

EARTHWORM ABUNDANCE AND DIVERSITY IN DIFFERENT CROPPING SYSTEMS UNDER ORGANIC AND CONVENTIONAL FARMING

Thesis

**Submitted to the Punjab Agricultural University
in partial fulfilment of the requirements
for the degree of**

**MASTER OF SCIENCE
in
ZOOLOGY
(Minor Subject: Botany)**

**By
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(L-2019-BS-325-M)**

**Department of Zoology
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LUDHIANA-141004**

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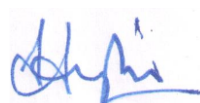
This is to certify that the dissertation entitled, “**Earthworm abundance and diversity in different cropping systems under organic and conventional farming**” submitted for the degree of Master’s in the subject of **Zoology** (Minor subject: **Botany**) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Arshpreet Kaur (L-2019-BS-325-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE –II

This is to certify that the thesis entitled, “**EARTHWORM ABUNDANCE AND DIVERSITY IN DIFFERENT CROPPING SYSTEMS UNDER ORGANIC AND CONVENTIONAL FARMING**” submitted by **Ms. Arshpreet Kaur (L-2019-BS-325-M)** to the Punjab Agricultural University, Ludhiana in partial fulfillment of the requirements for the degree of **M.Sc.** in the subject of **Zoology (Minor field: Botany)** has been approved by the Student’s Advisory Committee along with Head of the Department and External Examiner after an oral examination on the same.



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ABSTRACT

Earthworms form an integral part of the soil ecosystem, contributing to the development of soil structure, nutrient cycling, pedogenesis, water regulation and pollution remediation. The present study is an attempt to assess the earthworm abundance and diversity in the different cropping systems (i.e. basmati-wheat, basmati-chickpea, soybean-wheat, moong-wheat) under organic and conventional farming systems in the fields of Punjab Agricultural University, Ludhiana. The four earthworm species found during the study period are *Metaphire posthuma*, *Lampito mauritti*, *Amyntas morissia* and *Travoscolides chengannure*. which belong to two families i.e. Megascolicidae and Octochateidae. Out of these *Travoscolides chengannure* was reported for the first time in Punjab. The results indicate that richer earthworm diversity is found in the organic farming systems as compared to the conventional farming systems. The overall abundance of earthworms (60.4%) in organic farming system was more as compared to the conventional (39.5%) farming system. The present study reveals that the management practices adopted for conventional farming like chemical fertilizers and pesticides had a negative effect on the earthworm population which might be the reason for less abundance and diversity of earthworm species in conventional fields. The correlation analysis of earthworm abundance with the physicochemical parameters of soil in different farming systems has revealed that the abundance of earthworms in organic farming system shows positive but non-significant correlation with pH, nitrogen and potassium levels while negative correlation was observed for electric conductivity, organic carbon and phosphorus. In conventional farming system, significant positive correlation ($p \leq 0.01$) was found for organic carbon, electric conductivity and nitrogen.

Keywords: Earthworms, Diversity, Abundance, Organic, Conventional, Cropping System

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ਸਾਰ ਅੰਸ਼

ਧਰਤੀ ਦੇ ਗੰਡੋਏ ਵਾਤਾਵਰਣ ਪ੍ਰਣਾਲੀ ਦਾ ਇੱਕ ਅਟੁੱਟ ਅੰਗ ਹੁੰਦੇ ਹਨ। ਇਹ ਮਿੱਟੀ ਦੀ ਬਣਤਰ, ਪੌਸ਼ਟਿਕ ਆਹਾਰ ਚੱਕਰ, ਜਰਾਸੀਮ, ਪਾਣੀ ਮਿੱਟੀ ਦੀ ਗੁਣਵੱਤਾ ਅਤੇ ਪ੍ਰਦੂਸ਼ਣ ਉਪਚਾਰ ਦੇ ਵਿਕਾਸ ਵਿੱਚ ਯੋਗਦਾਨ ਪਾਉਂਦੇ ਹਨ। ਮੌਜੂਦਾ ਅਧਿਐਨ ਦੇ ਚਲਦਿਆਂ ਪੰਜਾਬ ਖੇਤੀਬਾੜੀ ਯੂਨੀਵਰਸਿਟੀ ਲੁਧਿਆਣਾ ਦੇ ਖੇਤਾਂ ਵਿੱਚ ਜੈਵਿਕ ਅਤੇ ਰਿਵਾਇਤੀ ਖੇਤੀ ਪ੍ਰਣਾਲੀਆਂ ਅਧੀਨ ਵੱਖ-ਵੱਖ ਫਸਲੀ ਪ੍ਰਣਾਲੀਆਂ (ਜਿਵੇਂ ਕਿ ਬਾਸਮਤੀ-ਕਣਕ, ਬਾਸਮਤੀ ਛੋਲੇ, ਸੋਇਆਬੀਨ-ਕਣਕ, ਮੂੰਗੀ ਕਣਕ) ਵਿੱਚ ਗੰਡੋਇਆਂ ਦੀ ਭਰਪੂਰਤਾ ਉਤੇ ਵਿਭਿੰਨਤਾ ਸਬੰਧੀ ਮੁਲਾਂਕਣ ਕਰਨ ਦਾ ਇੱਕ ਮਹੱਤਵਪੂਰਨ ਯਤਨ ਕੀਤਾ ਗਿਆ ਹੈ। ਇਹਨਾਂ ਸਬੰਧੀ ਆਰੰਭੀ ਖੋਜ ਦੇ ਦੌਰਾਨ ਗੰਡੋਇਆਂ ਦੀਆਂ ਚਾਰ ਕਿਸਮਾਂ ਹਨ ਜਿਹਨਾਂ ਵਿੱਚ ਮੈਟਾਫਾਇਰ ਪੋਸਥੂਮਾ, ਲੈਮਪੀਟੇ ਮੋਰੀਟੀ, ਐਮਿਥਸ ਮੋਰੀਸੀ ਅਤੇ ਟ੍ਰੈਵੋਸਕੋਲਾਈਡਜ਼ ਚੇਂਗੂਨੋਰ ਜੋ ਕਿ ਮੇਗਾਸਕੋਲੀਸੀਡੇ ਅਤੇ ਓਕਟੋਚੈਟੀਡਾਇ ਪਰਿਵਾਰਾਂ ਨਾਲ ਸਬੰਧਤ ਹਨ। ਇਹਨਾਂ ਵਿੱਚੋਂ ਟ੍ਰੈਵੋਸਕੋਲਾਈਡਜ਼ ਚੇਂਗੂਨੋਰ ਪੰਜਾਬ ਵਿੱਚ ਪਹਿਲੀ ਵਾਰ ਸਾਹਮਣੇ ਆਇਆ ਹੈ। ਨਤੀਜੇ ਦਰਸਾਉਂਦੇ ਹਨ ਕਿ ਰਿਵਾਇਤੀ ਖੇਤੀ ਪ੍ਰਣਾਲੀਆਂ ਦੇ ਮੁਕਾਬਲੇ ਜੈਵਿਕ ਖੇਤੀ ਪ੍ਰਣਾਲੀਆਂ ਵਿੱਚ ਅਮੀਰ ਗੰਡੋਇਆਂ ਦੀ ਵਿਭਿੰਨਤਾ ਪਾਈ ਜਾਂਦੀ ਹੈ। ਰਿਵਾਇਤੀ (39.5%) ਖੇਤੀ ਪ੍ਰਣਾਲੀ ਦੇ ਮੁਕਾਬਲੇ ਜੈਵਿਕ ਖੇਤੀ ਪ੍ਰਣਾਲੀ ਵਿੱਚ ਗੰਡੋਇਆਂ ਦੀ ਸਮੁੱਚੀ ਬਹੁਤਾਤ (60.4%) ਵਧੇਰੇ ਹਨ। ਮੌਜੂਦਾ ਅਧਿਐਨ ਇਹ ਦਰਸਾਉਂਦਾ ਹੈ ਕਿ ਰਿਵਾਇਤੀ ਖੇਤੀ ਲਈ ਅਪਣਾਏ ਗਏ ਪ੍ਰਬੰਧਨ ਅਭਿਆਸਾਂ ਜਿਵੇਂ ਕਿ ਰਸਾਇਣਿਕ ਖਾਦਾਂ ਅਤੇ ਕੀਟਨਾਸ਼ਕਾਂ ਦਾ ਗੰਡੋਇਆਂ ਦੀ ਅਬਾਦੀ ਤੇ ਮਾੜਾ ਪ੍ਰਭਾਵ ਪਾਉਂਦੇ ਹਨ ਜੋ ਕਿ ਰਿਵਾਇਤੀ ਖੇਤਾਂ ਵਿੱਚ ਗੰਡੋਇਆਂ ਦੀਆਂ ਕਿਸਮਾਂ ਦੀ ਘੱਟ ਵਿਭਿੰਨਤਾ ਦਾ ਕਾਰਨ ਹੋ ਸਕਦਾ ਹੈ। ਵੱਖ-ਵੱਖ ਖੇਤੀ ਪ੍ਰਣਾਲੀਆਂ ਵਿੱਚ ਮਿੱਟੀ ਦੇ ਭੌਤਿਕ ਰਸਾਇਣਿਕ ਮਾਪਦੰਡਾਂ ਦੇ ਨਾਲ ਗੰਡੋਇਆਂ ਦੀ ਭਰਪੂਰਤਾ ਦੇ ਸਬੰਧ ਦੇ ਵਿਸ਼ਲੇਸ਼ਣ ਤੋਂ ਪਤਾ ਲੱਗਾ ਹੈ ਕਿ ਜੈਵਿਕ ਖੇਤੀ ਪ੍ਰਣਾਲੀ ਵਿੱਚ ਗੰਡੋਇਆਂ ਦੀ ਬਹੁਤਾਤ ਪੀ ਐਚ, ਨਾਈਟ੍ਰੋਜਨ ਅਤੇ ਪੋਟਾਸ਼ੀਅਮ ਦੇ ਪੱਧਰਾਂ ਨਾਲ ਸਕਾਰਾਤਮਕ ਪਰ ਗੈਰ-ਮਹੱਤਵਪੂਰਨ ਸਬੰਧ ਨੂੰ ਦਰਸਾਉਂਦੀ ਹੈ ਜਦੋਂ ਕਿ ਇਲੈਕਟ੍ਰਿਕਲ ਕੰਡਕਟੀਵਿਟੀ ਲਈ ਨਕਾਰਾਤਮਕ ਸਬੰਧ ਦੇਖਿਆ ਗਿਆ ਹੈ। ਜੈਵਿਕ ਕਾਰਬਨ ਅਤੇ ਫਾਸਫੋਰਸ, ਰਿਵਾਇਤੀ ਖੇਤੀ ਪ੍ਰਣਾਲੀ ਵਿੱਚ, ਜੈਵਿਕ ਕਾਰਬਨ, ਇਲੈਕਟ੍ਰਿਕਲ ਕੰਡਕਟੀਵਿਟੀ ਅਤੇ ਨਾਈਟ੍ਰੋਜਨ ਲਈ ਮਹੱਤਵਪੂਰਨ ਸਕਾਰਾਤਮਕ ਸਬੰਧ ($P \leq 0.01$) ਪਾਇਆ ਗਿਆ ਸੀ।

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CONTENTS

CHAPTER	TOPIC	PAGE NO.
I.	INTRODUCTION	1-4
II.	REVIEW OF LITERATURE	5-21
III.	MATERIAL AND METHODS	22-30
IV.	RESULTS AND DISCUSSION	31-50
V.	SUMMARY	51-52
	REFERENCES	53-69
	VITA	

LIST OF TABLES

Table No.	TOPIC	PAGE NO.
1.	The sampling sites under organic fields.	23
2.	The sampling sites under conventional fields.	23
3.	Morphological characteristics of earthworm species.	32
4.	Management practices of organic field	37
5.	Management practices of conventional field	38
6.	Distribution of earthworm species and their annual abundance (%) of kharif and rabi seasons in organic and conventional field (a) Basmati-Wheat Cropping system (b) Basmati-chickpea cropping system (c) Soybean-Wheat cropping system (d) Moong-wheat cropping system	40-42
7.	Species present in different cropping systems in organic fields.	42
8.	Species present in different cropping systems in conventional fields.	43
9.	Relative abundance of earthworms in different cropping systems under organic and conventional farming	43
10.	Diversity indices of earthworm species in different cropping systems: Simpsons index(D), Shannon Wiener index(H) and Evenness (E)	45
11.	Correlational analysis of earthworm abundance with the soil parameters in different farming systems	48
12.	Analysis of soil parameters of organic and conventional farming systems.	49
13.	Soil Temperature and Moisture of different crop systems in both organic and conventional cropping system.	49

LIST OF FIGURES

Figure No.	TOPIC	PAGE NO.
1.	Earthworm gDNA sample agarose (1.5%) gel electrophoresis of PCR using LCO1490 and HCO2198 primers.	33
2.	Evolutionary relationships of earthworm E2 sample with other taxa.	35
3.	Bar graph showing earthworm population in different cropping systems under organic and conventional farming	44

LIST OF PLATES

Plate No.	TOPIC
1.	Earthworms preserved in 5 percent formalin solution in glass vials for further study of morphological characters
2.	Fixation of worms in cotton bed
3.	Using a stereomicroscope, the preserved samples of earthworms were examined for morphological characteristics such as total length (in cm), total number of segments, prostomium shape, position and type of clitellum, position and number of spermathecae, position of male pore, and so on.
4.	Identification of morphological characteristics of earthworms under stereomicroscope. The observations were recorded in a visualizer.
5.	Morphological characters of <i>Metaphire posthuma</i>
6.	Morphological characters of <i>Lampito mauritti</i>
7.	Morphological characters of <i>Amyntas morrisi</i>
8.	Morphological characters of <i>Travoscolides chengannur</i>

CHAPTER - I

INTRODUCTION

Earthworms belong to Phylum Annelida and Class Oligochaeta. Inside the phylum Annelida, three important categories have been traditionally acknowledged. These include Polychaeta, Oligochaeta and Hirudinea. Talking about their phylogenetic relationship, Oligochaeta have evolved from Polychaeta (Struck *et al.* 2011). While the Hirudineans have oligochaete as their ancestors. Therefore, according to phylogenetic categorization, only Oligochaeta and Polychaeta have been successfully maintained. However, Clitellata is a universally recognized taxa that covers all the Oligochaete and Hirudinea within it (Michaelsen 1919).

On the basis of size, the oligochaetes have been classified as Microdrili and Megadrili. As the names suggest, the Megadrili includes the large sized terrestrial as well as aquatic worms while the Microdrili involves small sized aquatic and terrestrial worms. The earthworms from Megadrili constitutes about 60-80% of the total soil fauna which means that they dominate in the soil. (Omodeo 2000). Therefore, being the significant members of the soil, they play a very important role in the synthesis of organic matter and thereby maintaining the best soil structure. The earthworms acquire food from the soil in the form of dead plant residues and while doing so they mix up the organic matter thoroughly with the soil particles. This is their way to improve the soil structure and infiltration of water. Hence, earthworms utilize a large variety of dead organic matter in their nutritive material.

On the basis of habitat, the earthworms are classified into three major categories – Epigeic, Anecic and Endogeic. (Tripathi and Bhardwaj 2004). The Epigeic form of earthworms are mainly found above the mineral soil surface generally the litter layers and are called as litter inhibitors. As, they do not possess the capacity of building burrows, therefore their abundance is qualified to the soil surface itself. They have relatively small size and their body is highly pigmented. They are typically reddish-brown worms with ephemeral life cycles. Also, their movement within the soil is quite fast and spontaneous. In conditions of severe drought, they cocoon themselves to survive the adverse environment. However, despite their rapid locomotion and cocoon formation, their predation pressure is high. Common examples of such earthworms include *Eisenia fetida*, *Perionyx excavates*, *E. andrei* and *Eudrilus eugeniae*. Then comes the Anecic worms or vertical burrowers that predominantly lives in the soil burrows created vertically up to 3 m deep soil. They also have a characteristic feature of obtaining nutrients from different zones of the soil. They have dark heads usually brown or red coloured and light tail endings. These species are generally long

lived. When they are on the surface of soil, the predation pressure on them is quite high but they remain secured inside their burrows. Also, under water deficient circumstances, they go in quiescent stage. Major examples of Anecic species cover *Lumbricus terrestris* and *Lampito mauritti*. The last category is Endogeic earthworms that live in horizontal burrows. They construct horizontal but temporary burrows within the 10-15 cm soil surface. Their body colour is light usually pink or greyish. Also, they are slow moving worms with intermediate life cycle. Under drought, the species show diapause stage. The example is *Allobophora chlorotica* (Tripathi and Bhardwaj 2004).

