	6.107	2.731	6.017
15.8	Sample 2	15				and
mg/kg	Sample 3	18				21.18
	Sample 4	20				
	Sample 5	5				

Table 4.18: Effect of different concentrations of Pendimethalin on biomass growth of *Eisenia fetida*

Chemical name and concentration	Replica no.	Biomass growth (%)	Mean growth (AvG)	Standard deviation (SD)	Std. error of mean (SE)	Lower and upper 95% CI mean
Control soil	Sample 1	161	153.3	16.81	7.52	132.4 and 174.2
	Sample 2	130.9				
	Sample 3	140				
	Sample 4	166.6				
	Sample 5	168				
Pendimethalin 0.474 mg/kg	Sample 1	31	38.88	9.218	4.122	27.43 and 50.33
	Sample 2	53				
	Sample 3	33.7				
	Sample 4	43.4				
	Sample 5	33.3				
Pendimethalin 1.5 mg/kg	Sample 1	27.4	25.48	1.843	0.824	23.19 and 27.77
	Sample 2	24				
	Sample 3	24.8				
	Sample 4	27.5				
	Sample 5	23.7				
Pendimethalin 4.74 mg/kg	Sample 1	19.2	20.54	3.787	1.694	15.84 and 25.24
	Sample 2	19.5				
	Sample 3	27.2				
	Sample 4	17.7				
	Sample 5	19.1				
Pendimethalin 15 mg/kg	Sample 1	28.5	19.68	5.117	2.289	13.33 and 26.03
	Sample 2	19.6				
	Sample 3	16				
	Sample 4	17.7				
	Sample 5	16.6				
Pendimethalin 47.4 mg/kg	Sample 1	9.1	10.2	3.874	1.732	5.39 and 15.01
	Sample 2	16.5				
	Sample 3	6				
	Sample 4	9				
	Sample 5	10.4				

Table 4.19: Effect of different combined concentrations of Triazophos and Pendimethalin on biomass growth of *Eisenia fetida*

Chemical name and concentration	Replica no.	Biomass growth (%)	Mean growth (AvG)	Standard deviation (SD)	Std. error of mean (SE)	Lower and upper 95% CI mean
Control soil	Sample 1	161	153.3	16.81	7.52	132.4
	Sample 2	130.9				and
	Sample 3	140				174.2
	Sample 4	166.6				
	Sample 5	168				
Triazophos	Sample 1	108	106.3	3.324	1.487	102.2
0.158 mg/kg	Sample 2	108.6				and
+	Sample 3	108.8				110.4
Pendimethalin	Sample 4	105				
0.474 mg/kg	Sample 5	101				
Triazophos	Sample 1	65.7	69.94	3.757	1.68	65.27
0.5 mg/kg	Sample 2	70				and
+	Sample 3	75				74.61
Pendimethalin	Sample 4	72				
1.5 mg/kg	Sample 5	67				
Triazophos	Sample 1	55	57	3.674	1.643	52.44
1.58 mg/kg	Sample 2	52				and
+	Sample 3	57				61.56
Pendimethalin	Sample 4	61				
4.74 mg/kg	Sample 5	60				
Triazophos	Sample 1	45	40	3.808	1.703	35.27
5 mg/kg	Sample 2	42				and
+	Sample 3	40				44.73
Pendimethalin	Sample 4	38				
15 mg/kg	Sample 5	35				
Triazophos	Sample 1	10	11	1.581	0.7071	9.037
15.8 mg/kg	Sample 2	12				and
+	Sample 3	13				12.96
Pendimethalin	Sample 4	11				
47.4 mg/kg	Sample 5	9				

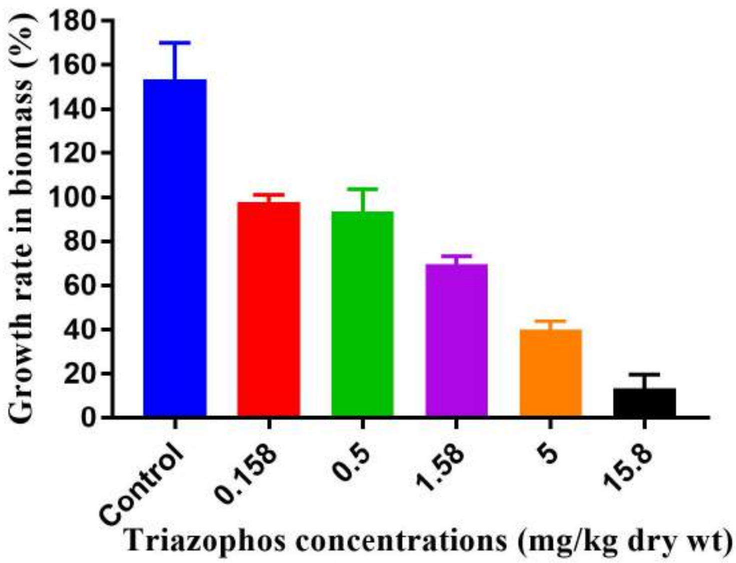

Figure 4.23: Growth rate of *E. fetida* for different concentrations of Triazophos

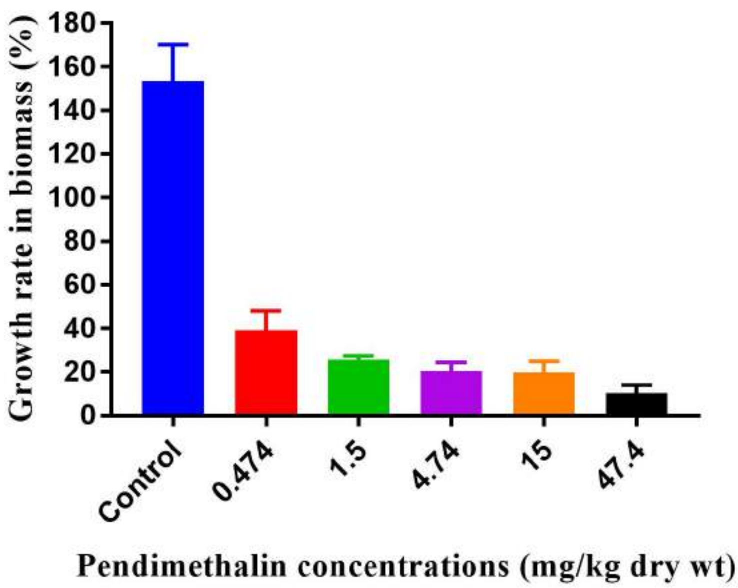

Figure 4.24: Growth rate of *E. fetida* for different concentrations of Pendimethalin

