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ORIGINAL RESEARCH ARTICLE

Nematode Fauna Associated with Kiwi (*Actinidia delicosa*, *Chev*.) Plants in Machchhegaun, Kathmandu, Nepal

Bijay Chhetri[1]*

[1]Central Department of Zoology, Tribhuvan University, Kirtipur, Kathmandu, Nepal

[1]Amrit Campus, Tribhuvan University, Lainchaur, Kathmandu, Nepal

Abstract

Plants harbor many trophic groups of nematodes in them. The plant production is also determined by the occurrence of nematodes adjacent to the rhizosphere of plants, such as parasitic, free-living etc. Altogether 40 samples from the 30 cm away from the Kiwi plants were examined to detect the nematode distribution in Kiwi plants in Machchhegaun, Kathmandu, Nepal. Total 10 genera of nematodes including both free living/beneficial and parasitic were identified. Overall, 880 individuals of 10 nematodes genera belonging to four orders were found to be linked with Kiwi plants, among them the highest report was of order Dorylaimida (40.91%) (*Dorylaimus* spp., *Cephalobus* spp., *Eucephalobus* spp. and *Discolaimus* spp.) which was followed by Mononchida (36.36%) (*Iotonchus* spp., *Parahadronchus* spp. and *Mononchus* spp.), Rhabditida (18.18%) (*Rhabditis* spp. and *Mesorhabditis* spp.) and Tylenchida (4.55%) (*Helicotylenchus* spp.). No any published data about study of plant nematodes was found from study area. So, these four order of plant nematodes have been reported for the first time associated with Kiwi plants from Machchhegaun in Nepal. The result specified no significant distribution of nematodes in all Kiwi plants. Proper management of manures and Kiwi plants treatment is recommended for more production of Kiwi fruits in study area by nitrogen reduction, phosphorus reduction, odor reduction, energy recovery and adding value to manure techniques.

Keywords: kiwi, nematode, herbivorous, soil, vine

***Corresponding Author**

Email: bjme686@gmail.com

Introduction

Kiwi (*Actinidia delicosa*) belongs to the order Ericales and family Actinidiaceae, which normally ripens within 25 weeks after the appearance of first flowers [1]. The Kiwi fruits are beneficial to the human health because it contains vitamin A, B, C, E and K, copper, potassium, fiber and folate [2]. The demand of Kiwi in the worldwide market is hiking due to these nutrients available in Kiwi fruit, which is mainly beneficial for the treatment of many diseases like cancer and heart disease, children and pregnant women [2].

Nematodes are classified into free-living and parasitic [3, 4]. Some soil nematodes increase the plant production, whereas some lower the production by causing harm to the plants. Harmful nematodes are more disadvantageous to the crop production and cause massive loss to the farmers [5]. About 100-157 US$ crop production is lost worldwide per year from the nematodes [6]. The crop rotation techniques are being practiced by farmers to lower the crop loss from disease and parasites [7, 8]. However, this technique won't be conceivable to perennial plants like Kiwi. Plant parasitic nematodes constitute one of the most important groups of organism inhabiting the soil and in and around the roots of plants. Plant parasitic nematodes lower the plant production by damaging crops. The most abundant plant parasitic nematodes fall under the orders like Tylenchida, Dorylaimida and Aphelenchida [9]. Furthermore, *Rhabditis* spp., *Mesorhabditis* spp., *Cephalobus* spp. and *Eucephalobus* spp., etc. are the free-living nematodes, which are common in the plants, and benefit plants by providing nutrients through decaying organic matters [10]. Likewise, *Aphelenchus* spp. is one of the fungivorous nematodes, which also turn organic matters into inorganic form [9]. However, despite of being least abundant predatory nematode in most of the soil contents, *Mononchus* spp. feeds indiscriminately both free-living and plant parasitic nematodes [10].

The Kiwi fruit production is declining, which may be due to the effects of nematodes such as *Xiphinema* spp., *Pratylenchus* spp., *Meloidogyne* spp., *Helicotylenchus* spp., *Paratylenchus* spp., *Psilenchus* spp. *Heterodera* spp., *Tylenchus* spp. and *Paratrichodorus* spp. [11]. In Nepal, till date very less research specified the occurrences/ distribution of soil nematodes in kiwi plants. Therefore, the present study aims to lessen this research gap.

Materials and Methods

Study area

The present study was carried out on the Kiwi plants at Machchhegaun, Kathmandu, Nepal on March to April 2017. Geographically, Machchhegaun lies in between 27˚47′ N latitude and 85˚79′ E longitude, at 1531 m of elevation [12]. The study area covers 1.6 hectors of land, and 10 farmers are commercially farming kiwi plants in the study area. Two hundred fifty Kiwi plants are planted in 16000 m^2 of Machchhegaun, Kathmandu.

Preliminary survey

Preliminary survey was done to acqire general information about study area. Interaction with concerned person was done to collect information about Kiwi plants farming in Machchhegaun.