On the basis of their feeding habits, earthworms can be categorized into three types- litter feeding, Geophagus and Limiphagous earthworms. Litter feeders feed on the mineral surface usually on plant litter, decayed plant roots, animal dung. The Epigeic and Anecic earthworms come under this category and are also known as humus formers. The Epigeic species absorb little or sometimes no soil. The species like *Lumbricus castaneus* and *Lumbricus rubellus* completely rely upon litter while almost all the *Dendrobaena* species prefer decomposed wood. Additionally, *E. fetida* and *E. andrei* consume compost which is full of organic matter. Geophagus on the contrary are deep feeders that take large amount of organic soil. These include Endogeic earthworms or humus feeders. Limiphagous are specifically found on mud. (Tripathi and Bhardwaj, 2004). Morphologically, the earthworms are classified on the basis of characters like number of segments, growth, body length and clitellum. The morphological attributes of earthworms are also dependent upon the climate and organic matter which forms a major part of their nutrition (Curry 2002). As they have the ability to process organic matter into a fertile compost so, they are successfully sold for vermiculture and also as a bait. Hence, the process of composting or cultivating earthworms, especially in order to use them to convert organic waste into fertilizer is called Vermiculture. (Dey and Chaudhuri 2016).

The various land use patterns have affected the diversity and abundance of earthworms. The attention of many researchers has been drawn to aboveground biodiversity, but scientific knowledge on soil species has remained ignored and their possible benefits have not been completely achieved (Rossi and Blanchart, 2005; Decaens, 2010). Different studies have shown that changes in land use structures have impacted the structure, functionality and dynamics of soil species with a major effect on critical soil functions (Decaens 2010; Bartz *et al.* 2014). Normal and relatively undisturbed habitats foster a wide range of earthworms due to the availability of adequate niches for them. The appropriate niches for their growth, natural and relatively undisturbed habitats promote a rich diversity of earthworms (Barros *et al.* 2002; Birang *et al.* 2003). Owing to habitat alteration and food distribution, the disturbed

or fragmented soils typically have low earthworm populations (Lavelle 1992). In most degraded soils, their activities are usually poor, mainly the ones associated with agriculture and forest (Lavelle 1994). Recent studies have shown that land use intensification and anthropogenic intervention cause random shifts in soil communities that have a long-term effect on ecological services (Dupouey *et al.* 2002).

Earthworms are very old organisms that are known to have existed on earth 600 million years ago (Lavelle *et al.* 1994). India comprises mainly 11.1% of total global earthworm's diversity, that includes about 505 species placed belonging to 10 families and 69 genera (Suthar 2011). However, in Punjab, a total of 21 species of earthworm have been recorded (Dhiman and Batish 2006). In the tropical regions where humidity is high, earthworms with soil processing activity are more predominant. But, as the earthworms are highly responsive to immediate change in climate and food sources, therefore the practices related to conventional management has reduced density as well as diversity of these organisms (Suthar 2011). In addition to providing shelter for other soil species, they have also been involved in altering the physical, chemical and biological characteristics of the soil since its formation (Shipitalo and Le, 2004).

Earthworm populations significantly improve the soil structure by various mechanisms like provision of adequate aeration to the soil, creating deep burrows and mixing the organic residues well with the soil particles. They actively participate in nutrient cycling thus increasing the overall fertility and productivity of agricultural soils. Soil structure is the fundamental property of soil that affects the soil physical properties like water infiltration, plant nutrient uptake, soil porosity, hydraulic conductivity, water holding capacity etc. Therefore, it is right to say that the abundance or even the presence of earthworms in the soil profile indicates a positive sign for the soil health. Not only this, they also play a vital role in the biological degradation of organic fuel which consequently leads to nutrient rotation. The earthworms feeding only on litter have massive amounts of plant nutrients inside their castings. The greater availability of nitrogen, phosphorus, potassium and calcium in the casts of earthworms enriches the soil with substantial amounts of major nutrients than the surrounding soil (Tiwari *et al.*, 1989).

Earthworms also stimulate microbial species in their gut and casts, such as nitrogen fixing bacteria, which promote soil fertility, increasing plant growth and crop yield (Edwards and Bohlen 1996). Hence, all these activities of earthworms engender their name as 'indicator' organisms to assess the biological effect of soil contaminants (Rochfort *et al.* 2009). Also, the soil management practices heavily intervene the activities of earthworms

within the soil. Any modification in their activity thus indicates the fertility and quality aspects of the soil. Furthermore, there are some earthworms that feed on toxic soil nematodes. Such species of earthworms help in reducing the population of poisonous nematodes from the soil. (Arancon *et al.* 2005; Marhan and Scheu, 2005). The scientists show considerable interest to exploit earthworms for breaking down complex organic waste into simpler forms (Karmegam and Daniel, 2009; Alagesan and Dheeba, 2010). Via decomposing organic materials, the earthworms run the energy transformation cycles through mineralization of organically bound nutrients such as lignocellulose (Bhadauria T and Ramakrishnan P.S 1989). This deterioration of organic materials by earthworms is due to the presence of various degrading microorganisms in their guts (Edwards and Bohlen, 1996). Earthworms have procured increased scientific attention in India as well as other countries due to their broad utilization in the bioremediation of soil, vermicomposting and as an important source of easily digestible animal protein for livestock. Many species of earthworms are also bio-indicators for detecting chemical toxicity in the soil. This is because the earthworms tend to accumulate considerable amounts of such chemicals inside their tissues. With their unique food and feeding habits, earthworms are the most useful converters of organic waste in nature therefore, earthworms are very beneficial to humans in many aspects.

Objectives

- i. To study earthworm diversity and abundance in different cropping systems.
- ii. To evaluate physico-chemical parameters of soil in different cropping systems.

CHAPTER-II

REVIEW OF LITERATURE

Representatives of class Oligochaeta has an elongated and cylindrical body with approximately 15 cm length. Their body has segments on both dorsal and ventral sides which possess distinctive grooves. Earthworms are very useful soil invertebrates that form a major part of soil. They are suitably adapted to the soil environment typically found in decaying organic material, rotted vegetation, compost, manure etc. (Giraddi *et al.* 2014). Aristotle (324-322 BC), termed earthworms as 'Intestines of Earth' due to their unique behavior of ingesting and egesting soil. They play a vital role in agro-ecosystems. Their mode of feeding and burrowing soil give rise to many structural changes within the soil (Joshi and Aga 2009). The presence of high levels of organic matter in the soil determines the abundance of earthworms. The fertility of soil depends upon the physical, chemical and biological factors of soil, all of which are improved by the activities of earthworms. They are very well known for feeding, burrowing as well as for casting activities, and are therefore given the name of "Ecosystem engineers". They improve soil aeration, drainage, mineral and nutrients intake of plants. All of these modifications are favourable for the growth and production of crops.

The quality of soil depends upon the population density and diversity level of earthworm's community (Karmegam and Daniel 2007). Because of their large body size, high soil consumption rates and other burrowing activities, these organisms also help in supporting the habitats for other soil biota. Earthworms affect plant growth indirectly by changing soil structure, process of mineralization, hormonal effects which stimulates the microorganisms and tends to undergo antagonistic changes to the roots. They not only modify the availability of nutrients to the plants but also alter the whole sequence of rhizosphere environment (Makin *et al.* 2014). These organisms secretly govern the distribution of organic matter within the soil profile, therefore affecting the root foraging. It has also been documented that they protect the environment by reducing the chances of soil - borne diseases by altering the soil microbial communities (Mohan *et al.* 2013). The species composition as well as population dynamics of earthworms get affected due to the modifications in the land utility patterns followed in various agro-climatic areas of India.

Also, organic growers show a keen interest in determining the impact of farming methods on earthworms and their population dynamics. Due to various services provided by earthworms, these terrestrial annelids are renowned by several names like "intestines of earth", "farmer's friend" and "Cindrella of organic farming". Abundance of earthworm species is highly beneficial to agriculture. As the earthworms have a loop link with agriculture

ecosystem, any kind of cultural activity aimed to manage the soil or crop affects the density and population of earthworm adversely. The species richness, size and structure of populations (number of individuals, ratio of juveniles/adults), and their biomass are all dependent upon the crop management practices. The tillage which destroys the soil structure has a worst effect on earthworm species composition. However, the diversity is vaguely dependent upon the density and scheduling of tillage practices (Pelosi *et al.* 2009). There are about 7,000 species of earthworms, out of which only 150 species are found to be distributed all around the world. Generally, these are peregrine or cosmopolitan but India has the richest species diversity. The western Ghats, eastern Himalayas and regions of northeast zone are regarded as "Biodiversity hotspots" of earthworm species. According to the available resources, it has come into notice that the species of earthworms undergo certain behavioural, physiological as well as morphological adaptations.

Earthworms increasingly contribute to soil structure and rebuilding through processes such as carbon turnover, humification, mineral weathering and mixing of organic-mineral compounds with the soil particles. With their burrowing activities, they also affect mechanical nature of soil and produces large sized pores that enhances hydraulic conductivity and water infiltration which are of utmost importance for adequate water supply to the crops. In addition, they also control the surface runoff of water (Larsen *et al.* 2012).

Earthworm diversity-

Diversity of earthworms: World

Csuzdi and Pavlicek (1999) organized a research on earthworm genera: *Dendrobaena* and *Bimastos* in Israel. There are twelve taxa, out of which the newly described ones are *Bimastos jordanis*, *Dendrobaena negevis* spp. *Dendrobaena semitica*, *Dendrobaena nevoi*, *Dendrobaena orientalis* and *Dendrobaena veneta kervillei*. The species *Dendrobaena hortensis* and *Dendrobaena veneta* can be uniquely related with human activity. Further, James *et al.* in 2005 described different species of earthworm from Taiwan. The new species isolated were *A. nanrenensis*, *A. hengchunensis*, *A. corticis*, *A. monsoonus*, *A. huangi*, *A. chaishanensis*, *A. kaopingensis* and *A. ailiaoensis* and *A. aelianus*. Out of all, *Amyntas chaishanensis* varies from other proandric species in case of having the spermathecal pores at some different site.

Celis and Rangel-Ch in 2015 discovered two new earthworm species *Periscolex malibu* sp.nov and *Righiodrilus lebrijae* sp. nov. in the residues of tropical dry forests in the Cesar's southern province which is situated along the Colombia's Caribbean coast. In both

the species, the clitellum and tubercula pubertatis is located at the same place. The major difference between the two is the existence of five pairs of spermatheca and clitellum extension. Sun *et al.* (2015) from China isolated three distinguished kinds of earthworm species that include *Amyntas trapezoides*, *Amyntas dongfangensis* and *Amyntas conchicapillatus*. The species *Amyntas trapezoides* has no body pigment and possess two sets of 6/7 and 7/8 spermathecal pores. After two years, Chanabun *et al.* (2017) introduced various semiaquatic earthworm species which were seven in total. These species were isolated from Thailand and Laos. The species include *Glyphidrilus nanensis*, *Glyphidrilus chiangraiensis*, *Glyphidrilus sekongensis*, *Glyphidrilus namdonensis*, *Glyphidrilus namphao* and *Glyphidrilus satunensis*, *Glyphidrilus champasakensis*.

Diversity of earthworms: India

The Indian diversity of earthworms is very rich. About 450 or above species from various districts of different states have been reported till date. Nijhawan and Kanwar (1952) examined the morphological features such as form and structure of castings of earthworm species namely *Eutyphoeus waltoni*. Soota and Julka (1972) documented two new earthworm species made *Howascolex Stephensoni* and *Howascolex Bahli*. Julka (1976) conducted a study in Arunachal Pradesh in the middle of subansiri expedition. The species *Drawida tihunensis*, *Plutellus mishmiensis* and *Tonoscolex michaelsoni* were successfully reported.

Furthermore, in 1981 Julka organized some taxonomic surveys on earthworms inhabiting within and near the state of Arunachal Pradesh. In this report, he identified different species namely *Drawida aruna*, *Plutellus bahli*, *Drawida duttai*, *Plutellus richikensis*, *Plutellus taksingensis*, *Plutellus daflaensis*, *Plutellus daminensis*, *Plutellus daminensis*, *Plutellus vidakensis*, *Tonoscolex indicus* and *Tonoscolex kabakensis*. Tripathi and Bhardwaj (2004) recorded six new species Jodhpur district, Rajasthan. The species like *Metaphire posthuma* and *Lampito mauritii* were known to exist in all the sites of study. The earthworms identified in this survey were eco-morphologically distinct. Also, they demonstrated a positive correlation between the earthworm species number with that of soil organic carbon. Joshi and Aga (2009) reported the species of families: Octochaetidae and Megascolecidae. The species *beatrix*, *Eutyphoeus incommodus*, *Eutyphoeus waltoni*, *Eutyphoeus nicholsoni* and *Eutyphoeus*. *Orientalis* belonged to the family Octochaetidae while *Amyntas alexandri* of Megascolecidae was documented.

Najar and Khan (2011) studied the relative distribution, diversity, abundance and seasonal richness of earthworms. The survey took place in Kashmir and submitted report of eight different earthworm species belong to three different families namely Moniligastridae,

Lumbricidae and Megascolecidae. In this study, the species, *Aporrectodea caliginosa*, *E. fetida* and *Octolasion cyaneum* were identified for the very first time.

Diversity of earthworms: Punjab

Mohan *et al.* (2013) from Guru Nanak Dev University, Punjab reported the diversity and distribution of earthworm species. A total of six species of earthworms were identified. These species are from three different families. The species *Metaphire posthuma*, *Lampito mauritii* and *Amyntas morrisi* belong to the family Megascolecidae. Then, the species *Octochaetona beatrix* and *Eutyphoeus incommodus* are derived from Octochaetidae family while the *Bimastos parvus* belong to the family Lumbricidae. In his report, he also documented They also reported *Metaphire posthuma* as one of the most widely distributed earthworm species. Studying the relationship between earthworms and various physicochemical attributes of soil, they successfully obtained a positive relation with soil organic carbon, temperature, nitrogen level, soil moisture and pH. Paliwal (2014) submitted a report on the diversity of earthworms in the Gobindsagar and Nangal dam. The various species identified in the experiment were associated with families such as Tubificidae, Octochaetidae, Lumbricidae and Megascolecidae. The species i.e. *Bimastos parvus*, *Octochaetona Beatrix* and *Eutyphoeus waltoni* were found to be indigenous to Gobindsagar and Nangal dam wetlands. Singh *et al.* (2015) from Amritsar introduced the incidence of *Eutyphoeus waltoni* as well as *Metaphire houlleti* for the very first time.

Panjgotra *et al.* in 2016 conducted an experiment to study thoroughly the diversity and relative abundance earthworms residing in the wheat sown crop fields of Punjab. The experiment started in November 2014 and was completed by April 2015. Three sperate species of earthworm including *Lampito mauritii*, *Amyntas morrisi* and *Metaphire posthuma* were discovered. It was also reported that the abundance and diversity of *Metaphire posthuma* was relatively higher as compared to *Lampito mauritii*. Singh *et al.* (2018) studied the diversity and abundance of earthworm populations. He included all the districts of Punjab. In his survey, the earthworm species *Pontoscolex corethrurus* was documented for the very first time from the District Roopnagar. Further, the occurrence of earthworms in distinct land use patterns was studied by Singh *et al.* (2020). He also included the relationship of earthworms with the various soil parameters.