Table 4.20: Effect of different concentrations of Triazophos on reproduction of *Eisenia fetida*

Chemical name and concentration	Replica no.	No. of juveniles/ earthworm	Mean no. of juv. (AvJ)	Standard deviation (SD)	Std. error of mean (SE)	Lower and upper 95% CI mean
Control soil	Sample 1	20.1	20.2	2.586	1.156	16.99
	Sample 2	21.2				and
	Sample 3	16				23.41
	Sample 4	23.4				
	Sample 5	20.7				
Triazophos	Sample 1	7.1	8.68	1.139	0.5093	7.266
0.158	Sample 2	9.3				and
mg/kg	Sample 3	9				10.09
	Sample 4	10				
	Sample 5	8				
Triazophos	Sample 1	7.5	7.5	0.3808	0.1703	7.027
0.5	Sample 2	8				and
mg/kg	Sample 3	7				7.973
	Sample 4	7.3				
	Sample 5	7.7				
Triazophos	Sample 1	5.5	5.9	0.6519	0.2915	5.091
1.58	Sample 2	5				and
mg/kg	Sample 3	6.5				6.709
	Sample 4	6				
	Sample 5	6.5				
Triazophos	Sample 1	4	4.04	0.3647	0.1631	3.587
5	Sample 2	4.2				and
mg/kg	Sample 3	4.5				4.493
	Sample 4	4				
	Sample 5	3.5				
Triazophos	Sample 1	2	2.1	0.4301	0.1924	1.566
15.8	Sample 2	2.2				and
mg/kg	Sample 3	2.1				2.634
	Sample 4	1.5				
	Sample 5	2.7				

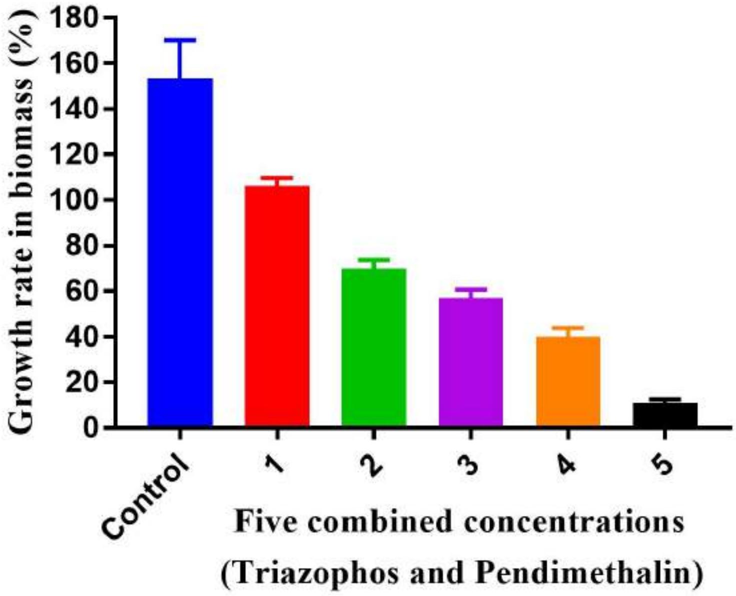

Figure 4.25: Growth rate of *E. fetida* for different combined concentration labels of Triazophos and Pendimethalin

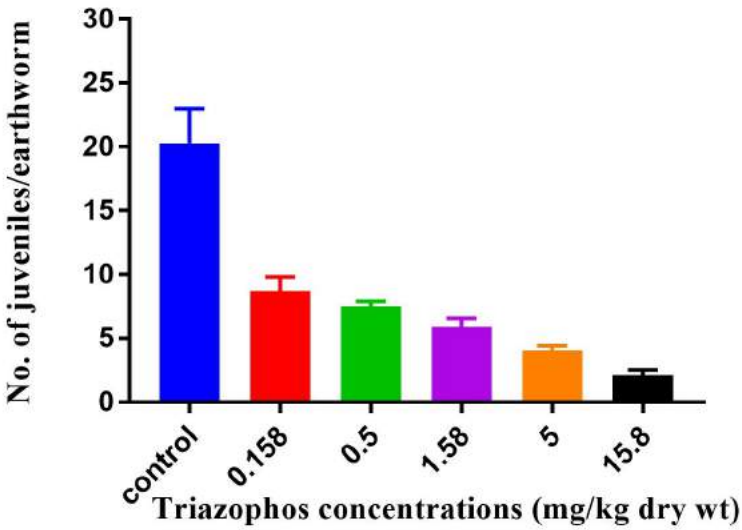

Figure 4.26: No. of juveniles produced per earthworm (*E. fetida*) for different concentrations of Triazophos

Table 4.21: Effect of different concentrations of Pendimethalin on reproduction of *Eisenia fetida*