Soil sampling

Total 40 Kiwi plants randomly selected to collect Kiwi plants related soil nematodes. About 20 cm was dug on the side of kiwi plants, and 500 gm of soil sample was taken. The soil was kept in plastic bags and tagged and brought to the lab of Central Department of Zoology for further processing.

Processing of soil samples

Processing Soil SamplesIn laboratory, the chunks were smashed and pebbles were removed from soil samples by hand picking method. Processing of samples were done by Cobb's sieving and decantation techniques [13] (mesh size: 53µm and 200 mm) with continuous wash with fresh water. The nematode suspension acquired from soil sample was transferred to Baermann's funnel [14].

Isolation of nematodes from soil

The funnel was left for 24 hours for the movement of nematodes to the bottom of the funnel.

Counting of nematode species

Total 10 ml of nematode suspension was prepared and 1 ml out of 10 ml suspension was taken into a counting chamber for counting of nematodes and result obtained was multiplied by 10.

Killing and fixation of nematodes

The killing and fixing of nematodes were done by Seinhorst's process [15] by using 4% formaldehyde heated at 60-70°C.

Dehydration of nematodes

Dehydration of nematodes was done by keeping them in a desiccator for about 1 week [16].

Mounting and sealing

The slides of nematodes were prepared by using glycerin, purified wax and hot plate.

Observation and identification

The slides were then observed under the low power and high power magnification. i.e. 10× (eye piece) and 10×, 20× and 40× (nose piece) from left to right side of the slide. The nematodes were identified up to genus level by comparing the characters of observed nematodes with available taxonomic keys [17].

Statistical method

The distribution of Kiwi nematodes was analyzed in R Program 3.4.1 version [18].

Table 1: Genus wise nematodes identified in rhizosphere soil of Kiwi plant.

S.N.	Nematodes	Frequency (N)	Absolute Frequency (AF %)	Total no. of each genus of nematode	Mean Density	*p*- value
1	*Helicotylenchus* spp.	20	50	40	1	
2	*Iotonchus* spp.	40	100	200	5	
3	*Dorylaimus* spp.	40	100	120	3	
4	*Parahadronchus* spp.	20	50	80	2	
5	*Mononchus* spp.	18	45	40	1	
6	*Rhabditis* spp.	24	60	120	3	2.2×10^{-16}
7	*Cephalobus* spp.	20	50	80	2	
8	*Eucephalobus* spp.	20	50	120	3	
9	*Mesorhabditis* spp.	10	25	40	1	
10	*Discolaimus* spp.	8	20	40	1	
	Total	313		880		

Results

Isolation and identification of different types of nematodes found in rhizosphere of Kiwi plant.

Nematode species belonging to four orders were found in the present work. Among them Dorylaimida (40.91%) (*Dorylaimus* spp., *Cephalobus* spp., *Eucephalobus* spp. and *Discolaimus* spp.) order was significantly higher, which was followed by order Mononchida (36.36%) (*Iotonchus* spp., *Parahadronchus* spp. and *Mononchus* spp.), Rhabditida (18.18%) (*Rhabditis* spp. and *Mesorhabditis* spp.) and Tylenchida (4.55%) (*Helicotylenchus* spp.).

Out of 40 soil samples, all soil samples were positive (100%) for the prevalence of free living nematodes. However, plant parasitic nematodes were documented only from 50% soil samples. There was high prevalence of *Iotonchus* spp. (22.73%) and low prevalence of *Helictylenchus* spp., *Mononchus* spp., *Mesorhabditis* spp. and *Discolaimus* spp. (4.55%).

The number of *Iotonchus* spp. was highest (22.73%) followed by *Rhabditis* spp. (13.64%), *Dorylaimus* spp. (13.64%), *Eucephalobus* spp. (13.64%), *Parahadronchus* spp. (9.09%), *Cephalobus* spp. (9.09%), *Mononchus* spp. (4.55%), *Mesorhabditis* spp. (4.55%) *Helichotylenchus* spp. (4.55%) and *Discolaimus* spp. (4.55%) (**Table 1**). There was significant difference ($\chi^2_{cal} > \chi^2_{tab}$) between the total number of nematode species in samples studied in Machchhegaun study area ($P < 0.05$).

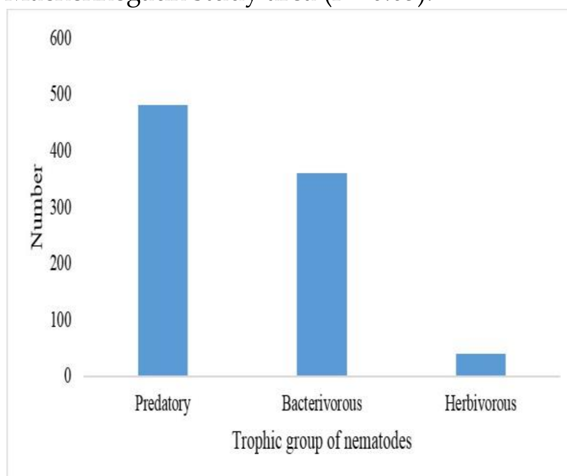

Figure 1: Tropic groups of soil nematodes.