Earthworms in organic field-

Organic crop farming has a plethora of objectives of system sustainability and environmental sensitivity. In order to attain these two objectives, there are some rules and

standards which must be strictly adhered to. There are two broad cropping patterns which can be identified. These are:

- (i) Kharif crops which are sown early in the rainy season. The rainy season in India is generally from June to September. Paddy, maize, soyabean, groundnut and cotton are some examples of kharif crops.
- (ii) Rabi Crops, which are grown in the winter season (October to March). Examples of rabi crops include wheat, gram, pea, mustard and linseed. Besides these, pulses and vegetables in some places are preferred to be sown in summer season. The use of chemical fertilizers has helped a lot to farmers to get better crop yield such as of wheat, rice and maize (Sharma and Chetani, 2017). But due to excessive use of fertilizers soil has become less fertile. Fertilizers have also become a source of water pollution. Therefore, in order to maintain the fertility of the soil, there is a need to substitute fertilizers with organic manure or leave the field to be uncultivated (fallow) in between two alternate crops (Le Bayon *et al.* 2002).

In India, the casting activities of earthworms were first recorded by (Chaudhuri and Bhattacharjee 2011) in pasture lands. They surveyed Natural forest lands, coffee plantation soils, Acacia plantation grasslands and agro-forestry areas. The earthworm castings can be destroyed by heavy rainfall or soil aggregation. Due to continuous water infiltration, earthworms are known for reducing the amount of soil surface runoff. The requirements of soil moisture may vary with different earthworm species. There are several peregrine species which adapt themselves to the wide range of moisture conditions. Most of the worms prefer moist soils other than dry ones during the rainfall. The temperature and moisture varies inversely with each other. Dry soils remain earthworm deficient than less temperature and water logged areas. The work on earthworm showed that these organisms prefer neutral pH. On the basis of dispersal Paliwal and Julka (2005) grouped these species under i.) Endemic (native species) which are found in India and in other regions of country as well. A study on oligochaete family revealed most of the exotic species in soil, or in the roots of some plants.

Peregrine vs Exotic Earthworms

Michaelsen (1903) referred to earthworm species as "peregrine" because they had spread all over the world. More than 100 earthworm species were classified as peregrine globally (Blakemore, 2002). In India, their number is estimated to be 52, accounting for approximately 12.1 percent of the country's earthworm diversity (Julka and Paliwal (2005) classified Indian earthworms into three groups based on endemism and dispersal: I endemic

or native species that were restricted to India; ii) exotic peregrine species that originated in other biogeographical regions; and iii) native peregrine species that evolved in India and now have widespread distribution in the country and in other biogeographical regions. The extinction of native species, followed by the colonisation of exotic species, was associated with severe ecosystem disturbance and intensification of land use (Geissen *et al.* 2009). Nonetheless, coexistence of native and exotic species was more common than native species extinction (Marichal *et al.* 2010).

According to Edwards and Bohlen (1996), earthworms moved from one location to another through both active and passive dispersal. According to Addison (2009), active dispersal in earthworms is very slow and limited to 5-10 m/year. The majority of peregrine earthworms appeared to have dispersed passively along various pathways, including cocoon transportation in soil attached to the feet of animals and birds and around plant roots, in ballast and fishing bait, and other human activities (Gates, 1972). Gates (1976) listed several exotic peregrine species of earthworms in soil around plant roots or otherwise during a study on oligochaetes intercepted by the US Bureau of Plant Quarantine. The majority of these were also discovered in various parts of India over time (Gates, 1972; Julka, 1988). Exotic earthworms colonised disturbed habitats after being introduced, from which they expanded their range (Julka *et al.* 2004). The majority of exotic earthworm introductions in India were associated with the arrival of Europeans around 300 years ago (Gates, 1972), though Arabian and Chinese traders had also visited the country with rooted plants prior to the arrival of Europeans (Julka, 2008).

Several studies have found that introduced earthworms do not disperse into nearby undisturbed areas, even decades after their introduction (Kalisz and Dotson, 1989; Hendrix *et al.*, 2006). Other studies, however, have found that the introduction of exotic species may create hotspots that serve as hubs for further invasion into surrounding areas (Holdsworth *et al.* 2007). Bowie *et al.* (2016) investigated exotic earthworms' ability to move from pasture into adjacent fields with native vegetation, particularly leached agricultural lands. They also believed that exotic earthworms displaced native species through competitive interactions or occupied niches vacated by extinct native species. The introduction of exotic grasses and crops, as well as the associated soil disruption caused by agricultural practices, are thought to be the primary causes of endemic earthworm extinction (Lee, 1961). Increased anthropogenic activity has resulted in the spread of exotic earthworms all over the world (Reynolds 1995). It was determined that habitat fragmentation was the primary driver of exotic earthworm establishment and dominance in habitats occupied by native species (Kalisz and Wood, 1995). Exotic earthworm survival and establishment have also been linked to climatic

changes caused by global warming (Addison 2009). The changing climatic conditions were blamed for the range expansion of exotic earthworm species (Ahmed and Julka, 2017). Forest degradation and abandoned agricultural lands may have favoured exotics and resulted in the extinction of endemic earthworms (Sinha *et al.* 2003). Endemics outnumbered exotics in the rehabilitated agricultural land site, which had a more stable and resource-rich soil environment (Bhadauria *et al.* 2009). Boyer *et al.* (2016) discovered that by restoring native vegetation, the biomass of endemic earthworm species increased significantly, but exotic earthworm species did not disappear even after thirty years of restoration of native vegetation.

EARTHWORM ECOLOGICAL GROUPS

Evans and Guild (1947) divided earthworms into two groups: those that live on the surface of the soil and those that live deeper in the soil. Byzova (1965) was the first to distinguish surface-dwelling smaller worms from deep-dwelling larger worms based on metabolic rates. Bouché (1977) classified earthworms into three broad ecological categories based on their habitat, burrowing habits, and reproductive potential: i) Epigeic: litter or dung dwellers on the soil surface with a high rate of cocoon production; ii) Endogeic: living within soil in horizontal burrows with a moderate to high rate of cocoon production; and iii) Anecic: deep soil dwellers with a low rate of cocoon production.

Lavelle (1983) classified earthworms into five ecological groups: Epigeic, Anecic, oligo-, meso-, and poly-humic endogeic. Each functional group had a distinct impact on soil structure and biogeochemical processes (Lee, 1985). Klemmedson (1992) discovered that an increase in the accumulation of forest leaf litter layer favoured the population of Epigeic and Anecic earthworms. Kretzschmar (1998) demonstrated the adaptability of earthworm populations in terms of activities, fecundity, growth rate, and mortality in response to environmental changes. Several studies found that conversion of vegetation from forest to agroecosystems resulted in a decrease in Anecic earthworm populations and an increase in Endogeic earthworm populations in most tropical countries (Zou *et al.* 2006; González *et al.* 2008). Lavelle *et al.* (2004) discovered that a faster rate of organic matter decomposition reduced litter availability, which harmed the populations of Epigeic and Anecic earthworms while increasing the abundance of Endogeic species. In agroecosystems where surface organic litter was burned during field preparation, Epigeic earthworms were eliminated (Didden 2001). Zou *et al.* (2006) discovered that the diversity of Endogeic worms was greater in pastures than in forests in a study on the influence of land use changes on earthworm communities in Puerto Rico.

Bhadauria and Saxena (2007) linked changes in earthworm functional guilds to changes in land use and land management practices. Chaudhuri *et al.* (2008) found that Endogeic earthworms predominated in rubber plantations, with Epigeic species playing a minor role. Bhadauria *et al.* (2009) discovered lower earthworm diversity in natural and less disturbed ecosystems, as well as the dominance of Endogeic earthworm species in Indo-Gangetic Plains agro-ecosystems. The accumulation of organic matter on the soil surface in forests was thought to be beneficial to the development of Epigeic species (Salomé *et al.* 2011). Endogeic earthworms predominated in cultivated soils due to the abundance of plant residues that were ploughed back into the soil (Edwards, 1998). The incorporation of organic matter during ploughing benefits these worms by increasing food availability (Van Capelle *et al.* 2012). Endogeic worms were also discovered to be more tolerant of disturbed soil conditions (Jouquet *et al.* 2010). Singh *et al.* (2016) discovered increased populations of Endogeic earthworms and decreased diversity of Epigeic species in cultivation as a result of physical disturbance and chemical input.

EARTHWORMS POPULATION DENSITY IN NATURAL AND MAN-MADE ECOSYSTEMS

The pattern of land use and the type of vegetation not only influenced microhabitat conditions, but also altered the physicochemical properties of soil, which in turn influenced earthworm communities. The density of earthworms varied according to soil type, with lower density recorded in mor and fallow soils than in mull soils (Edwards and Bohlen, 1996). Abrahamsen (1972) found 10-40 m⁻² earthworms in ironpodsol and 60-260 m⁻² worms in semipodsols and brown earth. Mele and Carter (1999) discovered an average density of 89 m⁻² earthworms in cropping and pasture soils in Australia, with exotic lumbricid earthworms predominating. According to several studies, oak woodlands have higher earthworm densities than pine woodlands (Reddy and Alfred, 1978; Bhadauria *et al.* 2000). Earthworm density estimates for some European habitats ranged from 254-848 m⁻² in orchards to 18.5-33.5 m² in fallow lands (Dzangaliev and Belousova, 1969).

Earthworm densities were also found to be low in habitats affected by anthropochorous activities such as cattle trampling (Ismail *et al.* 1990), as well as in a South American tropical rain forest (Fragoso and Lavelle, 1992). Haynes *et al.* (2003) found 230-310 m⁻² earthworms in pastures and 60 m⁻² worms in maize and sugarcane cultivation in their study on the effects of land use on earthworm communities in South Africa. Zhipinga *et al.* (2006) discovered seven earthworm species at a density of 105 m⁻² in highly fertile soils under agricultural intensification in east China. Smetak *et al.* (2007) discovered significant

differences in earthworm population density and diversity in several urban areas in Idaho State, USA, including an urban park with the highest density (437 m⁻²), an old residential area with a medium density (121 m⁻²), and a young residential site with the lowest density (26 m⁻²).

Smith *et al.* (2008) discovered that the most intensively managed agricultural land-use and coniferous forest had the lowest population of earthworms, the intermediate population in no-till and tilled organic cropping systems, and the highest population in old deciduous forest systems. They also concluded that land-use intensity and land-use type are important drivers of earthworm community abundance and composition in agricultural ecosystems. Walsh and Johnson-Maynard (2016) discovered a higher population of 151.2 m⁻² earthworms in the driest crop-fallow than in the transition and 86.4 m⁻² in annual cropping fields. Kamdem *et al.* (2018) reported earthworm density and biomass from woody savannah (63 no. m⁻² and 22 gm⁻²), pasture (45 no. m⁻² and 25.2 g m⁻²), grass savannah (36 no. m⁻² and 25.4 g m⁻²) and agricultural land in a study on earthworm population in four land use types in Cameroon (21 no. m⁻² and 12.1 g m⁻²). A comparison of population size in different Indian ecosystems revealed that grasslands had the highest population of 936.5 m⁻² among natural ecosystems (Kale and Krishnamoorthy, 1978); 12-617 m⁻² in organic waste deposit sites among human interfered ecosystems (Sahu *et al.* 1988); 38.2-483.5 m⁻² in an orchard with grasses, 138.6-398.3 m⁻² in a barren land, and 51.9-274.5 Reddy *et al.* (1997) evaluated effect of periodic tillage on earthworm populations.

Blanchart and Julka (1997) reported that densities of earthworms ranged from 35.3 to 545.3 individuals m⁻² a mosaic of more or less disturbed forests, pastures and mainly paddy crops in the western Ghats (south India) where the natural evergreen forest had been highly disturbed and fragmented for 150 years. Sinha *et al.* (2003) determined an earthworm population of 395 m⁻² in a west Himalayan pine forest. During their studies on population of earthworms in rubber plantations in Tripura, Chaudhuri *et al.* (2008) reported different species of earthworm with mean density values of 108.6 m⁻². Mohan *et al.* (2013) reported predominance of exotic *Metaphire posthuma* at 4 sites including houses, nursery crop field and near water treatment plant in Guru Nanak Dev University campus Amritsar (Punjab). Prasad *et al.* (2016) studied the mixed forest of Kumaun Himalaya, Uttarakhand and reported that earthworm density ranged between 0-21 individuals m⁻² and biomass 0-9.8 gm⁻². In a comparison of earthworm density under different agroecosystems, Akilan and Nanthakumar (2017) recorded higher earthworm density in paddy field than in turmeric field.

SEASONAL VARIATION IN EARTHWORM'S POPULATION DENSITY AND BIOMASS

Earthworm density and biomass varied seasonally, and both physical and organic soil factors influenced earthworm abundance and distribution (Callaham and Hendrix, 1997). The seasonal abundance of earthworms was influenced by a number of interconnected ecological parameters, the most important of which were edaphic factors such as soil temperature and moisture (Julka, 1988). During the wet season, earthworms attained high densities and biomass (Rahman *et al.* 2012). Due to the high density of exotic lumbricids in the western Himalaya, earthworm activity peaked during early summer and winter rains (Julka and Paliwal 1990b), whereas maximum activity of earthworms in the plain region was observed during the summer rainy season (Reddy and Alfred 1978; Chauhan 1980). Earthworm activity and rainfall were found to be related by Fragoso and Lavelle (1992). According to Callaham and Hendrix (1997), earthworms are most abundant in the spring. Martinez *et al.* (2006) discovered an abundance of earthworms in the upper layers of soil during the rainy season, followed by their migration to the lower layers during the dry season. Joshi and Aga (2009) discovered increased earthworm activity in areas with high rainfall. Najar and Khan (2011) also found seasonal variations in earthworm density and biomass, ranging from 11 no. m⁻² at maize cultivation sites in summer to 159 no. m⁻² at pasture sites in spring. According to Mariappan *et al.* (2013), earthworm population density is higher during the rainy season than in the summer and winter.

ENVIRONMENTAL FACTORS INFLUENCING DISTRIBUTION OF EARTHWORM SPECIES

According to Evans and Guild (1947), earthworms were not uniformly distributed in the soil ecosystem, and their distribution and abundance were influenced by a variety of abiotic factors (Reynolds 1973). Certain parameters such as organic matter, soil type, soil moisture content, temperature, and soil pH were thought to be the most important in regulating earthworm populations (Werner *et al.* 2005). Various climatic factors and soil chemical parameters, such as dry and/or cold periods, variation in vegetation, organic carbon, nitrogen, phosphorus, and potassium, were also discovered to be responsible for the distribution and abundance of earthworms (Baker *et al.* 1993). Julka (1988) discovered that earthworms doubled their population in one month under ideal conditions of soil temperature, moisture, and food availability. Joshi and Aga (2009) discovered that soil moisture, temperature, pH, oxidizable organic matter, nitrogen, phosphorous, potassium, and calcium all influenced the abundance and distribution of earthworms in Shiwalik hills subtropical

forests. Abiotic factors such as total nitrogen, available phosphorous, available potassium, pH, soil moisture, soil texture, and organic carbon, according to Chauhan (2014) and Singh *et al.* (2016), have a strong positive effect on the distribution of earthworms.

SOIL TEXTURE

The distribution of earthworms in many ecosystems was influenced by soil texture (Huerta *et al.* 2007). Its influence on earthworm distribution was primarily explained by its impact on other edaphic properties such as soil moisture relationships, nutrient status, electrical conductivity, and cation exchange capacity (Edwards and Bohlen, 1996). Earthworm populations were higher in light and medium loam soils than in alluvium and gravelly soils (Guild 1948). Boyd (1957) discovered more earthworms in both light and dark peaty soils. *Aporrectodea caliginosa* and *Lumbricus castaneus* were common in light soils, while *Allolobophoridella eiseni* and *Dendrobaena octaedra* were common in dark peaty soils. Earthworm populations were denser in mull soils than in moder and mor soils (Alphei, 1998). *Aporrectodea rosea* abundance in alluvial soil was attributed to higher clay content in soil (Nordstrom and Rundgren 1974), whereas *Octochaetona serrata* preferred clay loam soils (Ismail and Murthy 1985). In relation to soil texture, earthworms frequently displayed clumped spatial distributions (Hendrix and Bohlen 2002). In a tropical pasture system, there was an inverse relationship between earthworm abundance and soil sand content, and in rain forest soils, there was a positive relationship between earthworm density and clay content (Huerta *et al.* 2007). Parthasarathi *et al.* (2015) discovered that clay loam and sandy loam soils with optimal moisture (17.6-23.8 percent), pH (6.7-7.3), organic carbon content (0.61-78 percent) and temperature (17.3-18.5°C) had the highest earthworm populations.