Chemical name and concentration	Replica no.	No. of juveniles/ earthworm	Mean no. of juv. (AvJ)	Standard deviation (SD)	Std. error of mean (SE)	Lower and upper 95% CI mean
Control soil	Sample 1	20.1	20.2	2.586	1.156	16.99 and 23.41
	Sample 2	21.2				
	Sample 3	16				
	Sample 4	23.4				
	Sample 5	20.7				
Pendimethalin 0.474 mg/kg	Sample 1	6.5	5.84	0.555	0.2482	5.151 and 6.529
	Sample 2	5				
	Sample 3	5.7				
	Sample 4	6.1				
	Sample 5	5.9				
Pendimethalin 1.5 mg/kg	Sample 1	4.2	4.1	0.2915	0.1304	3.738 and 4.462
	Sample 2	4.5				
	Sample 3	4				
	Sample 4	3.7				
	Sample 5	4.1				
Pendimethalin 4.74 mg/kg	Sample 1	2.4	3.28	0.6979	0.3121	2.413 and 4.147
	Sample 2	2.7				
	Sample 3	4				
	Sample 4	3.5				
	Sample 5	3.8				
Pendimethalin 15 mg/kg	Sample 1	1.3	2.82	0.9834	0.4398	1.599 and 4.041
	Sample 2	3				
	Sample 3	2.5				
	Sample 4	3.8				
	Sample 5	3.5				
Pendimethalin 47.4 mg/kg	Sample 1	1.9	1.38	0.6301	0.2818	0.5977 and 2.162
	Sample 2	1				
	Sample 3	0.5				
	Sample 4	2				
	Sample 5	1.5				

Table 4.22: Effect of different combined concentrations of Triazophos and Pendimethalin on reproduction of *Eisenia fetida*

Chemical name and concentration	Replica no.	No. of juveniles/ earthworm	Mean no. of juv. (AvJ)	Standard deviation (SD)	Std. error of mean (SE)	Lower and upper 95% CI mean
Control soil	Sample 1	20.1	20.2	2.586	1.156	16.99
	Sample 2	21.2				and
	Sample 3	16				23.41
	Sample 4	23.4				
	Sample 5	20.7				
Triazophos	Sample 1	8.7	7.26	1.704	0.762	5.144
0.158 mg/kg	Sample 2	8.6				and
+	Sample 3	4.5				9.376
Pendimethalin	Sample 4	7				
0.474 mg/kg	Sample 5	7.5				
Triazophos	Sample 1	7	6.2	0.5701	0.255	5.492
0.5 mg/kg	Sample 2	6				and
+	Sample 3	6.5				6.908
Pendimethalin	Sample 4	5.5				
1.5 mg/kg	Sample 5	6				
Triazophos	Sample 1	3.8	3.32	0.497	0.2223	2.703
1.58 mg/kg	Sample 2	3.9				and
+	Sample 3	2.8				3.937
Pendimethalin	Sample 4	3				
4.74 mg/kg	Sample 5	3.1				
Triazophos	Sample 1	2.5	2.5	0.2739	0.1225	2.16
5 mg/kg	Sample 2	2.4				and
+	Sample 3	2.7				2.84
Pendimethalin	Sample 4	2.8				
15 mg/kg	Sample 5	2.1				
Triazophos	Sample 1	1.7	1.7	0.4062	0.1817	1.196
15.8 mg/kg	Sample 2	2.3				and
+	Sample 3	1.8				2.204
Pendimethalin	Sample 4	1.5				
47.4 mg/kg	Sample 5	1.2				

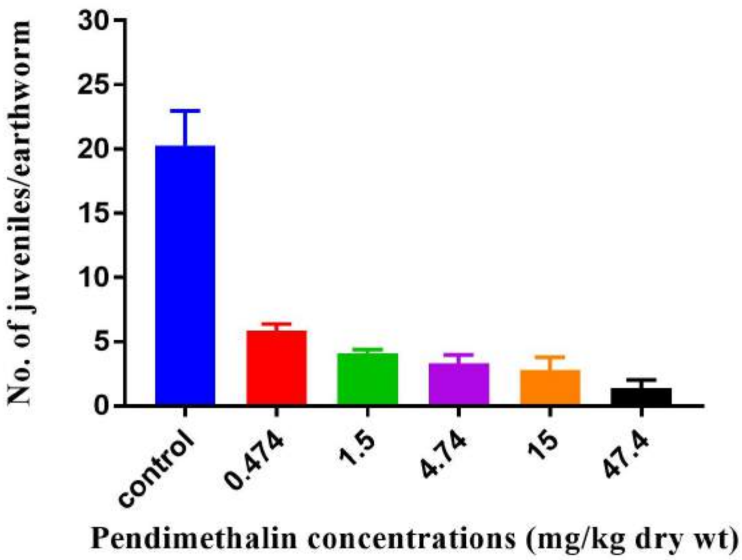

Figure 4.27: No. of juveniles produced per earthworm (*E. fetida*) for different concentrations of Pendimethalin

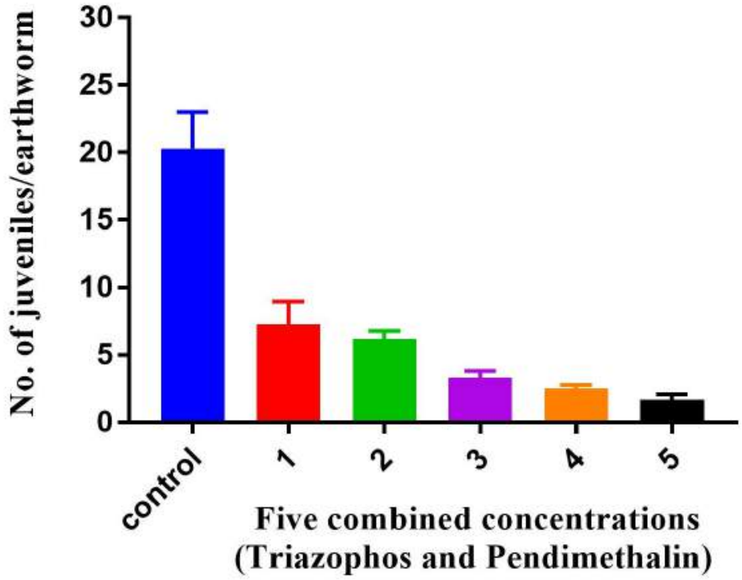

Figure 4.28: No. of juveniles produced per earthworm (*E. fetida*) for different combined concentration labels of Triazophos and Pendimethalin

Figure 4.29: Earthworm swelling due to the effect of Triazophos

Figure 4.30: Earthworm showing lesions due to the effect of Pendimethalin

Figure 4.31: Earthworm cocoons showing emergence of juvenile (view 2)

Figure 4.32: Earthworm juveniles (view 3)

Figure 4.33: Earthworm juveniles (view 4)

4.3.4 Statistical analysis of effect of Triazophos and Pendimethalin

The statistical data of growth rate under influence of Triazophos, Pendimethalin and their combined concentration are presented in Table 4.23. Data shows that effect on growth rate is significant in Triazophos and combined concentrations for 95% confidence interval (CI) of mean. Whereas for Pendimethalin, results are not significant for 95% CI of mean. Correlation value for these chemicals lies in the range of -0.986 to -0.7947. This again ascertains the adverse effect on growth rate.

