Vegetable Science (2023) 50(1): 88-94
doi: 10.61180/vegsci.2023.v50.i1.13
ISSN- 0970-6585 (Print), ISSN- 2455-7552 (Online)
www.isvsvegsci.in

RESEARCH PAPER OPEN ACCESS

Quantitative assessment and seasonal incidence of soil invertebrates in okra (*Abelmoschus esculentus* L.) crop

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Abstract
The present study on population dynamics and diversity of soil invertebrates was carried out at Vegetable Research Farm, Punjab Agricultural University, Ludhiana during 2019. The study revealed that okra crop harboured 14 different soil invertebrate species belonging to nine orders and 13 families dominated by order coleoptera (five species) dominated by ground beetles. Interestingly, higher relative abundance (%) was recorded in order hymenoptera (80.72%) followed by polyzoniida (10.41%) among which higher dominance (%) was recorded in little black ants (80.78%). Species richness, species evenness, Shannon-Wiener index (H') and Simpson's index were calculated to be 14.0, 0.094, 0.098 and 0.051, respectively, indicating high degree of diversity among soil invertebrates in okra crop. Interestingly, an increase of 88.84 and 69.64% in population was recorded in root-knot and free-living/predatory nematodes at harvest time compared to the okra crop sowing time. A positive correlation (r=+0.06 to + 0.83) was recorded between soil temperature and soil moisture with soil invertebrate population. Okra crop fields survive soil fauna, which is a key factor for increasing soil fertility and productivity and its physical characteristics, which should be increased using soil amendments.

Keywords: Abundance, diversity, earthworm, soil fertility, soil invertebrates, okra.

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Citation: Kaur, R. and Singh, R. (2023). Quantitative assessment and seasonal incidence of soil invertebrates in okra (Abelmoschus esculentus L.) crop. Vegetable Science 50(1): 88-94.

Source of support: Nil

Conflict of interest: None.

Received: February 2023 **Accepted:** May 2023

Introduction

Soil has a diverse range of arthropods and annelids and is largely made up of invertebrate excrement and these soil invertebrates perform a variety of vital functions in maintaining the fertility, aeration and texture of soil. Microbe-grazing by soil invertebrates mineralizes nutrients, completing the nutrient recycling process. Extensive research may aid in identifying ecological factors behind the dynamics of insect populations in an agroecological system, rendering less adverse impact (Domene 2016). Soil fauna can be classified into three groups-microfauna, mesofauna and macrofauna (Ghosh *et al.* 2020), among them macro-fauna attains main focus *that comprises of invertebrates which can disrupt soil particles by feeding and burrowing (Stork and Eggleton 1992).* Soil invertebrates participate in a number of processes that enable soil to interact with plants, atmosphere and water. Therefore, increasing the diversity of invertebrate species is beneficial to maintain nutrient cycles, regulating organic matter dynamics, fix carbon and convert soil structural components (Urbano *et al.* 2019). The terrestrial environment has greatest diversity of arthropods, mollusks, annelids and crustacean species that inhabit the surface litter (Lavelle *et al.* 2006). Springtails are soil invertebrates that play a key role in the breakdown of organic matter. Smaller springtail species aid in humification by combining mineral soil particles with organic matter, whereas larger springtail species aid in mineralization by feeding on fungi (Van *et al.* 1988). Termites have a negative impact on soil fertility and global carbon cycling because of the changes they make to soil texture, the type and distribution of organic matter, the disturbance they bring to the soil profile, and the changes to the soil's chemistry (Jones 1990).