SOIL TEMPERATURE

Edward and Lofty (1977) discovered that temperature had a significant impact on earthworm activity. Bodenheimer (1935) discovered that the activity of the lumbricid *Allolobophora samarigera* was normal at 14.7°C-27.8°C, inactive at -13°C, and paralysis at 32.4°C. At higher temperatures, these organisms' sexual maturity decreased (Evans and Guild 1948). *Aporrectodea caliginosa* laid more cocoons as temperature increased from 6°C to 16°C, and hatching was also improved as temperature increased (Evans and Guild, 1948). The nocturnal surface activity of Anecic *Lumbricus terrestris* was highest at 10.5°C (Kollmannsperger 1955). According to various studies, different species became active at different temperatures: *Amyntas hupiensis* at 15 to 23°C, *Eisenia fetida* at 15.7 to 23.2°C, and *Aporrectodea caliginosa* at 10 to 23.2°C (Grant 1955); *Aporrectodea rosea* at 24.1 to 25.6°C (Reinecke, 1983). Voisin (1961) discovered that different soil temperatures suited for

optimal earthworm activity: 15°C for *Octolasion cyaneum* and *Allolobophora chlorotica*, 12°C for *Aporrectodea rosea* and *Aporrectodea caliginosa*, 18°C to 20°C for *Dendrodrilus rubidus*, 25°C for *Eisenia fetida* and 15°C to 18°C for *Lumbricus rubellus*. Earthworm temperature tolerance varied by species: 37°C to *Pheretima californica* - 37.75°C, *Aporrectodea caliginosa* - 39.55°C to 40.75°C (ElDuweini and Ghabbour, 1968); *Hyperiodrilus africanus* - 34°C to 38.5°C (Madge 1969); *Aporrectodea rosea* - 29.7°C (Reinecke 1983). *Eisenia fetida* and *Dendrobena rubida* required a higher optimal temperature for development, according to Reynolds and Jordan (1975). Several studies have revealed that the populations of some Indian earthworms have decreased as soil temperatures have decreased (Ismail and Murthy 1985; Karmegam and Daniel 2007).

SOIL MOISTURE

Earthworms can survive in poor soil moisture conditions by migrating to moist areas (Valle *et al.* 1997) or by aestivation (Baker *et al.* 1992). Preventing body water loss was critical for earthworm survival because water made up 75-90 percent of their body weight (Grant, 1955). The greatest population of earthworms was found in soil with moisture levels ranging from 12 to 30 percent in the Ohio region of the United States (Olson, 1928). Several studies have found that earthworm density is directly related to soil moisture (Satchell 1967; Reynolds 1972). Auerswald *et al.* (1996) discovered that the soil moisture requirements of earthworms differed by region. Peak earthworm populations were recorded in subtropical India during the monsoon season, while the lowest earthworm populations were recorded during the dry summer season (Choudhuri and Mitra 1983). According to El-Duweini and Ghabbour (1968), the population of *Aporrectodea caliginosa* increased with an increase in soil moisture from 15% to 34%. Madge (1969) discovered that the main activity of *Hyperiodrilus africanus* in Nigeria coincided with the onset of the wet season, when soil moisture levels ranged from 12.5% to 17.2%. Low soil moisture, according to Reynolds (1972), has a negative impact on earthworm density and biomass. *Millsonia anomala*, a surface-dwelling West African earthworm, went into dormancy in response to extreme draughts and reawakened at 8% to 10% soil moisture (Lavelle *et al.* 1988). In Orissa, earthworms went dormant when soil moisture fell to 10% or less (Dash *et al.* 1980). Kale and Krishnamoorthy (1981) linked the increase in adult population of *Lampito mauritii* with rainfall in a study on seasonal changes in earthworm population.

Fragoso and Lavelle (1987, 1992) and Fragoso (1993) found that earthworms migrate upward to the upper layers of soil during the rainy season and downward to the lower layers during the dry season. During the dry season, Jimenez and Decaens (2000) observed

Ocnerodrilidae earthworms moving downward. Seasonal variations in soil moisture were found to have a significant positive correlation with earthworm population/ biomass (Ghosh, 1993). High earthworm activity species diversity was linked to higher soil moisture by Joshi and Aga (2009) and Najar and Khan (2011). Lalthanzara *et al.* (2011) found a link between an increase in earthworm population and rainfall in Mizoram agroforestry plantations. Earthworm populations of most species were affected by seasons, according to Bhadauria *et al.* (2012) and Mariappan *et al.* (2013), with the highest values during the rainy season. According to Kalu *et al.* (2015), there is a significant positive correlation between earthworm population density and soil moisture.

SOIL pH

Changes in soil pH influenced the structure, distribution, and population of earthworms. Several studies have found that earthworms prefer neutral soil (Arrhenius 1921; Petrov 1946). Wherry (1924) stated that these organisms preferred slightly acidic and neutral soils but could not tolerate pH levels below 6.0. Olson (1928) reported a pH range of 5.4 to 8.6 for Ohio earthworms in the United States. Evans and Guild (1947) discovered that European earthworms have a pH range of 6.3 to 8.1. Satchell (1955) classified lumbricid earthworms into four pH-tolerant groups: i) Acid-tolerant species: *Dendrobaena octaedra*, *Bimastos eiseni*, and *Dendrodrilus rubidus*; ii) Acid-intolerant species: *Aporrectodea caliginosa*, *Ap. rosea*, *Allolobophora longa*, *A. chlorotica*, and *A. nocturna*; iii) Ubiquitous species: *Lumbricus castaneus*. Lee (1959) discovered that pH had no effect on New Zealand earthworms. El-Duweini and Ghabbour (1968) discovered that earthworm populations in Egyptian soils decreased as pH increased from 7.25 to 8.25. Reynolds (1972) discovered that soil acidity reduced earthworm biomass. Several studies concluded that most earthworm species preferred soil pH levels ranging from 6.0 to 7.8 (Edwards and Bohlen, 1996; Chaudhuri and Bhattacharjee 1999). Raty and Huhta, 2003. demonstrated that soil pH was critical for controlling earthworm populations. The diversity of acid-tolerant species was drastically reduced in soils with pH levels greater than 7.0 (Lee 1985). *Lampito mauritii* and *Octochaetona serrata* were found in higher densities by Ismail and Murthy (1985) in neutral sandy loams and medium acidic clay loams, respectively. Seasonal abundance of *Octochaetona philloti* juveniles and subadults correlated positively with soil pH in a semi-arid tropical grassland in southern Peninsular India (Reddy and Pasha 1993). According to Edwards and Bohlen (1996), soil pH is a factor that limits earthworm distribution. It was discovered that there was a positive relationship between soil pH and earthworm population (both density and biomass) (Najar and Khan 2011). Earthworm diversity and activity decreased in both highly acidic (pH below 5.0) and highly alkaline (pH above 9.0) soils

(Werner *et al.* 2005). Chauhan (2014) also demonstrated that the majority of earthworm species did not prefer both low and high pH values. However, some research has found that earthworms prefer slightly acidic to moderately alkaline soils (Chaudhuri and Bhattacharjee 1999; Kumar and Singh, 2013). According to Karmegam and Daniel (2007), the majority of earthworm species preferred slightly acidic to neutral soils with pH levels ranging from 6.0 to 7.0. Chaudhuri *et al.* (2008) discovered that earthworms tolerated acidic soils with pH ranging from 4.1 to 6.8 in rubber plantations and mixed forests.

SOIL ORGANIC CARBON

According to Edwards and Bohlen (1996), organic matter had a large influence on earthworm distribution, and poor soils had low earthworm populations. For food, these organisms primarily consumed decaying plant material. Because of their limited mobility, they lived in areas very close to the source of their food (Reynolds and Jordan, 1975). Nonetheless, worms were found in lower numbers in undisturbed thick layers of decaying organic matter (Ismail and Murthy, 1985). There had been very few studies on the impact of organic matter on the diversity and abundance of soil organisms (Tian *et al.* 1997). Seasonal changes in earthworm population were determined by the periodic increase or decrease of organic materials in soil (El-Duweini and Ghabbour 1965). Reynolds (1972) preferred decreased earthworm density and biomass with a decrease in food source palatability. There was a significant correlation found between the population of deep burrowing anecic earthworms and the amount of organic matter in the soil (Nordstrom and Rundgren 1974). Because of the availability of higher organic matter in soil, Phillipson *et al.* (1976) and Baker *et al.* (1993) found a greater number of earthworms in forests. However, Kale and Krishnamoorthy (1978) discovered no relationship between population densities and soil organic matter levels.

The density of earthworms and the organic content of the soil were found to have a strong positive correlation (Karmegam and Daniel 2007). However, Bhadauria and Ramakrishnan (1989) linked a decline in earthworm population in shifting cultivation fallow lands to an increase in organic matter. Several studies have found a positive relationship between earthworm population density and soil organic carbon percentage (Brown *et al.* 2003; Lathanzara *et al.* 2011). *Perionyx excavatus* and *Eisenia fetida* were discovered to live in dung heaps with high organic carbon levels ranging from 27.93 to 57.67 percent (Paliwal and Julka 2007). Soil organic carbon was essential for earthworm abundance and distribution (Chan and Barchia 2007). According to Chaudhuri *et al.* (2008), earthworms in rubber plantations in Tripura, India, could tolerate a wide range of organic matter (0.67-8.46

percent). Earthworm density and biomass increased as soil available carbon increased (Suthar 2009). Mariappan *et al.* (2013) also linked an increase in earthworm density and biomass to an increase in soil organic carbon.

SOIL NITROGEN

Higher fecundity in earthworms was related to nitrogen rich food than to low nitrogen diets (Evans and Guild 1948). Bhatnagar (1975) attributed high earthworm population to low C/N ratio due to increased activity of nitrogen fixers in drilosphere. Edwards and Bohlen (1996) also observed that earthworms favored litter with C/N ratio of 20:1. Scheu (1987) found a linear correlation between earthworm population and mineralization of nitrogen. Rate of nitrification in the walls of earthworms' burrows and castings were greater than I surrounding soil (Kharin and Kurakov 2009). Kale and Krishnamurthy (1981) recorded higher. Yadav *et al.*, 2015 linked higher macroinvertebrate density and biomass to high soil nitrogen content. Iordache and Borza (2010) discovered a link between earthworm biomass and total nitrogen. Moghimian *et al.* (2013) concluded that total nitrogen in soil regulated the distribution of macrofauna based on PCA studies. Parthasarathi *et al.* (2013) discovered that nitrogen plays an important role in regulating the abundance and distribution of earthworms. According to Chauhan (2014), earthworms do not prefer organic residues with a high C/N ratio because they are less palatable.

PHOSPHORUS AND POTASSIUM

The long-term effects of earthworm feeding behaviour improve soil ecosystem nutrient cycling processes (Lavelle *et al.* 1988). Earthworm casts, according to Sabrina *et al.* 2009, have a significant impact on plant growth due to their effects on microorganisms, soil aggregation, and nutrient supply. The presence of a large number of micro-flora in the earthworm's gut was important in increasing the phosphorus and potassium content of the vermicompost (Bisen *et al.* 2011). Casts of the geophagous earthworm *Pontoscolex corethrurus*, for example, had higher phosphate availability than the bulk soil of an Oxisol (Chapuis-Lardy *et al.* 1998). Increased phosphorus availability in earthworm casts boosts phosphatase activity (Satchell and Martin 1984). Enzymes like -glucosidase, alkaline phosphatase, dehydrogenase, and protease were discovered to be more abundant in the castings than in the surrounding soil (Tao *et al.* 2009). Soil phosphorus and earthworm density were found to have significant positive correlations in a variety of agroforestry ecosystems and cultivable soils (Parthasarathi *et al.* 2013). Earthworms converted potassium into a form that plants could use (Zhu *et al.* 2013).

EARTHWORM DIVERSITY AND LAND USE SYSTEMS

The transformation and intensification of various land use systems had a significant impact on the distribution and diversity of earthworms (Hedlund *et al.* 2004). The transformation of natural ecosystems to man-made systems, according to Geissen *et al.* (2009) involved changes in edaphic attributes as well as soil biodiversity. Land use changes frequently resulted in a decrease in the activity and diversity of soil macro-invertebrates, including earthworms (Hedlund *et al.* 2004). According to Scheu (1992), stable ecosystems have greater species diversity than unstable environments. Earthworm species composition and population structure in natural forests have been altered as a result of interference and degradation (Fragoso and Fernandez 1994).

A lowland broadleaf forest had a higher species richness than a high-altitude silver beech stand (Moeed and Meads, 1986). González *et al.* (1996) discovered that exotic earthworms inhabited both plantation and secondary forests in a study on earthworm habitat preference in Puerto Rico, whereas native earthworms were only found in secondary forests. Chandran *et al.* (2012) discovered a greater diversity of earthworm species in tropical rain forests than in grasslands or moist deciduous forests in Nilgiri Biosphere Reserve. The highest species richness was found in natural mixed forest, followed by oak-dominated disturbed forest and managed oak plantation in Manipur's northeastern hills (Haokip and Singh, 2012). The number of peregrine earthworms increased in tropical rain forests as a result of deforestation, and one or more peregrine species became dominant as land use systems changed (González *et al.* 2007). Rathinamala *et al.* (2011) found that earthworm species diversity was lower in arable land than in uncultivated land, whereas Najar and Khan (2011) found that earthworm species diversity was high in anthropogenic systems. When a traditional pure crop system was converted to a traditional agroforestry system, earthworm density and biomass increased rather than species richness (Bhadauria *et al.* 2012). There was an increase in earthworm density and biomass as the age of rubber plantations increased, but there was a decrease in species diversity, species evenness, and species richness (Chaudhuri *et al.* 2013).

Earthworm densities and diversity were generally lower in agro-systems. Nonetheless, earthworm populations were high in both continuously cropped and conventional corn fields (Whalen *et al.* 1998), and they fluctuated in response to changes in vegetation and soil characteristics (Valckx *et al.* 2011). Earthworm populations and species richness were lower in arable lands than in pastures (Nunes *et al.* 2006). Earthworm populations, according to Smith *et al.* (2008), varied depending on the type of land use. Earthworm density was lowest in intensively managed agricultural systems and coniferous forests, intermediate in no-till and

tilled organic cropping systems, and highest in old deciduous forest systems. Kumari *et al.* (2012) discovered significant differences in earthworm population densities between tilled and non-tilled lands. According to Somniam and Suwanwaree (2009), the highest density of earthworms was found in residential areas, followed by arable and forested lands.

Arable soils had a higher index of earthworm species diversity and evenness than gardens, grasslands, and wastelands (Tripathi and Bhardwaj 2004). Najjar and Khan (2011) found the highest earthworm species diversity index in flower gardens and the lowest in paddy cultivation; the highest and lowest species richness index in *Populus* and paddy plantations, respectively; and the highest species evenness in flower gardens and the lowest in paddy cultivation in a comparative study on various earthworm diversity indices.

CHAPTER-III

MATERIALS AND METHODS

The present research was done to determine the abundance and diversity of earthworm species in different cropping systems under organic and conventional farming. The different farming systems surveyed were basmati-wheat, basmati-chickpea, soybean-wheat, moong-wheat. All these fields are in the experimental area of School of Organic Farming, Punjab Agricultural University, Ludhiana. Earthworm collection was carried out from July, 2020 to March, 2021 in the assigned fields of the experimental Area.