The statistical data showing the number of juveniles produced per earthworm under influence of Triazophos, Pendimethalin and their combined concentrations is given in Table 4.24. Results shows that effect on number of juveniles produced per earthworm under influence of Pendimethalin is not significant with 95% CI of mean. Whereas for Triazophos and combined concentrations, results are significant with 95% CI of mean. Correlation values between number of juveniles produced per earthworm and concentration lies in the range of -0.9714 to -0.8403. This ascertains the adverse effect on number of juveniles produced per earthworm in our experiments.

Most of the P (two-tailed) values are closer to zero as reflected in Tables 4.23 and 4.24. This result ascertains negative effect of chemicals over important life history parameters of earthworm. While $R_{squared}$ values are nearer to one. This shows that data is closer to the fitted regression line around its mean.

The combined plot showing correlation between growth rate of earthworms and number of juveniles produced per earthworm for different concentrations of Triazophos, Pendimethalin and their combined concentrations are shown in Figures 4.34, 4.35 and 4.36.

Table 4.23: Statistical analysis of biomass growth in *Eisenia fetida* due to Triazophos and Pendimethalin

Chemical name	Correlation Concentration v/s Biomass growth (%)	95% CI	R squared	P (two-tailed)	Significant (alpha=0.05) Confidence 95%
Triazophos	-0.9172	-0.9946 to -0.1832	0.8413	0.0282	Yes
Pendimethalin	-0.7947	-0.9858 to 0.2930	0.6316	0.1082	No
Triazophos and Pendimethalin	-0.986	-0.9991 to -0.7977	0.9722	0.0020	Yes

Table 4.24: Statistical analysis of reproduction in *Eisenia fetida* due to Triazophos and Pendimethalin

Chemical name	Correlation Concentration v/s Juvenile/earthworm	95% CI	R squared	P (two-tailed)	Significant (alpha=0.05) Confidence 95%
Triazophos	-0.9005	-0.9935 to -0.0887	0.8109	0.0371	Yes
Pendimethalin	-0.8403	-0.9892 to 0.1622	0.7061	0.0747	No
Triazophos and Pendimethalin	-0.9714	-0.9982 to -0.6229	0.9435	0.0058	Yes

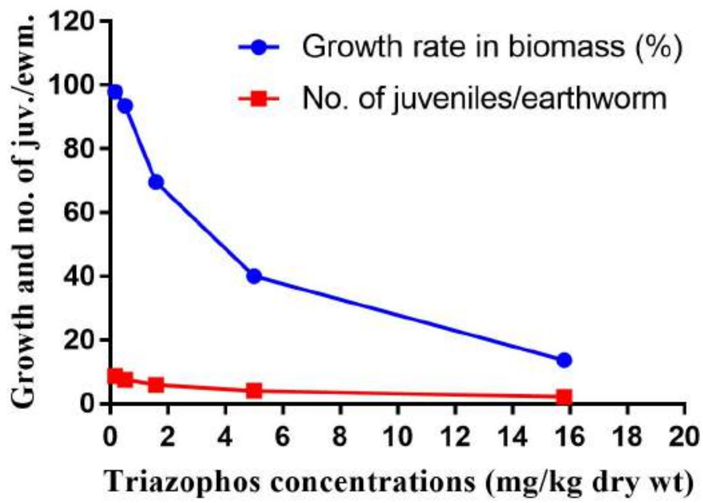

Figure 4.34: Correlation between growth rate and no. of juveniles produced per earthworm for different concentrations of Triazophos

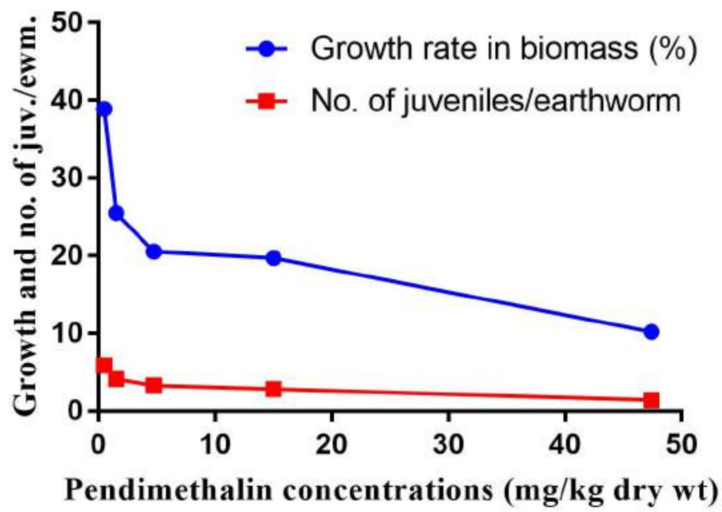

Figure 4.35: Correlation between growth rate and no. of juveniles produced per earthworm for different concentrations of Pendimethalin