Collembolans are the most common soil invertebrates that play a significant part in the food webs of decomposers. *In some hydromorphic soils, earthworms can deposit as much as 200 tonnes per hectare of castings on the soil's surface to increase soil fertility (*Jin *et al.* 2022*).* Earthworms, sometimes known as the bowels of the earth, play an important role in soil health, primarily by digesting organic matter, fragmenting it, and combining it intimately with mineral particles to produce aggregates. Earthworms dramatically promote microbial activity while feeding, speeding up organic material decomposition (Potvin and Lilleskov 2017). However, there is relatively little information accessible about the eco-toxicological effects of pesticides on soil fauna. The usage of commercial inputs and agrochemicals in agriculture increased a thousandfold with the advent of the green revolution and high-yielding cultivars. However, indiscriminate application of agrochemicals reduces soil fertility because soil is a repository for all types of chemical inputs, including insecticides used to combat crop insect pests (Ramanujam and Jha 2011). In agriculture, insecticides not only enhance crop productivity but they also attributed with enhancing food production and helping to prevent against crop loss caused by insect pests and diseases. Despite their importance, pesticides have a negative impact, such as harmful residues in food, water, air, soil, resurgence, resistance and non-target species (Roarty *et al.* 2017) due to their lethal or sub-lethal activity. Insecticide hazards in soil are mostly determined by their persistence. The longer they persist, the higher the odds of harming the fauna present and contaminating the environment, which eventually leads to soil health distortion. Due to the application of insecticides, a large amount of insecticide and its degraded products are mixed or accumulated in the top 10 to 15 cm layer of soil, which is also the region of greatest activity for microorganisms and soil fauna, setting the stage for interactions between insecticides and fauna in soil ecosystem (Kibblewhite *et al.* 2007) thus, generate both qualitative and quantitative changes in fauna, which further impact functioning of soil (Cardoso *et al.* 2013). Biodiversity inventories must be developed with good sampling and estimating methodologies, particularly for hyperdiverse groups of terrestrial animals such as the arthropod community (Zhang *et al.* 2014). The sustainability of agricultural practices depends upon soil health and the soil organisms respond sensitively to land management practices and climate. Many agronomical and breeding studies have been conducted to enhance the production and quality of okra crop (Bahadur *et al.* 2020, Vinay *et al.* 2021a, 2021b, Singh *et al.* 2021, Maurya *et al.* 2021). There is a paucity of information about soil invertebrates present in okra crop under Punjab conditions. The Present study was conducted to estimate abundance and diversity of invertebrates within soil in okra crop fields, which may provide information about their inventory and help us illustrate land management decisions for crop health.

Materials and Methods

Field surveys were carried out at Vegetable Research Farm, Punjab Agricultural University, Ludhiana from July to December 2019 on okra crop (variety Punjab Suhawani, recommended by Punjab Agricultural University, Ludhiana for Punjab region). The selected experimental fields were well pulverized by ploughing and weeds were removed manually. The soil type of selected experimental fields was loamy-sand. Okra crop was raised using agronomic practices as recommended by Punjab Agricultural University, Ludhiana. Surveys were done regularly at fortnight intervals and pooled at month level (from germination till harvest) to study the abundance and diversity of soil invertebrates. To record observations on count of different soil invertebrates present in/on soil, the samples were collected from four corner sides as well as from the centre of selected field plot with required sample size (30×30×15 cm) after digging with spade using visual search, handpicking and sieving methods. Each plot of size 10×10 m was selected randomly and taken as one replication and there were total six replications. Dug soil was collected in a transparent plastic bag which was further sifted through a wire sieve to count macroinvertebrates (Paquin and Coderre 1996). Collected soil invertebrates were killed using a killing bottle, having a cotton swab dipped in ethyl acetate and then preserved in 70% alcohol in glass vials and insect collection box as per desired methods. Invertebrates were identified by experts from the Zoological Survey of India, Kolkata (ZSI).

For nematode extraction 250 g soil samples were taken at sowing and harvest times and further processed by Cobb's sieving and decanting technique (Cobb 1918). The meteorological data regarding soil temperature (^{0}C) and soil moisture (%) was recorded using thermo-hygrometer (R-tek ™) at soil depth of 15 cm and correlated with soil invertebrate population. Soil invertebrate population was analysed using one way of variance (ANOVA) using SPSS software. To calculate biodiversity indices following formulae were used:

- Relative abundance

It was used to measure percentage of individuals of a particular species:

RA = $\frac{n_i}{N}$ ×100 (where, RA= relative abundance, n_i= total number of individuals in a particular sample, N = total population of all species)

- Simpson index

$$D = \frac{\sum_{i=1}^{S} n_i (n_i - 1)}{N (N-1)}$$

(where, D = Simpson index, ni = total number of individuals of a particular species, N = total population of all species)

- Shannon-Wiener index

$$H' = - \sum_{i=1}^{S} [\{ \frac{n_i}{N} \} \times \log \{ \frac{n_i}{N} \}]$$

(where, H' = Shannon-Wiener index, ni = total number of individuals in a particular sample, N = total population of all species)

- Species evenness

$$J = \frac{H'}{\log S}$$

(where, J = Species evenness, H'= Shannon-Wiener index)