3.1 EARTHWORMS SAMPLING AND IDENTIFICATION

3.1.1 Collection, preservation and population count of earthworms

The four different organic and conventional farming systems studied were basmati-wheat, basmati-chickpea, soybean-wheat, moong-wheat at the experimental area of School of Organic Farming, College of Agriculture, Punjab Agricultural University, Ludhiana. The fields had a plot size of 15m × 9m. These fields were surveyed at monthly intervals from June, 2020 to March 2021. The sampling of earthworms was done on monthly basis using hand sorting method. Moreover, the GPS coordinates of each sampling site were also recorded (Table1 and 2). Earthworm sampling method of Singh *et al.* (2016) was followed for sampling.

3.1.2 Collection and Identification of Earthworms

Earthworms were hand-sorted up to 50 cm deep (25 cm x 25 cm) at each study site under organic and conventional farming systems. The earthworms were separated from the above-mentioned block. The same area was also dug deeper with a spade to collect the deep burrowing earthworm individuals. The extracted earthworms were washed thoroughly with tap water and dried on filter paper. Further, the earthworms were killed using 70% ethanol and preserved in a 5% formalin solution. Then, using a stereomicroscope, all the preserved earthworms were examined for various characteristics such as total number of segments, prostomium shape, position and type of clitellum, position and number of spermathecae, position of male pore, and total length (in cm) using measuring scale. These external characteristics were investigated by examining earthworm samples under a microscope. Eventually, a pointed needle was used to count the number of segments from prostomium to anus.



Plate 1. Earthworms preserved in 5 percent formalin solution in glass vials for further study of morphological characters



Plate 2. Fixation of worms in cotton bed



Plate 3. Using a stereomicroscope, the preserved samples of earthworms were examined for morphological characteristics such as total length (in cm), total number of segments, prostomium shape, position and type of clitellum, position and number of spermathecae, position of male pore, and so on.



Plate 4. Identification of morphological characteristics of earthworms under stereomicroscope. The observations were recorded in a visualizer.

Table 1. The sampling sites under organic fields.

S. No.	Sampling Sites	Vegetation	Latitude	Longitude
1	ORGANIC FIELDS	Basmati-Wheat	30°90'13"	75 ° 78'77"
2		Basmati-Chickpea	30 ° 90'48"	75 ° 78'50"
3		Soybean-Wheat	30 ° 90'93"	75 ° 78'05"
4		Moong-Wheat	30 ° 90'17"	75 ° 78'22"

Table 2. The sampling sites under conventional fields.

S. No.	Sampling Sites	Vegetation	Latitude	Longitude
1	CONVENTIONAL FIELDS	Basmati-Wheat	30 ° 91'06"	75 ° 78'60"
2		Basmati-Chickpea	30 ° 91'32"	75 ° 78'68"
3		Soybean-Wheat	30 ° 91'44"	75 ° 78'61"
4		Moong-Wheat	30 ° 91'68"	75 ° 78'15"

3.2 EARTHWORM ECOLOGICAL INDICES

3.2.1 Relative abundance

Individual earthworms from both organic and conventional fields were identified to species level and classified into their ecological groups, namely Anecic, Epigeic, and Endogeic. The relative abundance of each earthworm species was calculated in percentage. The below given formula was used to measure the relative abundance of each earthworm species:

$$\text{Relative abundance(\%)} = \frac{\text{Number of earthworm individuals in each species}}{\text{Total number of Individuals}} \times 100$$

Photographs of each earthworm sample were also taken with a MIPS camera mounted on a microscope. Following a thorough examination, the earthworm samples were transferred to their respective vials. The various external morphological characteristics of each earthworm specimen were compared with Julka's (2008) earthworm taxonomic keys to identify the earthworms to species level. Dr. J.M. Julka with a designation of Former

Scientist at the Zoological Survey of India, Department of Zoology, Shoolini University, Himachal Pradesh, India, confirmed the earthworm species.

3.2.2 Simpson index (D)

The Simpson index measures the degree to which a species outnumbers its competitors in an ecological community. Its value ranges from 0 (no species present) to 1 (one species dominating completely). As a result, higher the values of D, the lower the diversity, and vice versa. The Simpson Index was computed using the following formula:

$$D = \sum \left(\frac{n}{N} \right)^2$$

n = Number of earthworm individuals in each species,

N = Whole number of earthworm individuals

3.2.3 Shannon-Wiener Diversity Index (H')

The Shannon and Wiener index (1949) measures the earthworm species diversity index. Its formula is:

$$(H') = -\sum p_i \times \ln p_i$$

p_i = Proportion of total sample represented by a species i,

ln = logarithm to base e

3.2.4. Species evenness index (E)

Pielou's evenness is related to species evenness and therefore Pielou's evenness index is known for calculating the species evenness index (Pielou, 1966). Its formula is:

$$E = \frac{H'}{\ln S}$$

H' = Shannon and Weiner diversity index,

S = number of species.

3.3 MOLECULAR CHARACTERISATION OF EARTHWORMS

Different species of earthworm were collected from four different conventional and organic cropping systems: basmati-wheat, basmati-chickpea, soybean-wheat, moong-wheat. The collected earthworm species were preserved in 100% ethanol. Extraction of DNA and their amplification for mtCO- I gene was carried out. Earthworms total genomic DNA was

isolated with modified CTAB method and the quantity was measured using NanoDrop Spectrophotometer. Further, the quality was determined using 0.8% agarose gel. Again, the PCR of the mtCO- I gene with universal primers namely **LCO 1490 (5'-GCTCAACAAATCATAAAGATATTGG-3')** and **HCO 2198 (5'-TTTCAGGAAACGTGACCAAAAAATCA -3')** was carried out.

The amplifications of PCR were done in an **Veriti™ 96-Well Thermal Cycler** (Applied Biosystem, USA) with the following parameters: three minutes of denaturation at a temperature of 94°C, 38 cycles at 94°C for 30 seconds, annealing at 52°C for 45 seconds, and 1 minute at 72°C, followed by an ultimate elongation step of 10 minutes at a temperature 72°C, and held at 4°C. A single discrete PCR amplicon band of 750 bp was observed when resolved on agarose gel (1.5%). The 'barcode fragment'/ mtDNA COI 3' fragment gene PCR amplicon was eluted and purified using NucleoSpin Gel and PCR Clean-up kit according to instructions of the producer. The LCO1490 forward and HCO 2198 reverse DNA sequencing reaction of PCR amplicon was done using a forward and a reverse primer respectively. BDT v3.1 was also used along with cycle sequencing kit on ABI 3730xl Genetic Analyzer which brought out the consensus sequence of barcode fragment gene.

Finally, the insect DNA barcode fragment gene sequence was exploited to perform the NCBI-Basic Local Alignment Search Tool database. Based on maximum identity score, the first ten sequences were selected and aligned using multiple alignment software program i.e. ClustalW multiple alignment. Distance matrix was generated and the phylogenetic tree was constructed by using MEGAX.

3.4 PHYSICO-CHEMICAL ANALYSIS OF SOIL

The analysis of soil temperature, moisture content and other physico-chemical attributes of soil such as N, P, K, OC, EC and pH of both organic and conventional farming systems were done. After burning the soil samples in a muffle furnace at a temperature of 550 °C, the Organic carbon was computed using the Walkely and Black method (1934). The N content in soil was calculated using Kjeldahl assembly. The soil sample was digested with concentrated H₂SO₄, it was then made to run through the Kjeldahl assembly, and eventually the titration was carried out using 0.01 N HCl, according to the Subbiah and Asija (1956). Further, the soil phosphorous was measured by spectrophotometer method. It included the digestion the soil using perchloric acid as well as nitric acid in a ratio of 1:4, respectively. Then with a colorimeter, Olsen's method was used to estimate phosphorus. The assessment of soil temperature and moisture are given below:

3.4.1 Soil temperature

Soil temperature in the field was computed by an apparatus known as digital thermometer. The thermometer was inserted directly in the soil and was kept for one minute before the temperature was noted. Soil temperature was recorded at monthly interval after sowing of each crop.

3.4.2 Soil moisture

Soil moisture was measured by gravimetric method. In this method, a fresh sample of soil was sieved and core weight was taken. It was then oven dried and reweighed.

3.4.3 Evaluation of pH, Electrical conductivity (EC)

After air drying the soil sample, 5 gm of soil was made to dissolve in 50 ml of distilled water (1:10 w/v) and vigorously shaken for 40 minutes using an orbital shaker. The supernatant was taken to measure the pH (Jackson, 1973) and EC (Richard, 1954) using a pH metre. Prior to use, the pH metre was calibrated with buffer solutions with pH values of 4, 7, and 9.2. In contrast, the standard potassium chloride (0.1N) was used as an EC standard. The EC was measured in dS/cm.

3.4.5 Nitrogen (N)

The total nitrogen in soil was determined using the Subbiah and Asija (1956) method.

Reagents

- a) 0.32% KMnO_4 (potassium permanganate) solution: This solution is made by dissolving 3.2g of pure KMnO_4 in distilled water. The final volume was made up to one litre.
- b) 2.5% NaOH (sodium hydroxide) solution: This alkali solution was prepared using 25 g of NaOH in distilled water. Again, the final volume was one litre.
- c) 4% H_3BO_3 (boric acid): The 4% boric acid was NaOH solution was made by combining 40g of boric acid with distilled water to make a final volume one litre.
- d) 0.01 N H_2SO_4 (Sulphuric acid): It was prepared by adding 2.71 ml concentrated sulphuric acid into distilled water to make one L final volume. This mixture was standardized with 0.01 N Na_2CO_3 along with methyl orange indicator. The dilution of the solution was done ten times for the ultimate preparation of 0.01N H_2SO_4 .
- e) Indicator mixture: 0.5 gm of bromo cresol green along with 0.1 gm of methyl red was diluted in 100 ml 95% ethanol to prepare the mixed indicator.

Procedure

In a distillation flask, 20 gm air dried soil sample and 1 g digestion mixture was placed. The soil sample was moistened using 20 ml distilled water. It was carried out to assemble all the apparatuses for distillation. Distillation was performed and the ammonia released from the components of distillation assembly was captured in a 250 ml conical flask. Now, 20 ml of H_3BO_3 and about 4-5 drops of mixed indicator was taken. After dipping one end of the delivery tube, the colour of the solution changes from pink to greenish. The change in colour occurs due to the absorption of the NH_3 .

Calculation:

$$\text{Nitrogen (kg/ha)} = \frac{R(a-b) \times N \times 14 \times \text{weight of one hectare of soil}}{\text{Weight of soil sample} \times 1000}$$

Where, a = volume of HCl used in titration for sample (ml),

b = volume of HCl used in titration for blank (ml),

N = Normality of HCl acid,

14 = atomic weight of Nitrogen

N (kg/ha) = N (%) $\times$ 10, where 10 is conversion factor.

3.4.6 Organic carbon (OC)

The organic carbon content was determined using the Walkely and Black method (1934).

Reagents

- Potassium dichromate solution (0.166M): To make the 0.166M potassium dichromate solution, dissolve 48.83g of potassium dichromate in 1 litre of distilled water.
- Ferrous sulphate solution (1M): A 1M solution of ferrous sulphate was made by combining 151.90g of ferrous sulphate in 800ml of distilled water, adding 20ml of conc. H_2SO_4 , to make the final volume 1 litre.
- Conc. H_2SO_4 (sulphuric acid -AR grade)
- Barium diphenylamine sulphonate
- Orthophosphoric acid

Procedure

Wet combustion of OC with a mixture of potassium dichromate ($K_2Cr_2O_7$) and sulfuric acid (H_2SO_4) was used in this method. 1 g soil sample was made to put in a 500 ml flask with 10 ml solution of potassium dichromate (0.166 M) and 20 ml of concentrated sulphuric acid (H_2SO_4). It was allowed to stand for 30 minutes before adding 250 mL of distilled water and 10 mL orthophosphoric acid. As an indicator, 1 ml of Barium diphenylamine sulphonate was added and titrated with 1M ferrous sulphate (Fe_2SO_3) solution. At the final stage, the colour of the solution changed from purple to green. The blank sample was also prepared in the same manner, but without the addition of soil.

Calculations:

$$OC (\%) = M \times \frac{V_1 - V_2}{S} \times 0.39$$

Where M= Molarity of Fe_2SO_3 solution,

V_1 = ml of Fe_2SO_3 solution used for blank,

V_2 = ml of Fe_2SO_3 solution used for sample,

S= weight of soil sample,

0.39 = correction factor.

3.4.7 Phosphorus (kg/ha)

Estimated along with the extraction of sodium bicarbonate (Olsen *et al.* 1954)

Reagents

- a) Charcoal
- b) 0.5 N Sodium Bicarbonate ($NaHCO_3$)
- c) 5 N Sulphuric Acid (H_2SO_4)
- d) Reagent A: 12 g ammonium molybdate + 300 ml of distilled water. In a separate beaker, put 0.2908 g antimony potassium tartarate + 300 ml hot water. This solution was cooled and mixed well with 1 litre 5N sulphuric acid (H_2SO_4).
- e) Reagent B: 1.056 g L- ascorbic acid and dissolve in 200 ml of reagent A

Procedure

1 gram of soil was weighed and transferred to a 100 ml of a conical flask. A small pinch of charcoal along with 20 ml of 0.5 N NaHCO_3 solution were added. For half an hour, the sample was shaken at a constant speed on an electrical shaker. Whattmann No. 1 filter paper was used. Then, 5 mL of extract was pipetted into a 25 mL volumetric flask and 0.5 mL 5N H_2SO_4 was added into it, and the flask was shaken until the carbon dioxide evolution stopped. Also, 10 mL of distilled water was added to thoroughly wash the flask's neck and sides. Finally, 4ml of reagent B was added to bring the volume up to the mark. At 760 m μ , the colorimeter recorded the percent transmittance. Preparation of standard curve: For standard curve, pipette out 0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 ml of 5 ppm. Phosphorous soln. was taken in seven different volumetric flasks each of 25ml capacity. Then graph was plotted by P concentration versus absorbance/transmittance reading taken on single graph paper.

Observations and calculations

Weight of soil = 1 g

Volume of 0.5 N NaHCO_3 solution= 20 ml

1st dilution= 20 times

Volume of filtrate taken for colour development=5 ml

Final volume = 25 ml

2nd solution= 5 times

Total dilution= 20 times $\times$ 5 times = 100 times

Transmittance of test sample=T

Concentration of Phosphorous read from standard curve against T= X ppm

Available P in soil (ppm) =X $\times$ 100

Available P in soil (kg/ha) = ppm $\times$ 2.24=A

Available P in soil (kg/ha) = A/2.5

3.4.8 Potassium (kg/ha)

Determined by ammonium acetate method preferably Simmard (1993).

Reagents

1. Ammonium Acetate (1 N)

Procedure

5 gm of soil was weighed and transferred to a conical flask of 150 ml. Then a 25 ml solution of Ammonium Acetate was put into that flask and shaken for 5 minutes. This suspension was passed through Whatman No. 1 filter paper. Now, 5 ml of the extract was pipetted out into 25 ml volumetric flask and volume added with distilled water. The solution was aspirated into automizer of calibrated flame photometer and readings were taken.

Observations and Calculations

Weight of soil = 5g

Volume of Ammonium acetate solution added=25 ml

1st dilution = 5 times

2nd dilution =5 times

Total Dilution= 5times $\times$ 5times = 25 times

Reading of the flame photometer for the test sample=X

Available potassium in soil (kg/ha) = X $\times$ total dilution =A

Available potassium in soil (kg/ha) = A $\times$ 2.24 =B

Available potassium in soil (kg/ha) = B/2.5

3.5 Statistical analysis

To compare the abundance of earthworms in organic and conventional farming systems, a two -way ANOVA followed by a Tukey post hoc test was used. The soil data included physico-chemical characteristics of soil. Further, the t-test analysis was done to analyze the soil parameters of the two selected farming systems i.e. Organic and Conventional farming systems.

CHAPTER-IV

RESULTS AND DISCUSSION

Morphological characteristics

Earthworms collected from different areas of four fields i.e. basmati-wheat, basmati-chickpea, soybean-wheat, moong-wheat at the experimental area of School of Organic Farming, Punjab Agricultural University, Ludhiana. These earthworm species were identified on the basis of key morphological features such as colour, number of segments, number of segments of clitellum, and spermathecae pairs, family, body length, prostomium shape, clitellum position, male pore and other features have been tabulated (Table3). In the current study, four earthworm species were identified using Julka's (2008) earthworm taxonomic keys based on morphological characteristics. The identified species belonged to families: Megascolecidae and Octochaetidae.