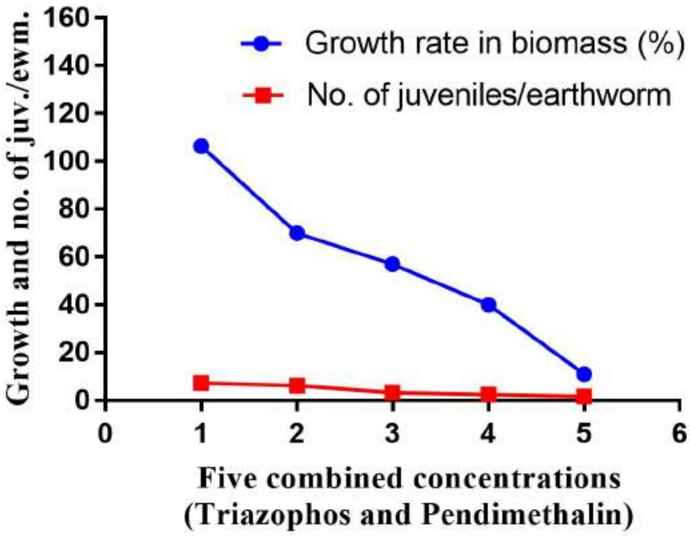

Figure 4.36: Correlation between growth rate and no. of juveniles produced per earthworm for different combined concentration labels of Triazophs and Pendimethalin

Chapter 5

Discussion

5.1 Identification of earthworm species in Kota

Survey of the areas revealed that earthworms were found only in the agricultural areas of Kota i.e., Bundi road, Rangpur road and Baran road. As Rawatbhata road, Jhawalwar road and Abhera area constitute the rocky terrain and also because of closed proximity of Abhera to the Thermal plant, no earthworms were found. *Eisenia fetida* was found to be the prominent species in all the agricultural areas around Kota.

Eisenia fetida also known as the red wiggler, brandling worm, dung worm, or the tiger worm, is found extensively not only in the ground but also in various habitats around the world. This is a common worm associated with garbage and waste and used also as a fishing bait. It is also probably the most widely used worm for vermicomposting. *Eisenia fetida* is the standard test organism used in terrestrial ecotoxicology, because it can easily be bred on a variety of organic wastes with short generation times **(ISO 1998, OECD 1984 and 2004)**. Although, earthworm species vary in their tolerance, reports have shown a decline in the earthworm populations in response to large amounts of organic chemical deposition **(Bayer and Foy 1982)**.

The anecic earthworm, i.e. *Lampito mauritii*, is commonly found in Indian soils, used as an efficient tool for organic waste reduction **(Tripathi and Bharadwaj 2004)**. The composting efficiency and biology of *Lampito mauritii*, is well documented in literature. Several authors have reported the vermicomposting potential of *Lampito mauritii* by using a variety of organic wastes **(Manivannan 2005, Suthar and Singh 2008)**. It can withstand wide range of temperature, soil moisture and various other physical factors **(Kale 1988)**.

Perionyx excavatus (Perrier, 1872), is a beautiful worm with an iridescent blue or violet sheen to its skin clearly visible under bright light. This species is mainly found in tropical regions, especially in Asia **(Blakemore et al., 2006)** and is also present in Europe and North America **(Edwards et al., 1998)**. Although primarily a compost worm, it is commonly found in the top soil layer (0-15 cm) at temperatures ranging between 20.8^oC and 28.8^oC and pH of 6.4-7.4 as discussed by **Bhattacharjee and Chaudhuri (2002)**. **Hallatt et al. (1990)**, **Joshi and Dabral (2008)** and **Reinecke and Hallatt (1989)** studied the life cycle and biology of this species extensively. This species makes excellent fishing bait. Like all tropical worm species, this species has a very poor tolerance for low temperatures, fluctuations in their environment or disruption to the system.

5.2 Avoidance behaviour of *Eisenia fetida*

It has been well established that earthworms are suitable biomarkers for the assessment of soil quality. The receptors present on their body are extremely sensitive to the changes in pH, temperature and chemicals. Hence, avoidance behaviour of earthworms for the two chemicals used for wheat crop and the two chemicals for soybean crop were conducted. Results show that at the lowest concentration of Triazophos and lower two concentrations of Pendimethalin, attraction towards the chemicals was noticed but the earthworms exhibited a very strong avoidance at higher concentration of all four chemicals. Avoidance response was higher in

Chlorpyrifos and Triazophos in comparison to 2,4-DE and Pendimethalin for higher concentration values. The results show a direct link between the concentrations of chemicals and avoidance behaviour. In general, there is a gradual increase in avoidance behaviour with increase in dose concentration. Numerous research papers have presented study on avoidance behaviour of *Eisenia fetida* for Chlorpyrifos. Whereas, avoidance behaviour of *Eisenia fetida* for 2,4-DE chemical has not been attempted much by researchers.

For the organophosphate Chlorpyrifos, *Eisenia fetida* proved to be a potentially suitable species to assess the soil contamination. In our study, Chlorpyrifos showed increased avoidance response with higher contaminant levels. **Garcia-santos et al. (2011)** reported that *Eisenia fetida* showed increased avoidance response with higher contaminant levels of Chlorpyrifos. Comparable trends of avoidance behaviour was reported at higher levels of concentrations of Chlorpyrifos using *Eisenia fetida* **(Zhou et al., 2007)** and by **De Silva et al. (2009)** using *Eisenia andrei* in a two compartment system for natural tropical soil from Sri Lanka. **De Silva et al. (2009)** observed that *Perionyx excavatus* was significantly attracted by Chlorpyrifos at the lowest three concentrations (1-10 mg a.i./ kg dry soil), whereas such an effect was not seen for *Eisenia andrei*. When neglecting this negative avoidance behaviour and focusing on the avoidance of Chlorpyrifos in **OECD (1984)** artificial and natural soil, *Eisenia andrei* was 2.5 and 2.0 times more sensitive, respectively, than *Perionyx Excavatus*.

In case of 2,4-DE, we found that *Eisenia fetida* showed decrement in avoidance behaviour as the concentration of 2,4-DE increases upto 10 mg/kg. Avoidance behaviour at this concentration was only 5%. Beyond this concentration, the avoidance behaviour increased as the concentration of 2,4-DE increased and observed value was 90% at 1000 mg/kg concentration. It seems that the concentration of 2,4-DE at around 10 mg/kg is favourable to *Eisenia fetida* as comparison to other concentration in our experimentation.