Results and Discussion

Diversity of soil invertebrates

During survey, 14 species of soil invertebrates belonging to phylum *arthropoda, annelida, mollusca and nemathelminthes, six classes namely,* insecta, diplopoda, clitellata, gastropoda, secernentea and enoplea and nine orders namely, dermaptera, hymenoptera, coleoptera, stylommatophora, tylenchida, rhabditida, mononchida, opisthopora and polyzoniida belonging to 13 families were recorded in okra crop (Table 1) among which order coleoptera represented the highest number of species (five) followed by order dermaptera (two). Various coleopterans recorded were, dung beetle, whiplash rove beetle, red tail rove beetle, ground beetle and black spruce beetle, among which ground beetle, *Bembidion tetracolum* (60.03%) was predominant one (Table 2) as compared to other coleopterans (12.42–7.64%) as identified by experts from Zoological Survey of India, Kolkata (ZSI).

Interestingly, two species of rove beetles, whiplash rove beetle (*Paederus riparius*) and red-tail rove beetle (*Hesperus apicialis*) having total population ranged from 0.56–0.48 individuals/m^2 were recorded during survey in crop period of okra crop. Out of which, whiplash rove beetle was most abundant one with pre-dominance of 53.84% as compared to red-tail rove beetle species (Table 2). Similarly, two species of earwigs, common earwigs (*Forficula auricularia*) and striped earwigs (*Labidura riparia*), were recorded with a total population ranging from 1.39–3.51 individuals/m^2 during crop period. Common earwig species was most abundant, with pre-dominance of 71.6% compared to striped earwig species (Table 3). Interestingly, higher relative abundance (%) was recorded in order Hymenoptera (80.72%) followed by Polyzoniida (10.41%) as compared to other species (Figure 1). Among all soil invertebrates recorded, higher dominance (%) was recorded (Table 3) in little black ants, *Formica rufa* (80.78%) whereas all other invertebrates ranged from 0.17-3.10% with lower dominance recorded in earthworm

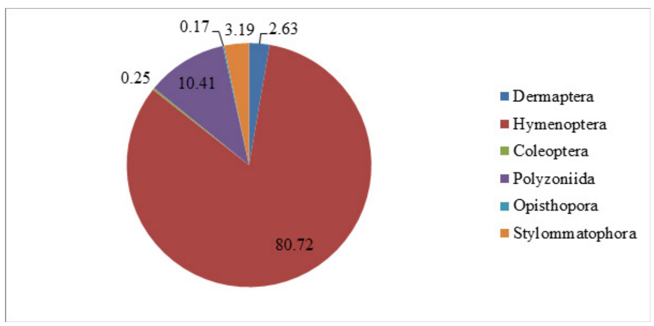

Figure 1: Relative abundance of different orders of soil invertebrates recorded in okra crop

Table 1: Inventory of different soil invertebrates recorded in okra crop

S. No.	*Common name*	*Scientific name*	*Phylum*	*Class*	*Order*	*Family*
1	Common earwig	*Forficula auricularia*	Arthropoda	Insecta	Dermaptera	Forficulidae
2	Striped earwig	*Labidura riparia*	Arthropoda	Insecta	Dermaptera	Labiduridae
3	Little black ant	*Formica rufa*	Arthropoda	Insecta	Hymenoptera	Formicidae
4	Dung beetle	*Onitis fabricius*	Arthropoda	Insecta	Coleoptera	Scarabidae
5	Ground beetle	*Bembidion tetracolum*	Arthropoda	Insecta	Coleoptera	Carabidae
6	Black spruce beetle	*Tetropium castaneum*	Arthropoda	Insecta	Coleoptera	Cerambycidae
7	Whiplash rove beetle	*Paederus riparius*	Arthropoda	Insecta	Coleoptera	Staphylinidae
8	Red tail rove beetle	*Hesperus apicialis*	Arthropoda	Insecta	Coleoptera	Staphylinidae
9	Millipede	*Octoglene sierra*	Arthropoda	Diplopoda	Polyzoniida	Hirudisomatidae
10	Earthworm	*Eisenia fetida*	Annelida	Clitellata	Opisthopora	Lumbricidae
11	Snail	*Succinea putris*	Mollusca	Gastropoda	Stylommatophora	Succineidae
12	Root knot nematode	*Meloidogyne incognita*	Nemathelminthes	Secernentea	Tylenchida	Meloidogynidae
13	Free-living nematode	*Rhabditis* spp.	Nemathelminthes	Secernentea	Rhabditida	Rhabditidae
14	Predatory nematode	*Mononchid* spp.	Nemathelminthes	Enoplea	Mononchida	Mononchidae