Earthworms benefit from nearly all organic fertilisers. Organic material additions can double or triple earthworm populations in a single year. The ammonia and salt content of some liquid manure can be harmful to earthworms, but populations usually recover quickly and continue to grow (Edwards and Bohlen, 1996). In most cases, the use of inorganic fertilisers has a positive effect on earthworm populations. This is most likely an indirect result of increased crop biomass production and, as a result, increased organic residues (Edwards and Bohlen, 1996; Edwards *et al.*, 1995). Where moisture levels would otherwise be too low, irrigated soil can support high levels of earthworm activity. Irrigation also increases crop production, resulting in more food and a greater population of earthworms. Irrigation waters containing earthworms and their cocoons may serve as a source of inoculum for some species (Edwards *et al.*, 1995). By aerating the soil, draining poorly drained soils may provide a more favourable environment for earthworm activity.

Table 3. Morphological characteristics of earthworm species.

Species	Family	Body length	Total no. of segments	Prostomium	Colour	Clitellum type	Clitellum segments	Spermathecae number	Spermathecae segments	Male pore	Female pore
<i>Metaphire posthuma</i>	Megascolecidae	12 cm	90-134	Epilobous	Dark brown	Annular	14-17	4	5/6-8/9	18	14
<i>Lampito mauritti</i>	Megascolecidae	8 cm	165-190	Prolobous	Light pink	Annular	14-17	3	6/7-8/9	18	14
<i>Amyntas morissi</i>	Megascolecidae	9 cm	98	Epilobus	Dark red	Annular	14-16	2	5/6-6/7	18	14
<i>Travoscolids chengannure</i>	Octochaetidae	12 cm	230	Prolobous	Light brown	Annular	14-18	2	8 and 9	18	14



Adult Earthworm



Prostomium



Genital apertures

Plate 5: Morphological characters of *Metaphire posthuma*

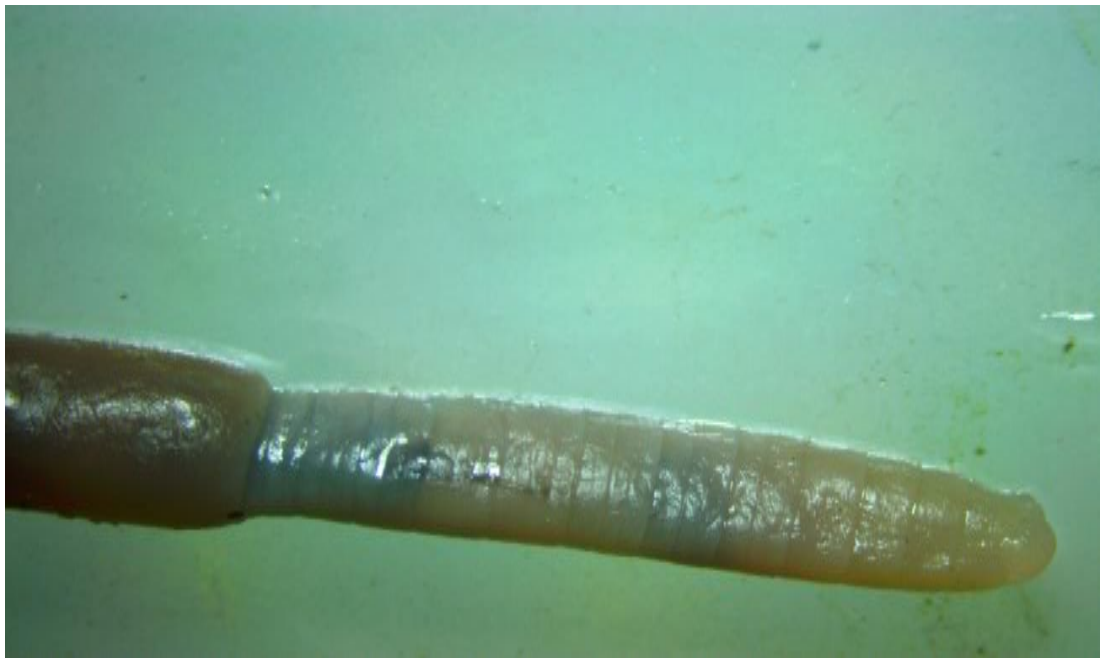


Adult Earthworm



Prostomium

Plate 6: Morphological characters of *Lampito mauritti*



Adult Earthworm



Male genital pores

Plate 7: Morphological characters of *Amyntas morrisi*



Adult Earthworm



Prostomium

Plate 8: Morphological characters of *Travoscolides chengannur*

Molecular characterization of earthworms

Identification of Earthworms using 'barcode fragment'/mtDNA COI 3' fragment based on DNA barcoding Technique

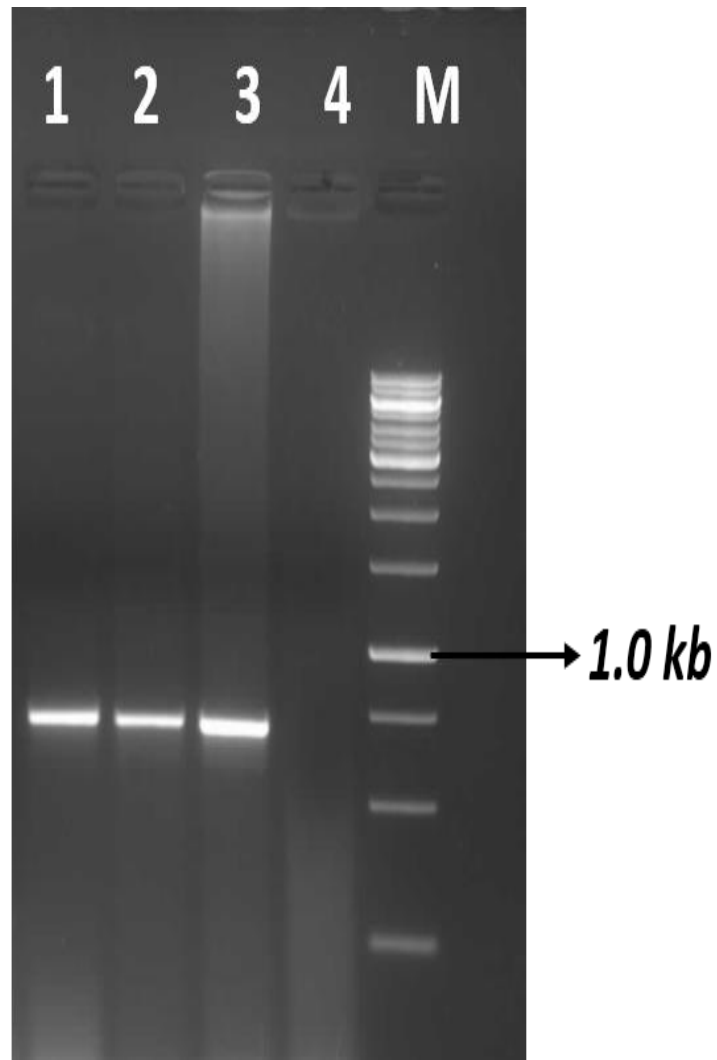


Figure 1: Earthworm gDNA sample agarose (1.5%) gel electrophoresis of PCR using LCO1490 and HCO2198 primers. Legends:

M-Molecular Weight Marker- 1.0 kb Thermo Scientific, USA,

1- E1 gDNA sample,

2- E2 gDNA sample,

3- E3 gDNA sample,

Based on sequence homology and phylogenetic analysis, the earthworm sample 1 (E1) and 2 (E2) has been found to be *Metaphire posthuma* while the 3 (E3) is found to be *Travoscolides chengannure*. (Figure1)

Based on sequence from where blast analysis is done from NCBI:

a) Mito1 LCO Sequence of *Metaphire posthuma*

TTAGAGCCGGAATAATTGGGAGCAGGAATAAGACTTCTTATTTCGAATTGAACTAA
GACAACCAGGATCCTTTCTAGGGAGAGACCAACTATATAACACTATTGTTACCGC
ACATGCGTTTTTAATAATTTTCTTTTAGTAATGCCCGTATTTATTGGTGGGTTTGG
TAATTGATTACTACCTCTTATACTTGGAAACCCAGACATAGCATTTCCACGACTAA
ATAACATAAGATTCTGATTGCTCCCGCCATCACTAATTTTACTCGTTAGGTCAGCC
GCTGTAGAAAAGGGAGCAGGTACTGGGTGAACAGTATACCCCCACTAGCAAGA
AATATTGCACACGCCGGACCGTCAGTAGATCTGGCAATTTTTTCACTTCACCTGG
CTGGTGCATCTTCAATTTTGTGAGCCATTAACCTTCATTACAACAGTAATTAATATA
CGATGATCTGGACTACGACTAGAACGAATCCCACTTTTTGTATGAGCAGTCGTAA
TACTGTAGTGCTACTATTACTATCATTACCAGCGTTAGCTGGTGCCATCACCATA
CTTCT

b) Mito1 LCO sequence of *Travoscolides chengannure*

TATAATTGGGAGCAGGTATAAAGACTTCTCATTCGAATCGAGCTAAGACAACCAG
GAGCATTTTTAGGAAGAGATCAACTATACAATAACAATTGTAACAGCACATGCATT
CCTAATAATTTTCTTCTTAGTTATAACCAGTATTTATCGGGGGCTTCGGAAATTGAT
TACTCCCTCTAATACTAGGTGCCCCTGACATAGCATTTTCTCGTCTCAATAACATA
AGATTTTGACTTCTCCCTCCATCCCTAATTCTTCTAGTATCCTCTGCAGCCGTAGA
AAAAGGAGCAGGAAGTGGATGAACAGTCTATCCGCCATTAGCAAGTAATATTGC
CCACGCTGGGCCATCAGTAGACCTAGCCATTTTTTCACTCCACTTAGCAGGAGCA
TCATCAATCTTAGGAGCAATTAACCTTTATTACTACAGTCATTAATATACGATGAG
CAGGACTACGACTCGAACGAATCCCACTATTTGTATGAGCAGTAGTAATTACAGT
AGTTCTATTACTACTATCGCTTCCAGTTCTAGCTGGTGCCATTACAATACTTCTCA
CAGATCGAAATCTAAATACCTCATTCTTTGATCCAGCTGGGGGTGGAGATCCCAT
TTTATATCAACACCTATTCTGATTTTTTGGTCACCCTGAAGTTTAAAT

Insect DNA barcode fragment gene sequence was used to carry out NCBI-Basic Local Alignment Search Tool database. Based on maximum identity score first ten sequences were selected and aligned using multiple alignment software program ClustalW multiple alignment. Distance matrix was generated and the phylogenetic tree (Fig 2) was constructed by using MEGAX

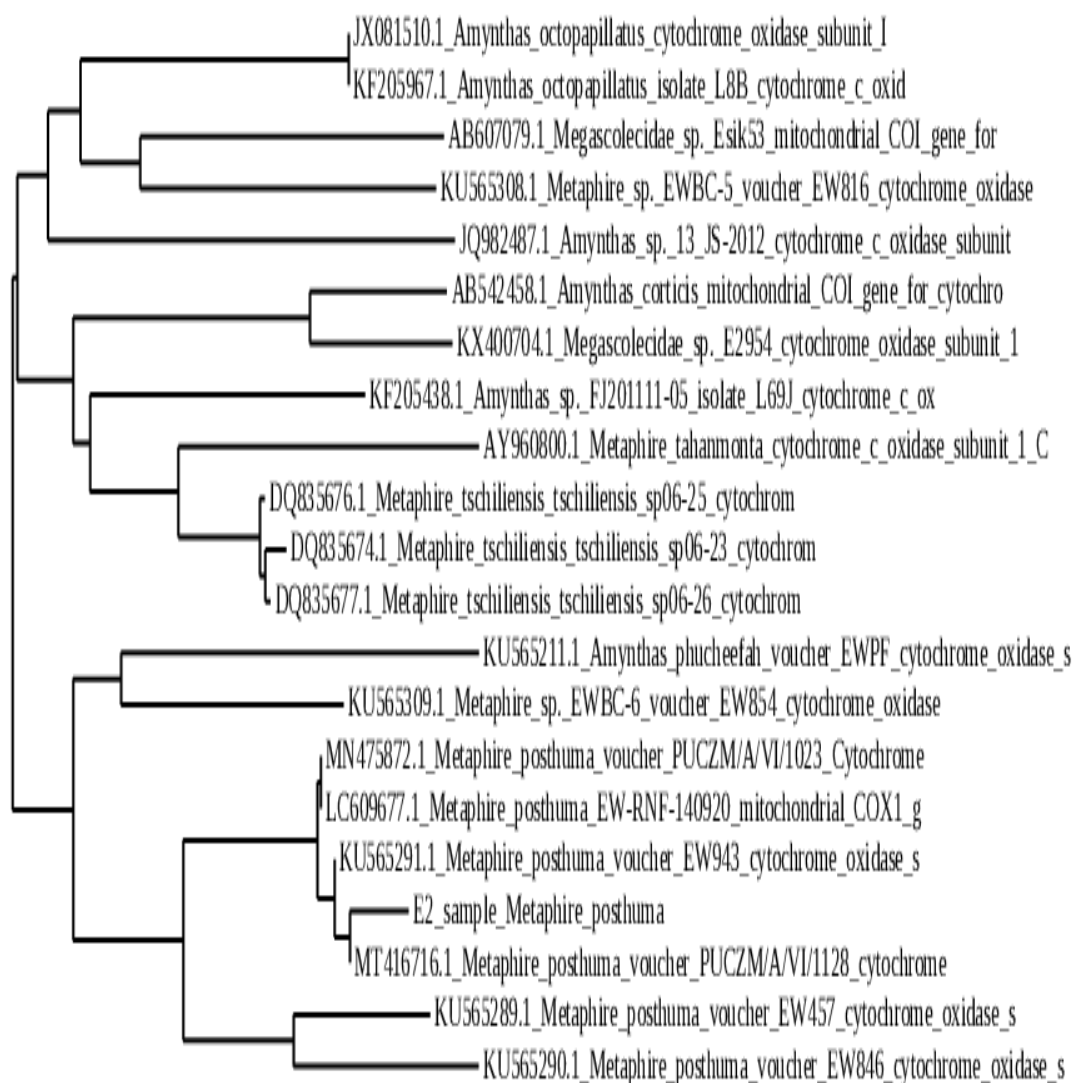


Fig.2 Evolutionary relationships of earthworm E2 sample with other taxa.

According to Panjgotra (2016) and Ahmed *et al.* (2020), Megascolecidae is the most diverse family in Punjab, Haryana, and Himachal Pradesh. The Megascolecidae family has a perichaetine arrangement of setae arranged in a ring around the segment, whereas the Octochaetidae family has a lumbricine arrangement with 8 setae closely paired around the segment. The earthworm species were identified using the steps in the keys that corresponded to the setae arrangement. The spermathecae pores differed according to species and family. The Megascolecidae family has 2 to 4 spermathecae pores, while the Octochaetidae family has only 1 to 2 spermathecae pores. Except for *L. mauritii*, whose clitellum was located at segments 14-17, the clitellum was annular in all reported earthworm species with positions at segments 14-16 in the Megascolecidae family. The clitellum position in Octochaetidae family species, on the other hand, was located at 13-17 segment. The male pore was located at segment 18 in the Megascolecidae family and varied from 17 to 18 in the Octochaetidae family. Several researchers have reported similar morphological features in earthworm specimens from the Megascolecidae and Octochaetidae families (Kumar and Singh 2013; Sharma and Garg, 2018).