Study on avoidance behaviour of *Eisenia fetida* for Triazophos and Pendimethalin is not available in literature. This may be due to heavy use of these chemicals in our study area as compared to other areas and other countries. At lowest concentration of Triazophos and Pendimethalin, attraction behaviour was observed in *Eisenia fetida*. This type of attraction behaviour in earthworms was also observed by other researchers. **Li et al. (2015)** reported that at concentration of 0.1 mg/kg of enrofloxacin, *Eisenia fetida* showed attraction behaviour. **Garcia et al. (2008)** observed that tropical *Eisenia fetida* in TAS soil and in LUFA soil indicate a significant avoidance behaviour of earthworms at concentrations $>$ 1.0 mg a.i./kg for fungicide carbendazim. While in artificial **OECD (1984)** soil, a significant attraction was observed at the lowest concentration (1.0 mg a.i./kg). **De Silva and Gestel (2009)** found same type of attraction behaviour with *Perionyx excavatus* at the lowest three concentrations (110 mg/kg dry soil) by Chlorpyrifos. *Eisenia andrei* also showed same attraction behaviour with carbofuran **(Bucha et al. 2013)**. **Marques et al. (2009)** found that *Eisenia andrei* showed attraction behaviour at concentration of 31.7 mg/kg of formulated herbicide Mikado (a.i. is sulcotrione). **Alves et al. (2013)** observed that more worms were found in the polluted compartments of thiametoxam, fungicides captan and carboxin plus thiram, at the lowest concentrations than in the control compartments. However, TAS treated with higher concentrations of these three pesticides was avoided by the worms. They also reported the same type of strong attraction by *Eisenia andrei* with fipronil, carboxin plus thiram, captan and thiametoxam chemicals. All these findings support our present study.

5.3 Impact of chemicals on the growth and reproduction of *Eisenia fetida*

The sensitivity of earthworm to chemicals is well established, hence the experiments were conducted to study the effect of most commonly used pesticides and herbicides for wheat and soybean crop on the growth and reproduction of *Eisenia fetida*. Since, meagre work is available on 2,4-DE, Triazophos and Pendimethalin, the results of the present study are discussed in light of the *Eisenia fetida* affected with other chemicals.

Statistical analysis of Chlorpyrifos showed that it's effect on growth rate and reproduction of *Eisenia fetida* is not significant for 95% confidence interval of mean, but Chlorpyrifos showed negative impact on growth rate and reproduction of *Eisenia fetida*. This type of finding were also discussed by other scientists in their research work. **Zhou et al. (2007)** observed that Chlorpyrifos has negative effect on growth and reproduction of *Eisenia fetida*. They also found that except 5 mg/kg Chlorpyrifos, in all concentrations (10, 20, 40 and 60 mg/kg) growth deffered from control, as the concentration increased growth was decreased. Effect of Chlorpyrifos was also negative on reproduction, with significant decrease in reproduction at the lowest concentration. Similar type of behaviour was also reflected from our study. **Zhou et al. (2011)** also reported that Chlorpyrifos siginificantly affected the growth rate and reproduction of earthworm *Eisenia fetida andrei*. Reproductive rates in all concentrations were found lower than the control group. These finding confirms the results of present investigation. **Booth et al. (2000)** also assessed the effect of Chlorpyrifos on *Aporrectodea caliginosa* and observed that earthworm weight was greatly reduced after four weeks of exposure of Chlorpyrifos. They also reported that cocoon production and hatching success were also reduced significantly by pesticide exposure which support the present findings. **Santos et al. (2012)** investigated the effect of Chlorpyrifos on *Eisenia andrei* which found similar type of observation as we found in present study with *Eisenia fetida* that there was gradual decrease in number of juveniles produced as the concentration of Chlorpyrifos in soil increased.

Statistical analysis of 2,4-DE showed that for *Eisenia fetida*, it's effect on growth rate is significant for 95% confidence interval of mean but it is not significant for reproduction. In case of 2,4-DE, we found in our study that at concentrations 0.75, 2.37 and 7.5 mg/kg, growth rate was almost similar. Such type of behaviour is also seen in case of reproduction where at concentrations values of 0.75 and 7.5 mg/kg, number of juveniles per earthworms produced were almost similar (approx. 9) while at 2.37 mg/kg it was 11.14. This seems that 2.37 mg/kg concentration of 2,4-DE is more favorable as compared to recommended agriculture dose, though even this concentration has negative impact on growth and reproduction of *Eisenia fetida* as compared to control. **Correia and Moreira (2010)** observed similar type of reduction in growth rate of *Eisenia fetida* as compared to control due to 2,4-D. They observed no cocoon or juveniles in any experiment using soil containing 2,4-D with concentrations of 10, 100, 500 and 1000 mg/kg. These findings are not in an ageement with the present study. In our experiment, we observed juveniles in all concentartions under study. This is due very high concentrations (100, 500 and 1000 mg/kg) used by the researchers. No juveniles were found even at concentration of 10 mg/kg in their experimentation. This is in contrast to our results. **Hattab et al. (2015)** studied the effect of 2,4-D on *Eisenia andrei* and observed that at concentration of 7 and 14 mg/kg 2,4-D significantly reduced the body weight of *Eisenia andrei* after exposure for 7 and 14 days. This result shows similarity with present study in which 2,4-DE also reduced the body weight of *Eisenia fetida* after exposure to the chemical.

Results showed that effect of 2,4-DE on growth of earthworms is more as compared to Chlorpyrifos for initial two lower concentrations and their combined effect is more severe as compared to their individual effect. Chlorpyrifos, 2,4-DE and their combined concentrations show gradual fall in growth rate of biomass with increase in level of concentration. In case of study on reproduction, Chlorpyrifos was found to be more toxic than 2,4-D except at the lower concentration. In case of 2,4-DE, lowest concentration (0.75 mg/kg) shows more negative impact than next concentration i.e. 2.37 mg/kg. Further increase in concentration of 2,4-DE decreases the number of juveniles produced per earthworm.