Table 2: Dominance (%) of different beetle and earwig species in okra crop

S. No.	*Common name*	*Scientific name*	*Total individuals*	*Dominance (%)*
	Beetles			
1	Dung beetle	*Onitis fabricius*	0.40	7.64
2	Ground beetle	*Bembidion tetracolum*	3.14	60.03
3	Black spruce beetle	*Tetropium castaneum*	0.65	12.42
4	Whiplash rove beetle	*Paederus riparius*	0.56	10.70
5	Red tail rove beetle	*Hesperus apicialis*	0.48	9.177
	Earwigs			
6	Common earwig	*Forficula auricularia*	3.51	71.6
7	Striped earwig	*Labidura riparia*	1.39	28.4

(*Eisenia fetida*). Species richness and species evenness of soil invertebrates were calculated to be 14.0 and 0.094, respectively in okra crop fields. Similarly, Shannon-Wiener index (H') and Simpson's index for the soil invertebrates was calculated to be 0.098 and 0.051, respectively indicating high degree of diversity among soil invertebrates in okra crop fields (Table 3). The analysis of soil samples obtained from okra fields revealed the presence of nematodes belonging to three trophic groups *viz*., predatory form: *Mononchids* sp., bacterial feeder: *Rhabditids* sp. and plant parasitic: *Meloidogyne incognita*. Before sowing and at harvest of okra crop, the soil collected from fields showed the population of root-knot nematode (*Meloidogyne incognita*) as 146.33 and 276.33/250 g of soil, respectively with increase in population as 88.84% during harvest time. Similarly, the population of predatory and free-living nematodes before sowing and at harvest of crop was reported to be 186.66 and 316.66/250 g soil, respectively with increase in population as 69.64% during harvest time (Figure 2).

In a study conducted by Santos *et al.* (2007) in olive grove, the most abundant epigeic taxa recorded were Formicidae

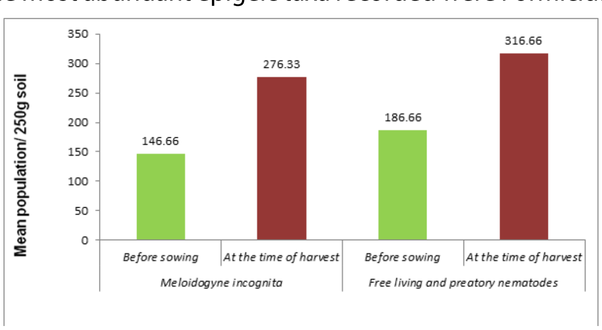

Figure 2: Population of plant parasitic (*Meloidogyne incognita*), free living and predatory nematodes in okra crop

(56.6%), belonging to 12 genera and 24 species. Gonçalves and Pereira (2012) studied the diversity and abundance of soil arthropods in olive groves in the northeast region of Portugal and recorded five classes of arthropods like Chilopoda, Malacostraca, Entognatha, Insecta and Arachnida where Collembola were predominating ones. In Nainital (India), Bisht *et al.* (2003) recorded six earthworm species from two families (Lumbricidae and Megascolecidae) during June to October. Uvarov *et al.* (2011) recorded maximum population of *L. rubellus*, *Dendrodril octaedra* and *D. usrubidus in forest soil.* Several scientists have thoroughly investigated the association of plant parasitic nematodes with different plants. (Sahu *et al.* 2011). Hussain and Muhktar (2019) reported that root-knot nematode (*M. incognita*) which is of phytophagous in nature and infest different vegetable crops was fairly distributed in soil of okra crop in all the districts of Punjab (Pakistan) in varying intensities. In a field study in Egypt, Ibrahim *et al.* (2021) reported five terrestrial snails (*Helicodiscus singleyanusinermis, Monacha obstructa*, *Oxyloma elegans*, *Cochlicella acuta and Eobania vermiculata*) and three slug species (*Lehmannia valentiana*, *Deroceras leave* and *Limax flavus*), out of which *M. obstructa was predominant one. Baker (1998) observed Cernuella virgata, Thebapis ana* and *Cochlicella* spp. as the major snail species found in Australia. In a study at Karnataka, Pallavi *et al.* (2018) reported that in vegetable crops the maximum snail population occur during September to November.