Management practices of Organic and conventional Farming

In organic farming system, the pesticides used were spray neem-based pesticides such as Neem kavach (*Trichogramma card*) in basmati. Further, green manure, FYM, vermicompost, Jeevamrit and ghanjeevamrit were used as fertilizers. Additionally, the fertilizers used in conventional farming system were Urea, N, P-AP, wheat consortium, moong rhizobium and bradyrhizobium (Table 4) Other weed control chemicals or weedicides in basmati rice used were Butachlor, insect pest- coragen and fame. For wheat – Stomp 30 EC was used as weedicide and pesticide was chloropyrifos and further for diseases- Tebucanazole and native etc. was used (Table 5). The tillage practices under organic and conventional field mainly included cultivator, rotavator, ploughing and manual weeding in all cropping systems i.e. Basmati-wheat, basmati-chickpea-moong-wheat, soybean-wheat. The crop rotation was followed by green manuring. Irrigation pattern for basmati crop was standing water for 15 days after transplanting, then irrigation was applied at the time when ponded water infiltrated. Similarly, wheat followed three irrigations.

According to Lee (1985), the gradual decline in earthworm abundance with successive tillage following the initial increase was due to adverse modifications in soil environment. All these changes were caused due to loss of soil aggregation and reduction in soil organic matter content. This suggests that the decline in earthworm abundance that is

frequently reported with conventional tillage is closely related to the soil degradation associated with exploitative farming systems.

Table 4: MANAGEMENT PRACTICES OF ORGANIC FIELD

CROPPING SYSTEMS	TILLAGE PRACTICES	CROP ROTATION	IRRIGATION PATTERN	PESTICIDES SPRAYED	FERTILIZERS/MANURE USED	OTHER
Basmati-Wheat	-Cultivator -Rotallator -Planking -Manual weeding	-Green manure - Green manure sunhemp	-Standing water in rice for 15 days after transplanting. Then apply irrig. When ponded water infiltrate -Wheat: 3 std irrigation	-Spray neem-based pesticides Tricho-gramma card in B.Rice.	-Green manure FYM and vermicompost Jeevamrit and Ghanjeevamrit	Seed treatment with beejamrit
Basmati-Chickpea	-Cultivator -Rotallator -Planking -Manual weeding	Green manure	Standing water in rice for 15 days after Then apply irrig. When ponded water infiltrate. -Chickpea: 1 irrig. According to rainfall.	-Neem based pesticide and Tricho-gramma card in B.Rice, Neem kavach etc.	Green manure FYM and vermicompost Jeevamrit and Ghanjeevamrit	Seed treatment with beejamrit
Soybean-Wheat	-Cultivator -Rotallator -Planking -Manual weeding		3-4 according to rainfall one at pod filling. Wheat: 3 std. irrigation		Green manure FYM and vermicompost Jeevamrit and Ghanjeevamrit	Seed treatment with beejamrit
Moong-Wheat	-Cultivator -Rotallator -Planking- Manual weeding	-Kharif moong -Summer moong	-Moong:only if rain fails -Wheat: 3 std irrign.		Green manure FYM and vermicompost Jeevamrit and Ghanjeevamrit	Seed treatment with beejamrit

Table.5: MANAGEMENT PRACTICES OF CONVENTIONAL FIELD

CROPPING SYSTEMS	TILLAGE PRACTICES	CROP ROTATION	IRRIGATION PATTERN	PESTICIDES SPRAYED	FERTILIZERS/MANURE USED	OTHER
Basmati-Wheat	-Cultivator -Rotallator -Planking -Manual weeding	-Green manure - Green manure sunhemp	-Standing water in rice for 15 days after transplanting. Then apply irrig. When ponded water infiltrate -Wheat: 3 std irrigation	butachlor, Stomp 30 EC	Basmati- <i>Azospirillum streptomyces</i> (Azo-S) N-Urea N, P- AP Green manure Consortium Wheat	insect pest – <i>chloropyrifos</i> , Coragen and fame.
Basmati-Chickpea	-Cultivator -Rotallator -Planking -Manual weeding	Green manure	Standing water in rice for 15 days after Then apply irrig. When ponded water infiltrate. -Chickpea: 1 irrig. According to rainfall.	butachlor	Basmati- <i>Azospirillum streptomyces</i> (Azo-S) N-Urea N, P- AP Green manure Chickpea- Urea, DAP Chickpea Rhizobium	Coragen and fame
Soybean-Wheat	-Cultivator -Rotallator -Planking -Manual weeding		3-4 according to rainfall one at pod filling. Wheat: 3 std. irrigation	Stomp 30 EC	Soybean-Brady rhizobium, Wheat consortium	chloropyrifos
Moong-Wheat	-Cultivator -Rotallator -Planking -Manual weeding	-Kharif moong -Summer moong	-Moong:only if rain fails -Wheat: 3 std irrign.	Stomp 30 EC	Moong rhizobium, Wheat consortium	chloropyrifos,

Deep ploughing, fertilizer, and pesticide application all have a direct impact on the earthworm species, which in turn affects the diversity indices. Bartz *et al.* 2014 also discovered that sites with minimal ploughing have higher earthworm richness than sites with

conventional tillage. On the other hand, conventional field practices such as application of fertilizers, pesticides, and tillage operations have devastating impact on the earthworm distribution. Organic carbon, according to Chan and Barchia 2007, is a critical factor in earthworm distribution because it determines the type and nature of food for an earthworm. Jänsch *et al.* 2013 and Bartz *et al.* 2014 also found a significant relationship between soil OC and earthworm abundance.

Earthworm communities in agro-ecosystems are generally very sensitive to soil physico-chemical properties. This is because these physico-chemical properties directly or indirectly influence the availability of resources for the growth and survival of earthworm species. The differences in soil physicochemical properties at different sites aided in the formation of population patches for earthworm species. Knowing the physicochemical properties of soil at a specific site could help to possibly predict the earthworm species at that site. It is also necessary to quantify the spatial distribution of earthworms in various agroecosystems in order to comprehend the impact of abiotic soil processes and to link earthworm abundance to the spatial distribution of macro-pores in the soil. The data collected on the types of earthworm species and their abundance in various habitats may also provide useful information on the efficiency and strength of that ecosystem. The purpose of this study was to look into the diversity, abundance, and distribution of earthworms in organic and conventional farming system, as well as the physicochemical properties that influence earthworm diversity and abundance. Further, the soil structure and pore size also play an important role in the distribution of earthworms within the soil. Indeed, minor disturbance to soil structure can have a negative impact on the earthworm's community structure. The richness and diversity of earthworm species have always been observed to be greater in organic soils than in conventional soils, and this is likely to increase under favourable soil conditions. Earthworms are benefitted from nearly all organic based amendments. The incorporation of various forms of organic fertilizers into the soil can double or triple the earthworm populations in a single year. Organic carbon, according to Chan and Barchia 2007, is a critical factor in earthworm distribution because it determines the type and nature of food for earthworm species.

However, the ammonia and salt content released from liquid manures can be harmful to earthworms, but the populations tend to recover quickly and continue to grow. In most cases, the use of organic fertilizers has an overall positive effect on earthworm populations. This is most likely an indirect result of increased crop biomass production and, as a result, increased organic residues (Edwards and Bohlen, 1996). Further, in fields where moisture levels are otherwise low, the irrigated soil can support high levels of earthworm activity. As

irrigation increases crop production, the resulting crop residues in the soil after harvesting engenders a greater population of earthworms. Further, the irrigation waters containing earthworms along with their cocoons may serve as a source of inoculum for some species (Edwards 1997). Additionally, provision of adequate aeration and drainage to poorly drained soils may provide a more favourable environment for the earthworm activity.

DISTRIBUTION OF EARTHWORMS IN FIELDS OF ORGANIC AND CONVENTIONAL FARMING

A total of four earthworm species belonged to two families i.e. Megascolecidae and Octochaetidae were reported in this study. The earthworm species *Amyntas morrissi*, *Lampito mauritii*, and *Metaphire posthuma* belong to the family Megascolecidae while *Travoscolides chengannur* belongs to the Octochaetidae. The species of earthworm were reported in different crop fields. The relative abundance of *L.mauritii* and *A.morissi* is relatively higher in organic fields than in conventional farming based fields (Table 6) However, the species *Travoscolides chengannur* was found only from organic fields. Furthermore, Dhar and Chaudhuri (2020) concluded that number of earthworm species is considerably affected by changes in the environment, soil properties and other agricultural management practices. Cai *et al.* (2020) has also reported negative effects of chemical fertilizers and pesticides on earthworm population. Therefore, the excessive use of chemical fertilizers and pesticides in the agriculture fields that adopt conventional farming techniques may reduce the relative abundance of earthworm species and their diversity.

Table 6. Distribution of earthworm species and their annual abundance (%) of kharif and rabi seasons in organic and conventional field

a) Basmati-Wheat Cropping system

SPECIES	Basmati				Wheat			
	Organic		conventional		Organic		conventional	
	Mean	AA	Mean	AA	Mean	AA	Mean	AA
<i>M. posthuma</i>	61.75	0.531183	50.75	0.604167	8.2	0.602941	7	0.714286
<i>L.mauritii</i>	38.25	0.329032	27.25	0.324405	3.4	0.25	1.8	0.183673
<i>A. morissi</i>	14	0.12043	6	0.071429	2	0.147059	1	0.102041
<i>T. chengannur</i>	2.25	0.019355	0	0	0	0	0	0

Simpson's index =	0.40	0.47	0.43	0.54
Shannon weiner diversity index=	1.03	0.85	0.93	0.78
Species evenness=	0.74	0.78	0.84	0.71

b) Basmati-chickpea cropping system

SPECIES	Basmati				Chickpea			
	Organic		conventional		Organic		conventional	
	Mean	AA	Mean	AA	Mean	AA	Mean	AA
<i>M. posthuma</i>	62.75	0.535181	56	0.632768	9.2	0.582278	5.4	0.675
<i>L.mauritii</i>	39.5	0.336887	25.75	0.29096	3.8	0.240506	2.4	0.3
<i>A. morissi</i>	15	0.127932	6.75	0.076271	2.8	0.177215	0.2	0.025
<i>T. chengannur</i>	0	0	0	0	0	0	0	0

Simpson's index = 0.41 0.48 0.42 0.53

Shannon weiner diversity index= 0.96 0.84 0.96 0.71

Species evenness= 0.87 0.76 0.87 0.65

c) Soybean-Wheat cropping system

SPECIES	Soybean				Wheat			
	Organic		conventional		Organic		conventional	
	Mean	AA	Mean	AA	Mean	AA	Mean	AA
<i>M. posthuma</i>	36.5	0.640351	23.75	0.629139	9.2	0.630137	5.8	0.58
<i>L.mauritii</i>	12.75	0.223684	6	0.15894	8.5	0.232877	1	0.1
<i>A. morissi</i>	7.75	0.135965	8	0.211921	2	0.136986	3.2	0.32
<i>T. chengannur</i>	0	0	0	0	0	0	0	0

Simpson's index = 0.47 0.46 0.46 0.43

Shannon weiner diversity index = 0.89 0.91 0.90 0.91

Species evenness= 0.81 0.83 0.82 0.82

d) Moong-wheat cropping system

SPECIES	Moong				Wheat			
	Organic		conventional		Organic		conventional	
	Mean	AA	Mean	AA	Mean	AA	Mean	AA
<i>M. posthuma</i>	43.5	0.5625	21.33	0.554113	8.2	0.577465	4.2	0.617647
<i>L.mauritii</i>	24.6	0.318966	11.5	0.298701	3.4	0.239437	1.4	0.205882
<i>A. morissi</i>	9.16	0.118534	5.67	0.147186	2.6	0.183099	1.2	0.176471
<i>T. chengannur</i>	0	0	0	0	0	0	0	0

Simpson's index = 0.43 0.41 0.41 0.43

Shannon weiner diversity index= 0.94 0.97 0.97 0.92

Species evenness= 0.85 0.88 0.88 0.84

Table7: Species present in different cropping systems in organic fields.

Cropping system (organic)	Species			
	<i>Metaphire posthuma</i>	<i>Lampito mauritii</i>	<i>Amynthas morissi</i>	<i>Travoscolides chengannures</i>
B-W	++	+	+	+
B-C	++	+	+	-
S-W	++	+	+	-
M-W	++	+	+	-

Table8: Species present in different cropping systems in conventional fields.

Cropping system (Conventional)	Species			
	<i>Metaphire posthuma</i>	<i>Lampito mauritii</i>	<i>Amyntas morissi</i>	<i>Travoscolides chengannures</i>
B-W	+	+	+	-
B-C	+	+	+	-
S-W	+	+	+	-
M-W	+	+	+	-

Table9: Relative abundance of earthworms in different cropping systems under organic and conventional farming

	Cropping System	<i>Metaphire posthuma</i>	<i>Lampito mauritii</i>	<i>Amyntas morissi</i>	<i>Travoscolides chengannure</i>
Organic	B-W	54.03	31.89	12.38	1.68
	B-C	54.19	32.29	13.5	0
	S-W	63.78	22.59	13.61	0
	M-W	57.19	31.25	11.55	0
Conventional	B-W	61.81	30.64	7.53	0
	B-C	63.7	39.34	7.1	0
	S-W	61.69	14.43	23.88	0
	M-W	54.77	27.94	17.27	0

The relative abundance of earthworms in different cropping systems under organic and conventional farming is represented in the graph. The relative abundance is denoted in percentage. (Table 9) Among all the species, the *Metaphire posthuma* shows maximum

relative abundance in organic soybean-wheat. *Lampito mauritii*, is higher in both organic and conventional fields in basmati – chickpea as compared to other cropping systems. However, the overall relative abundance is higher in organic cropping farming system than conventional fields. Further, the earthworm species, *Amyntas morissi* shows highest inhabitation in organic farming system. Least number of the same species has been found in basmati-chickpea system when put under conventional farming (=7.1). Interestingly, the species *Travoscolides chengannure* has only been isolated from the organic fields of basmati and wheat. It has a relative abundance of 1.68 which is overall the lowest among all other species.

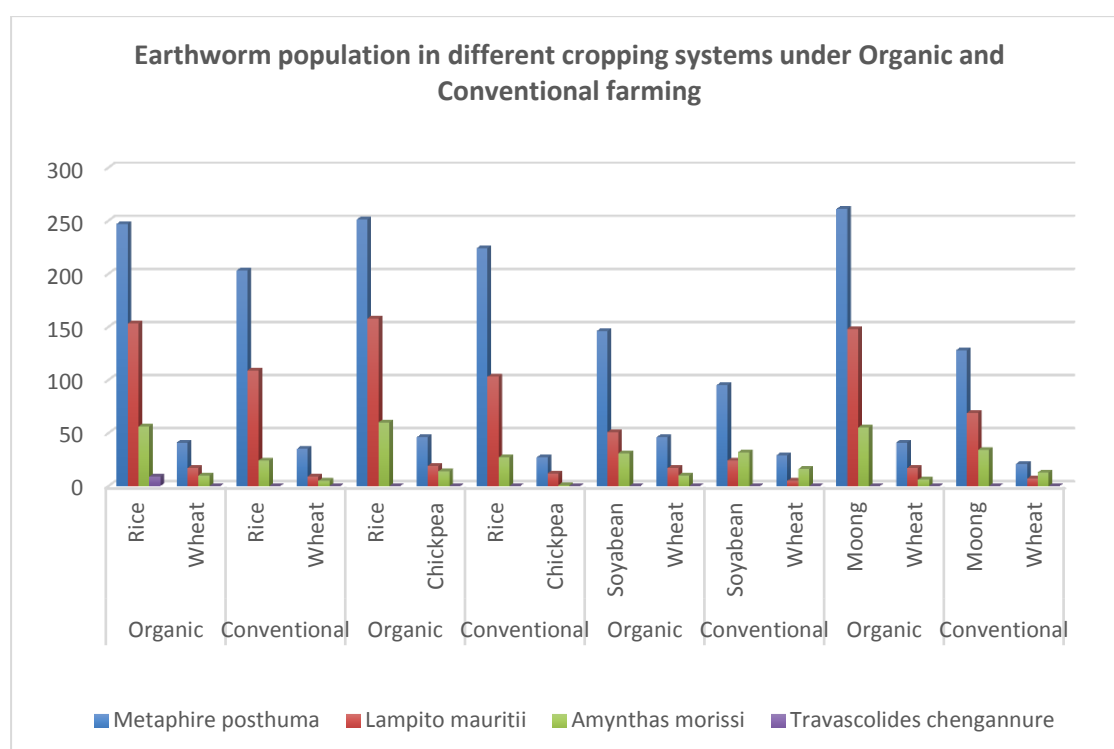


Fig.3: Bar graph showing earthworm population in different cropping systems under organic and conventional farming

The bar graph demonstrates the earthworm population in different cropping systems under organic and conventional farming. It displays that the population of all the earthworm species is relatively higher in organic system as compared to conventional farming (fig 3). Crittenden *et al.* (2014) also manifested that organic farming system have higher biomass concentration as well as Shannon diversity in contrast to conventional farming.