While studying the combined effect of Chlorpyrifos and 2,4-DE, it has been observed that their effect on growth rate and reproduction of *Eisenia fetida* is significant for 95% confidence interval of mean. Analysis of these results reflects that used insecticide and herbicide (Chlorpyrifos and 2,4-DE) showed a negative impact on growth rate and reproduction. Effect of their combined doses is more toxic in comparison to Chlorpyrifos and 2,4-DE individually. Overall, all these chemicals give negative values of correlation coefficient with respect to different values of chemical concentrations.

In the present study, combined effect of Chlorpyrifos and 2,4-DE was found more toxic than their individual effect on growth and reproduction of *Eisenia fetida*. This type of synergistic effects are supported by finding of **Yasmin and D'Souza (2007)**. They found that the combined effect of carbendazim, glyphosate and dimethoate was more toxic in comparison to their individual effect on the growth and reproduction of *Eisenia fetida*. This type of effect also confirmed by **Zhou et al. (2011)**. In their experiment, they found that combined effect of Chlorpyrifos and cypermethrin was more harmful than their individual effect on the growth and reproduction of *Eisenia fetida*. **Yang et al. (2015)** found that mixture of Chlorpyrifos and atrazine was antagonistic towards *Eisenia fetida*.

Statistical analysis of Triazophos showed that it's effect on growth rate and reproduction of *Eisenia fetida* is significant for 95% confidence interval of mean. On the other hand, effect of Pendimethalin on growth rate and reproduction of *Eisenia fetida* is not significant. In the study of combined effect of Triazophos and Pendimethalin, it has been observed that their effect on growth rate and reproduction of *Eisenia fetida* is significant. Analysis of these results reflects that used insecticide and herbicide (Triazophos and Pendimethalin) show a negative impact on growth rate and reproduction. Effect of Pendimethalin is more toxic in comparison to Triazophos and their combined doses. Overall, all these chemicals mostly give negative values of correlation coefficient with respect to different values of chemical concentrations. In our study, we observed that same level of concentration of Pendimethalin (when used as a sole chemical) shows more toxicity as compared to its combined dose with Triazophos. It might be due to masking effect of Triazophos over Pendimethalin. At Pendimethalin dose levels of 4.74, 15 and 47.4 mg/kg, growth rate was reduced significantly. On the other hand, Triazophos caused gradual fall in growth rate. Almost similar behaviour is observed with combined concentrations. Experiment on the study of number of juveniles produced per earthworm shows a sharp decline in soil treated with Triazophos and Pendimethalin as compared to control soil but there was no clear dose-response relationship between the substrate concentrations and the mean number of juveniles produced per earthworm.

The effect of these chemicals on earthworm species has not been discussed by other researchers in literature but they have discussed similar negative effect due to others chemicals on *Eisenia fetida*. **Vermulen et al. (2001)** found that mancozeb had no significant negative effect on either growth or reproduction of *Eisenia fetida* at recommended dose (8 mg/kg) or at an estimated environmental concentration (44 mg/kg), which is not in an agreement with present work. **Rai and Bansiwal (2009)** found that malathion also showed negative effect on growth and reproduction of *Eisenia fetida*, as we observed in our present study. Similar observations were reported by **Gobi and Gunasekaran (2010)** while studying effect of herbiside (butachlor) on *Eisenia fetida*. Biomass and cocoon production were decreased with increased herbicide concentration, which supports the present finding.

In case of studying the combined effect of Triazophos and Pendimethalin, we found that combined effect was more toxic in comparison to individual effect of Triazophos. On the other hand, combined effect of Triazophos and Pendimethalin was observed to be less toxic in comparison to individual effect of Pendimethalin. This type of antagonistic effect might be due to masking effect of Triazophos on

Pendimethalin. Many scientists studied the combined effect of different chemicals on toxicity of *Eisenia fetida*. **Chen et al. (2014b)** showed that combined toxicity of butachlor, atrazine and k-cyhalothrin on *Eisenia fetida* in which majority of mixtures except for combination of butachlor plus k-cyhalothrin showed synergism in artificial soil test. **Wang et al. (2015)** also studied combined effect of insecticides, herbicides and cadmium on *Eisenia fetida* in ternary combinations. They found that eleven combinations showed synergistic effects, four led to dual synergistic / additive behaviours and other five showed increasing antagonism within the entire range of effects.

In the present study, the synergistic effect was observed in case of combined effect of Chlorpyrifos and 2,4-DE, while antagonistic effect was observed in case of combined effect of Triazophos and Pendimethalin, which is in an agreement with the above mentioned works.

Chapter 6

Conclusion

Kota situated on the bank of river Chambal presents a diverse topographical features. Some areas, mainly along the bank of the river are agriculturally very productive and produce two seasons of crops; the Rabi and the Kharif. Whereas, other areas present a rocky terrain and have a flourishing stone mining business.

Earthworm species were abundantly found in the agricultural areas whereas their absence in rocky terrain and area in the vicinity of Thermal station was quite marked. Three species of earthworms were identified and all the species are vermicomposting. Of these, one species *Eisenia fetida* was predominantly found in all agriculturally inclined areas followed by *Lampito mauritii* and *Perionyx* was the least abundant. Presence of earthworms is beneficial to agro-ecosystem as earthworms are very efficient biomarkers to assess the soil quality and any contamination thereof.

Interactions with the local farmers in the survey areas and shopkeepers selling agricultural products revealed that the farmers are using pesticides excessively to enhance crop production. Chemicals used for Rabi crop (wheat) were Chlorpyrifos and 2,4-DE as insecticide and herbicide respectively. For Kharif crop (soybean), the chemicals used in practice are Triazophos and Pendimethalin as insecticide and herbicide respectively. The excessive use of chemicals is not only detrimental to the soil quality but also affects the diversity of earthworms and if discretion in use of these pesticides and concrete implementation are not enforced, the species of earthworms may soon be extinct.

Avoidance behaviour help us to determine the range of doses to be used for the study of the effect of chemicals on the growth and reproduction of earthworms. It can be concluded from the results that the concentration of Chlorpyrifos and avoidance behaviour are directly proportional. Increase in concentration resulted in increased avoidance behaviour. However, for 2,4-DE, avoidance decreases with increase in concentration value upto 10 mg/kg and thereafter the avoidance behaviour increases with the increase in the concentration of the chemical.