Population dynamics of soil invertebrates

The data on population dynamics of different soil invertebrates present in/on soil of okra crop revealed that the mean population of common earwig was evenly distributed from July to October months where the mean population ranged between 0.33-1.33 individuals/m^2 and total population during crop period as 3.51 individuals/m^2, with higher incidence during August (Table 3). In August, the mean population of striped earwig ranged from 0.08-0.66 individuals/m2 with a maximum incidence (0.66 individuals/m2). The mean population of little black ant was evenly distributed from July to October months where mean population ranged between 23.49-63.33 individuals/m^2 with maximum incidence in September. The population of dung beetle and ground beetle was distributed from July to October months where mean population ranged between 0.08–0.16 and 0.16–2.25 individuals/m^2, respectively and total population of 0.40 and 3.14 individuals/m^2, respectively with higher incidence during September. The population of black spruce beetle and whiplash rove beetle appeared in August and its mean population ranged between 0.08–0.24 individuals/m^2 with higher incidence during September (0.24 individuals/m^2) with total mean population recorded to be 0.65 and 0.56 individuals/m^2, respectively. The mean population of red-tail rove beetle ranged between 0.08–0.32 individuals/m^2 with higher incidence during September. The

Table 3: Diversity indices and correlation of total population with mean temperature and relative humidity of soil invertebrates in okra crop

S. No.	*Soil Soil invertebrates*	*July*	*Aug.*	*Sept.*	*Oct.*	*Total population (mean)*	*Dominance (%)*	*Correlation coefficient (r) with relation to temperature (^{0}C)*	*Correlation coefficient (r) with relation to soil moisture (%)*
1	Common earwig	1.06	1.33	0.79	0.33	3.51^a	1.88	+0.83	-0.54
2	Striped earwig	0.16	0.66	0.49	0.08	1.39^a	0.74	+0.18	+0.21
3	Little black ant	29.50	34.08	63.33	23.49	150.4^d	80.78	-0.25	+0.27
4	Dung beetle	0.00	0.16	0.16	0.08	0.40^a	0.21	-0.81	+0.70
5	Ground beetle	0.16	0.32	2.25	0.41	3.14^a	1.68	-0.52	+0.46
6	Black spruce beetle	0.00	0.16	0.41	0.08	0.65^a	0.34	-0.48	+0.57
7	Whiplash rove beetle	0.08	0.16	0.24	0.08	0.56^a	0.30	-0.26	+0.42
8	Red-tail rove beetle	0.00	0.08	0.32	0.08	0.48^a	0.25	-0.59	+0.59
9	Millipede	0.13	13	6.26	0	19.39^c	10.41	+0.18	+0.25
10	Earthworm	0.00	0.08	0.16	0.08	0.32^a	0.17	-0.71	+0.81
11	Snail	0.12	3.32	2.25	0.25	5.94^b	3.10	+0.06	+0.34
	Species richness	14.0							
	Species evenness	0.094							
	Simpson's index	0.051							
	Shannon-Wiener index	0.098							

*Mean population followed by same letter (a,b,c and d) in given table above are not significantly different

total mean population of red-tail rove beetle during the whole okra crop period was recorded to be 0.48 individuals/m^2. In case of millipede, as we move from July to October, there was an increase in the population of millipede which increased during July onwards and its mean population ranged between 0.13–13.00 individuals/m^2 with higher incidence during August with total mean population during the okra crop period recorded to be 19.39 individuals/m^2 whereas no individual was recorded in October. A few earthworm populations were observed, ranging between 0.08–0.16 individuals/m^2. Interestingly, the population of snails was recorded which ranged from 0.12–3.32 with higher incidence during August (Table 3).

Correlation of soil temperature and soil moisture with soil invertebrates

It is evident from the data that there is a positive correlation (r=+0.06 to +0.83) between the mean soil temperature (Table 3) and mean population of common earwig, striped earwig and millipede and snails whereas negative correlation (r=-0.25 to -0.81) with little black ant, dung beetle, ground beetle, black spruce beetle, whiplash rove beetle, red-tail rove beetle and earthworms. Interestingly, all the soil invertebrate population has a positive correlation (r=+0.21 to +0.81) with soil moisture (Table 3). Hence, soil moisture enhances the activity and survival of soil invertebrates as compared to soil temperature.