Table 10: Diversity indices of earthworm species in different cropping systems: Simpsons index(D), Shannon Wiener index(H) and Evenness (E)

ORGANIC				
Cropping System	No. of Species	D	H	E
B-W	4	0.409	1.025	0.739
B-C	3	0.416	0.967	0.881
S-W	3	0.477	0.984	0.814
M-W	3	0.438	0.932	0.849
CONVENTIONAL				
Cropping System	No. of Species	D	H	E
B-W	3	0.482	0.855	0.778
B-C	3	0.496	0.835	0.76
S-W	3	0.458	0.919	0.837
M-W	3	0.408	0.989	0.901

The table 10 displays the diversity indices of earthworm species in different cropping systems such as Simpsons index (D), Shannon Wiener index(H) and Evenness (E). The highest Shannon-weiner diversity is being demonstrated in organic basmati-wheat cropping system (1.025). Maximum evenness (0.881), is present in basmati-chickpea. However, the Simpson index among organic fields is fluctuating.

Physico-chemical properties of soil

The statistical analysis of pH, Electrical Conductivity (EC), nitrogen (N), phosphorous(P), potassium(K), organic carbon (OC) in soils of different cropping patterns were given in (Table 11). The pH was found in the range of (7.12-7.80) with an average value of (7.5) in organic soils. In conventional soils, pH ranged from (7.11-7.56). On the other hand, EC (mS) was ranged from (0.22-0.36) and (0.19 to 0.22) in organic and conventional farming systems respectively. The mean content of EC among organic and conventional fields was (0.19, 0.36) respectively. The OC ranged from (0.54 to 0.63) in organic fields, (0.41 to 0.46) in conventional soils. In contrast, the content of nitrogen was higher in organic soils

(362.40kg/ha) as compared to conventional soils (339.40kg/ha). Hackenberger and Hackenberger, 2014 also revealed that the physico-chemical attributes of soil such as organic matter as well as type of climatic influences the earthworm diversity. Further, Singh *et al.*, 2016 performed the principal component analysis and demonstrated that the physico-chemical parameters of soil such as pH, moisture content as well as organic carbon are firmly associated with the distribution of earthworm communities.

Highest pH in B-W was recorded in organic farming (7.7 ± 0.05) followed by conventional farming (7.1 ± 0.09). No significant variation was observed between the organic and conventional farming of B-W. Highest pH in B-C was observed in organic farming (7.6 ± 0.03) followed by conventional farming (7.2 ± 0.05). No significant variation was observed between the organic and conventional farming of B-C. Highest pH in S-W was recorded in organic farming (7.8 ± 0.05) followed by conventional farming (7.1 ± 0.05). Significant variation was observed between the organic and conventional farming of S-W. Highest pH in M-W was recorded in organic farming (7.1 ± 0.05) followed by conventional farming (7.3 ± 0.03). Significant variation was observed between the organic and conventional farming of M-W.

Highest EC in in B-W was recorded in organic farming (0.26 ± 0.005) followed by conventional farming (0.22 ± 0.005). A significant variation was observed between the organic and conventional farming of B-W. Highest EC in B-C was observed in organic farming (0.22 ± 0.005) followed by conventional farming (0.21 ± 0.005). No significant variation was observed between the organic and conventional farming of B-C. Highest EC in S-W was recorded in organic farming (0.22 ± 0.005) followed by conventional farming (0.19 ± 0.05). A Significant variation was observed between the organic and conventional farming of S-W. Highest EC in M-W was recorded in organic farming (0.36 ± 0.005) followed by conventional farming (0.20 ± 0.005). Significant variation was observed between the organic and conventional farming of M-W.

Highest OC in in B-W was recorded in organic farming (0.63 ± 0.005) followed by conventional farming (0.46 ± 0.005). A Significant variation was observed between the organic and conventional farming of B-W. Highest OC in B-C was observed in organic farming (0.54 ± 0.005) followed by conventional farming (0.44 ± 0.005). No significant variation was observed between the organic and conventional farming of B-C. Highest OC in S-W was recorded in organic farming (0.59 ± 0.005) followed by conventional farming (0.41 ± 0.005). A Significant variation was observed between the organic and conventional farming of S-W. Highest OC in M-W was recorded in organic farming (0.62 ± 0.005) followed by conventional

farming (0.0 ± 0.005). A Significant variation was observed between the organic and conventional farming of M-W. Further, Chan and Barchia, 2007 studied that the OC in soil is the most influential and critical parameter that affects not only the abundance but also the distribution of earthworms communities at any specific area.

Highest N in B-W was recorded in organic farming (356.7 ± 2.1) followed by conventional farming (339.4 ± 3.25). A Significant variation was observed between the organic and conventional farming of B-W. Highest N in B-C was observed in organic farming (362.4 ± 1.35) followed by conventional farming (317.2 ± 1.47). No significant variation was observed between the organic and conventional farming of B-C. Highest N in S-W was recorded in organic farming (360.6 ± 0.7) followed by conventional farming (314.8 ± 1.49). A significant variation was observed between the organic and conventional farming of S-W. Highest N in M-W was recorded in organic farming (324.1 ± 1.9) followed by conventional farming (311.5 ± 0.7). A significant variation was observed between the organic and conventional farming of M-W.

Highest P in B-W was recorded in organic farming (50.3 ± 0.7) followed by conventional farming (45.5 ± 1.65). A significant variation was observed between the organic and conventional farming of B-W. Highest P in B-C was observed in organic farming (44.2 ± 0.7) followed by conventional farming (41.4 ± 0.56). No significant variation was observed between the organic and conventional farming of B-C. Highest P in S-W was recorded in organic farming (48.2 ± 1.46) followed by conventional farming (42.6 ± 1.6). A significant variation was observed between the organic and conventional farming of S-W. Highest P in M-W was recorded in organic farming (50 ± 0.75) followed by conventional farming (44.2 ± 0.81). A significant variation was observed between the organic and conventional farming of M-W.

Highest K in B-W was recorded in organic farming (151.8 ± 0.7) followed by conventional farming (145.4 ± 2.33). A significant variation was observed between the organic and conventional farming of B-W. Highest K in B-C was observed in organic farming (152.3 ± 1.19) followed by conventional farming (145.4 ± 2.33). No significant variation was observed between the organic and conventional farming of B-C. Highest K in S-W was recorded in organic farming (143 ± 1.95) followed by conventional farming (137.3 ± 1.51). A significant variation was observed between the organic and conventional farming of S-W. Highest K in M-W was recorded in organic farming (151.8 ± 0.7) followed by conventional farming (144.9 ± 0.79). Significant variation was observed between the organic and conventional farming of M-W

Table11: Correlational analysis of earthworm abundance with the soil parameters in different farming systems

	Cropping System		pH	EC (dS/m)	OC	N (kg/ha)	P (kg/ha)	K (kg/ha)
Organic	B-W	Mean	7.7	0.26	0.63	356.7	50.3	157.80
		S.E.	0.15	0.03	0.02	9.02	1.4	3.06
	B-C	Mean	7.6	0.22	0.54	362.4	44.2	152.3
		S.E.	0.18	0.04	0.02	10.8	1.4	2.61
	S-W	Mean	7.8	0.22	0.59	360.6	48.2	143
		S.E.	0.2	0.04	0.08	9.16	1.1	2.27
	M-W	Mean	7.1	0.36	0.62	324.1	50	151.8
		S.E.	0.02	0.04	0.04	5.68	2.1	3.76
		r	0.318	-0.388	-0.171	0.462	-0.21	0.562
		p	0.312	0.211	0.594	0.13	0.51	0.056
Conventional	B-W	Mean	7.1	0.22	0.46	339.4	45.5	145.4
		S.E.	0.09	0	0	6.33	0.89	2.34
	B-C	Mean	7.2	0.21	0.44	320.1	41.4	136.8
		S.E.	0.1	0.005	0.005	1.43	0.7	2.26
	S-W	Mean	7.1	0.19	0.41	316.9	42.6	137.3
		S.E.	0.14	0	0	1.16	0.56	2.68
	M-W	Mean	7.5	0.2	0.42	311.5	44.2	144.9
		S.E.	0.1	0.07	0.14	110.13	15.6	2.22
		r	-0.466	0.733	0.808	0.813	0.1	0.147
		p	0.145	0.006**	0.001**	0.001**	0.75	0.648

**p<0.01(significant at 0.01 level)

The correlational analysis of earthworm abundance with the soil parameters in different farming systems i.e. Organic and Conventional farming systems has revealed that, the abundance of earthworms in Organic farming system shows slight positive correlation ($r = 0.31$, $p = 0.32$) with pH of the soil; slight negative correlation ($r = -0.38$, $p = 0.21$) with EC; very slight negative correlation ($r = -0.17$, $p = 0.59$); moderate positive correlation ($r = 0.46$, $p = 0.13$) with N; slight negative correlation ($r = -0.21$, $p = 0.51$) with P; moderate positive correlation ($r = 0.56$, $p = 0.05$) with K. For the Conventional farming system the abundance of earthworms shows a moderate negative correlation ($r = -0.46$, $p = 0.14$) with pH; high positive correlation which is statistically significant ($r = 0.73$, $p \leq 0.01$) with EC; high positive correlation which is statistically significant ($r = 0.808$, $p \leq 0.001$) with OC; high positive correlation which is statistically significant ($r = 0.81$, $p \leq 0.001$) with N; very slight positive

correlation ($r = 0.1$, $p = 0.75$); very slight positive correlation ($r = 0.14$, $p = 0.64$) with K has been found.

Table 12. Analysis of soil parameters of organic and conventional farming systems.

	Mean		SD		t	p
	Organic	Conventional	Organic	Conventional		
pH	7.51±0.08	7.19±0.04	0.29	0.13	3.48	0.002**
EC	0.265±0.017	0.205±0.004	0.604	0.014	3.347	0.002**
OC	0.595±0.01	0.43±0.006	0.037	0.021	12.968	0.0001**
N	350.9±4.07	320.7±2.9	16.27	11.6	6.048	0.0001**
P	48.17±0.84	43.42±0.71	2.91	2.46	4.31	0.0003**
K	151.22±1.73	141.1±1.47	6.002	5.11	4.44	0.0002**

** $p \leq 0.01$ (significant at 0.01 level) ** $p \leq 0.001$ (significant at 0.001 level)

Table 13. Soil Temperature and Moisture of different crop systems in both organic and conventional cropping system.

Sowing		
CROPPING SYSTEMS	Soil temperature (°C)	Soil moisture (%)
organic basmati	23.9	37
organic soybean	23.5	18.9
organic moong	23.7	19.2
organic wheat	21.8	19.3
organic chickpea	23.1	16.4
conventional wheat	21.2	18
conventional chickpea	22.1	16
conventional basmati	23.5	37.5
conventional soybean	23.1	18.2
conventional moong	23.5	17.5

The t-test analysis between different soil parameters of the two selected farming systems i.e. Organic and Conventional farming systems has revealed that, there is a significant difference in the pH of soil ($t = 3.48$, $p \leq 0.001$), EC of the soils ($t = 3.34$, $p \leq 0.001$), OC of the soil ($t = 12.96$, $p \leq 0.001$), N in the soil ($t = 6.04$, $p \leq 0.001$), P in the soil ($t = 4.31$, $p \leq 0.01$) and K levels of the soil ($t = 4.44$, $p \leq 0.001$) between the two farming systems. Therefore, there is a significant difference in the soil parameters of organic farming systems compared to the conventional farming systems (Table 12).

In our study, the highest soil temperature among organic crops was recorded in organic basmati. Whereas, the conventional moong as well as conventional basmati both demonstrated equal soil temperature. The soil moisture was highest in organic basmati and conventional basmati (Table13). Crusmey *et al.*, 2014 performed a thorough correlation between the overall density as well as biomass of earthworm population. It was also positively correlated with moisture content present in soil.

CHAPTER V

SUMMARY

The earthworms are one of the most important soil invertebrates that significantly affect the soil structure and other soil properties since its formation (Bhadauria and Saxena, 2010). Many scientists consider earthworms as predominant soil engineers that actively participate in the regulation of various soil processes. Various experiments have manifested that the diversity, abundance and species composition of earthworm species heavily relies upon the soil properties such as soil texture, pH, moisture content and organic matter (Curry, 2004). The present study provides information about the earthworm diversity and abundance in different cropping systems of organic and conventional farming systems at PAU Ludhiana. The four different cropping systems studied were basmati-wheat, basmati-chickpea, soybean-wheat, moong-wheat. Sampling of earthworms was done on monthly basis throughout the year. From the assigned fields, the population of earthworms was surveyed from July 2020 till March, 2021. In a particular field, random selection of five spots was done. These spots were dug to collect the earthworms through handpicking method. Their identification was done in accordance with the standardized keys (Gates 1972 and Julka 2008). The highest number of earthworms was found in the months of July and August. In contrast, least number of earthworms were observed during December and January. The reason behind this fact might be low temperature of soil.

The diversity and abundance of earthworms was much higher in organic as compared to the conventional farming system. The present study reveals that the management practices adopted for conventional farming included higher doses of chemicals such as consortium and pesticides had a negative effect on the earthworm population. Therefore, the presence of high content of chemical fertilizers and pesticides in conventional soil might be the reason for less abundance and diversity of earthworm species in conventional farming systems. Further, *Metaphire posthuma* was observed at maximum sampling sites due to its Endogeic ecological nature. The earthworm species *Amyntas morrisi*, *Lampito mauritii*, and *Metaphire posthuma* belong to the family Megascolidae while *Travoscolides chengannur* belongs to the Octochaetidae. *Travoscolides chengannures* has been recorded for the first time in Punjab from the fields of organic farming system, PAU Ludhiana. Its molecular characterisation and genome sequencing was also done. The physico- chemical parameters of soil such as pH, EC, OC, temperature and moisture were also higher in the organic as compared to conventional farming system. The pH was found in the range of (7.12-7.80) with an average value of (7.5) in organic soils. In conventional soils, pH ranged from (7.11-7.56). On the other hand, EC

(mS) was ranged from (0.22-0.36) and (0.19 to 0.22) in organic and conventional farming systems respectively. The mean content of EC among organic and conventional fields was (0.19, 0.36) respectively. The OC ranged from (0.54 to 0.63) in organic fields, (0.41 to 0.46) in conventional soils. In contrast, the content of nitrogen was higher in organic soils (362.40 kg/ha) as compared to conventional soils (339.40kg/ha).

Earthworms play an important role in the agro-ecosystem. They play an important role to increase the soil aeration. The more the soil becomes porous, the more is the infiltration rate of water. Earthworms also synthesize humus by mineralizing the soil. They ingest the soil along with litter and their casts are full of decomposed matter. Therefore, Farmers must be educated about the potential impact of agrochemicals and their effect on earthworms. They should be made aware of the roles of earthworms in soil. In this way, the farmers can be encouraged to switch from conventional to organic farming practices. These practices not only increase earthworm diversity and abundance, but enrich the soil with many major and micro-nutrients. The agriculture practices which are earthworm-friendly should be adopted for long-term soil productivity. The correlation analysis of earthworm abundance with the physic chemical parameter of soil in different farming systems has revealed that the abundance of earthworms in organic farming system shows positive but non-significant correlation with pH, nitrogen and potassium levels while negative correlation was observed in electrical conductivity, organic carbon and phosphorous. In conventional farming system, significant positive correlation ($p \leq 0.01$) was found for organic carbon, electrical conductivity and nitrogen. Therefore, there is a considerable difference between the soil parameters of organic farming systems as compared to the conventional.

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