In case of Triazophos, the attraction was found to be highest at lowest concentration (0.316 mg/kg). Beyond this concentration, the avoidance behaviour increased with the increased concentration of Triazophos. For Pendimethalin, it was seen that the lower concentration exhibited attraction behaviour but as the concentration increased, there was a gradual increase in the avoidance behaviour. The avoidance behaviour was higher for Triazophos as compared to Pendimethalin.

Study was conducted to know the impact of chemicals on growth and reproduction of *Eisenia fetida* by taking five different concentrations. Chlorpyrifos caused decline in the growth of earthworm as compared to the control. Similarly, reproduction also reduced drastically. 2,4-DE produced a sharp decline in the growth rate as compared to the control and also decline in the reproduction was observed. Combined effect of these chemicals caused a severe negative impact both on the growth and reproduction of earthworms.

Triazophos caused a drastic decline both in the growth and reproduction of earthworms. Similarly, Pendimethalin also caused a sharp decline both in the growth and reproduction of earthworms as compared to controls. The combined concentrations of these chemicals resulted in severe negative impact on both the growth and reproduction of earthworms.

Normally, the effect of the combined concentrations of chemicals was found to be more toxic as compared to their individual effect due to synergistic effect but in the case of Triazophos and Pendimethalin, the combined effect was less toxic to the individual effect of Pendimethalin. It may be concluded that it is due to the masking effect of Triazophos over Pendimethalin.

Conclusively, the toxicity of used chemicals for growth was in the order: Pendimethalin > 2,4-DE > Triazophos > Chlorpyrifos. Similarly, toxicity of the used chemicals for reproduction was in the order: Pendimethalin > Triazophos > 2,4-DE > Chlorpyrifos.

It has been established by the present study that the growth and reproduction parameters of earthworms exposed to agrochemicals are very efficient bioindicators of soil contamination. Study on the impact of all the four chemicals used on the above mentioned parameters showed a negative impact even at the recommended doses. Indications are that a long term exposure of earthworms to these chemicals will adversely affect their populations resulting in the low abundance of earthworm in the soil.

The present study was conducted on one of three species found in the study area. Additional research with the other species of the region is recommended. Further research on the chemical nature, mode of action and means of degradation of pesticides in soil is required so that the harm to the soil microorganisms as well as higher ups in the food chain can be contained and minimized. Choosing organic farming would be a very good method of achieving this goal. Implementation of the policies drafted by NPOF-ICAR as adopted by Sikkim would be a step towards protecting the earthworm populations, maintaining soil nutrients and leading to second green revolution in the country.

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Appendix

GOVERNMENT OF INDIA

ZOOLOGICAL SURVEY OF INDIA
Telegram: “Zoology” Kolkata
Phone: 033-22861608,
Fax: 033-22861610
E-mail: zsi-gnc@rediffmail.com

Zoological Survey of India,
General Non-Chordata Section,
27-J.L.Nehru Road,
Kolkata-700016.

F.No. GNC/I&A/2014-15/936 Date: 21.10.2014

To
Dr. Anuradha Singh
Research Supervisor
Deptt. of Zoology,
Govt. College
Kota, Rajasthan

Sub: Earthworm Identification report
Ref: Your letter, dated10.09.2014

Dear Dr. Anuradha,

With reference to the above mentioned request received from you by us on 01.10.2014 by hand, the specimen sent by you is examined and identified by group expert Dr. C.K.Mandal (e-mail: mandalsucker@gmail.com). The identification report is as follows.

Phylum	:	Annelida
Class	:	Clitellata
Order	:	Haplotaxida
Family	:	Megascolecidae

1. *Lampitomauritii*Kingberg, 1866. 3 ex.
2. *Perionyx*sp. 1 ex.

Family : Lumbricidae

3. *Eiseniafetida*(Savigny, 1826). 4 ex.

It is expected that the identification is duly acknowledged in your reports and publications. All the best for all your endeavours.

Yours faithfully

-sd-

(Dr. Ch. Satyanarayana)
Officer- in- charge,
General Non-ChordataSecion.
Zoological Survey of India
Kolkata: 700016

98
20-10-14

कार्यालय परियोजना निदेशक (शस्य), सी.ए.डी., नान्ता फार्म, कोटा
मृदा एवं जल परीक्षण प्रयोगशाला, सी.ए.डी., नान्ता फार्म, कोटा

	क्रम संख्या	प्र. शा. नं.	काश्तकार का नाम	गाँव का नाम	पंचायत समिति का नाम	फसल व किस्म जिसके लिए सिफारिश चाहिये	सिंचित या असिंचित	खसरा / चक नम्बर	विश्लेषणात्मक सूचना					सिफारिश प्रति हैक्टेयर				विशेष विवरण
		6941							पी. एच	कन्डक्टिविटी मि म्होज/ से.मी.	जैविक कार्बन प्रतिशत	फास्फेट किलो प्रति हैक्टेयर	पोटाश किलो प्रति हैक्टेयर	गोबर की खाद गाड़ी में	नत्रजन किलो में	फास्फोरस P_2O_5 किलो में	पोटाश K_2O किलो में	
सेम्पल नं	1	1714	श्रीमती अनामिका, 192 नान्ता नाड़ी, कोटा						8.04	0.320	0.48							
सेम्पल नं	2	1715	——"——						8.10	0.348	0.42							
सेम्पल नं	3	1716	——"——						7.89	0.475	0.60							
सेम्पल नं	4	1717	——"——						8.33	0.291	0.03							

(1) सभी नमूनों [illegible]
(2) [illegible] 1, 2, 4 में [illegible] की है [illegible]
(3) [illegible] आवश्यक है।

उप निदेशक कृषि (रसायन)
नान्ता फार्म, सीएडी, कोटा

सहायक कृषि अनुसंधान अधिकारी (रसायन)
नान्ता फार्म, सी.ए.डी., कोटा