Soil fauna (including earthworms, nematodes and soil-inhabiting invertebrates, etc.) are known to improve the structure of soil by decreasing bulk density, soil horizon mixing, litter decomposition, increased aeration and drainage, improving soil aggregate structure and increased water holding capacity (Curry and Good, 1992). In healthy soil invertebrate populations are abundant. Arthropods are "litter transformers" and "ecosystem engineers" in the soil food web (Lavelle *et al.* 1995), which humidify ingested plant debris, improving its quality as a substrate for microbial decomposition and promoting microbial growth and dispersal and regulating resource availability of other species (Jones *et al.* 1994). Collembola may play a larger role in the physical breakdown of organic matter in habitats without earthworms (Curry and Good, 1992). Saprophagous arthropods affect decomposition by feeding on litter and adhering microflora, converting energy into biomass and respiration, and indirectly by converting litter into faeces and re-ingesting faecal material, comminuting litter, mixing litter with soil, and regulating microflora. Only a small percentage of net primary production is assimilated by soil arthropods (Stork and Eggleton, 1992). Casts of earthworms work like time-released fertilizer, have nutrients readily available for plants, and increase plant yields (Whitford *et al.* 1995). According to Jin *et al.* (2022), about 50% of the soil will pass through the earthworm colon within 10 years and up to 90% will pass within 40 years. Soil microfauna and mesofauna is responsible for bioturbation, that is the process by which organic matter transport in the soil profile by mixing it with mineral particles by soil fauna through redistribution of ingested soil and by constructing subsurface burrows and surface mounds (Domene 2016). The present study concluded that okra crop fields survive soil fauna having 14 species among which coleopterans predominates. This may enhance soil fertility and productivity by increasing soil porosity and water filtration to increase crop yield, which will further increase farm income and food security.

Acknowledgment

The authors are highly thankful to the Head, Department of Zoology and Head, Department of Vegetable Science, Punjab Agricultural University, Ludhiana for providing the necessary facilities for conducting this research work.

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सारांश

वर्तमान शोध, वनस्पतिक अनुसंधान प्रक्षेत्र, पंजाब कृषि विश्वविद्यालय, लुधियाना (पंजाब) में वर्ष 2019 के दौरान जनसंख्या की गतिशीलता और मिट्टी के अकशेरूकीय की विविधता पर किया गया। परिणाम से स्पष्ट हुआ कि भिण्डी की फसल में 14 अलग-अलग मिट्टी के अकशेरूकीय किस्म हैं जो नौ ऑर्डर से संबंधित हैं और 13 कुलों के ऑर्डर कोलॉप्टेरा का प्रभुत्व है (पाँच किस्मों) जिनमें ग्राउंड बीटल अधिक है। दिलचस्प बात यह है कि हाइमेनोप्टेरा (80.72 प्रतिशत) में उच्च सापेक्ष बहुतायत (प्रतिशत) दर्ज की गई जिनके बीच उच्च प्रभुत्व (प्रतिशत) छोटी काली चींटियों (80.78 प्रतिशत) में दर्ज किया गया तथा इसके उपरान्त उच्च सापेक्ष बहुतायत पॉलीज़ोनिडा (10.41 प्रतिशत) में थी। किस्म की समृद्धि, किस्म की समरूपता, शैनन-वीनर इंडेक्स (एच.1) और सिम्पसन के मिट्टी के अकशेरूकीय सूचकांक की गणना क्रमशः 14.0, 0.094, 0.098 और 0.051 थी जो भिण्डी की फसल में मिट्टी के अकशेरूकीय के बीच उच्च स्तर की विविधता को प्रदर्शित करता है। भिण्डी की फसल के बुआई के समय की तुलना में कटाई के समय जड़ गाँठ और मुक्त रहने वाले/परभक्षी सूत्रकृमि में जनसंख्या में 88.84 प्रतिशत और 69.64 प्रतिशत की वृद्धि दर्ज की गयी। मिट्टी के अकशेरूकीय आबादी के साथ मिट्टी के तापमान और मिट्टी की नमी के बीच एक सकारात्मक सहसंबंध (आर.त $0.06 से $0.93) दर्ज किया गया। भिण्डी की फसल क्षेत्र में मिट्टी के जीवों को जीवित रहने में मदद करता है जो मिट्टी की उर्वरता और उत्पादकता और इसके भौतिक गुणों को बढ़ाने के लिए महत्वपूर्ण कारकों में से एक है। इस प्रकार मिट्टी के संसाधनों का उपयोग कर और बढ़ाया जाना चाहिए।