Different tropic groups of nematodes were selected for the study. In the study area predatory nematodes (54.55%) (*Mononchus* spp., *Parahadronchus* spp., *Iotonchus* spp., *Discolaimus* spp. and *Dorylaimus* spp.) comprised more than half of the soil nematode community in study area. Bacterivores (40.91%) (*Rhabditis* spp., *Mesorhabditis* spp., *Cephalobus* spp. and *Eucephalobus* spp.) and herbivores (4.54%) (*Helicotylenchus* spp.) were also represented (**Figure 1**).

Discussion

Many nematodes endeavor Kiwi plants for getting nutrition and for reproduction, such as *Pratylenchus vulnus* (walnut root lesion nematode) and *Meloidogyne* spp. (root knot nematode), both belonging to order Tylenchida is the main pest to Kiwi plants [19]. Among four orders of nematodes, order Dorylaimida (*Dorylaimus* spp., *Cephalobus* spp., *Eucephalobus* spp. and *Discolaimus* spp.) was dominant and order Tylenchida (*Helicotylenchus* spp.) was least found, probably due to the regular supplying of organic matters to the Kiwi plants. This assumption can be supported by the occurrence of nematodes belonging to orders Dorylaimida, Mononchida, Rhabditida and Tylenchida in Central Horticulture Centre, Kirtipur, Nepal [20].

Kiwi plants harbor several trophic groups of nematodes, namely, bacterivorous (*Rhabditis* spp., *Mesorhabditis* spp., etc.) and different stages of insects and animal parasites like *Deladinus* female, *Eudiplogaster* larva, *Sphaerularia* spp. and *Mermis* spp., etc. [21]. Among the identified nematodes in the Kiwi plants the free-living species such as *Iotonchus* spp. *Rhabditis* spp., *Dorylaimus* spp., *Eucephalobus* spp., *Parahdronchus* spp., *Cephalobus* spp., *Mononchus* spp., *Mesorhabditis* spp. and *Discolaimus* spp. were dominant, probably due to the regular supplying of organic matters to the Kiwi plants. This assumption can be supported by the occurrence of free-living nematodes *Iotonchus* spp. and *Rhabditis* spp. in Central Horticulture Centre, Kirtipur, Kathmandu [20], established grasslands [22, 23, 24]. The occurrence of predatory nematodes (*Mononchus* spp., *Parahadronchus* spp., *Iotonchus* spp., *Discolaimus* spp. and *Dorylaimus* spp.), were probably supported by the occurrence of numerous free-living nematodes. The dominance of predatory nematodes in study area may be due to the use of organic manure like cattle dung and plant leaves in 20:80 ratio on the rhizosphere soil of Kiwi plants. The composition of the soil nematode community depends on the vegetation present, as well as on soil type, season, soil moisture level, amount of soil organic matter, and many other factors. Because they are responsive to so many different factors, it is believed that nematodes may be useful bio-indicators of the condition of the soil environment [25].

The identified herbivorous nematode (*Helicotylenchus* spp.) in the Kiwi plants was less dominant, probably due to the presence of free-living nematodes like *Iotonchus* spp. *Rhabditis* spp., *Dorylaimus* spp., *Eucephalobus* spp., *Parahdronchus* spp., *Cephalobus* spp., *Mononchus* spp., *Mesorhabditis* spp. and *Discolaimus* spp. which graze on bacteria, fungi and other phyto-parasitic nematodes, thus they control the populations of harmful micro-organisms [20, 26]. Generally, plant parasitic nematodes can be found in less number in those plants where fungivorous *Eucephalobus* spp. are common are more common in homogeneous crop residues such as in maize (*Zea mays*) [27].

The predatory nematodes such as *Mononchus* spp., *Parahadronchus* spp., *Iotonchus* spp., *Discolaimus* spp. and *Dorylaimus* spp. were highest in kiwi plants of this study area which might be due to the high number of bacterivorous nematodes (*Rhabditis* spp., *Mesorhabditis* spp., *Cephalobus* spp. and *Eucephalobus* spp.). These genera are supposed to be common food of the predatory nematodes. Increase of predatory nematodes cause declines in prey populations, which in turn prevents further increase of the predator population [28]. Out of all identified nematodes from rhizosphere of kiwi plants, the bacterivorous nematodes like *Rhabditis* spp., *Mesorhabditis* spp., *Cephalobus* spp. and *Eucephalobus* spp. were dominant, probably due to the regular supplying of organic matters to the kiwi plants. Nematode occurrences can be supported by nematodes present in Central Horticulture Centre, Kirtipur, Kathmandu [20] and litter present in the soil [29].

Conclusion

The presence of predatory nematodes and bacterivore nematodes almost in equal number and also high number of herbivorous nematode species in Machchhegaun study area may be due to the use of organic manure like cattle dung and plant leaves as fertilizer in the rhizosphere soil of

Kiwi plants. Therefore, regular use of manures and treatment of Kiwi plants are recommended for increased yield of kiwi fruits.

Conflict of Interest

Declared none

Acknowledgements

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Photo Plate

Figure 2: Entire body of *Helicotylenchus* sp. through 10×40X magnification.

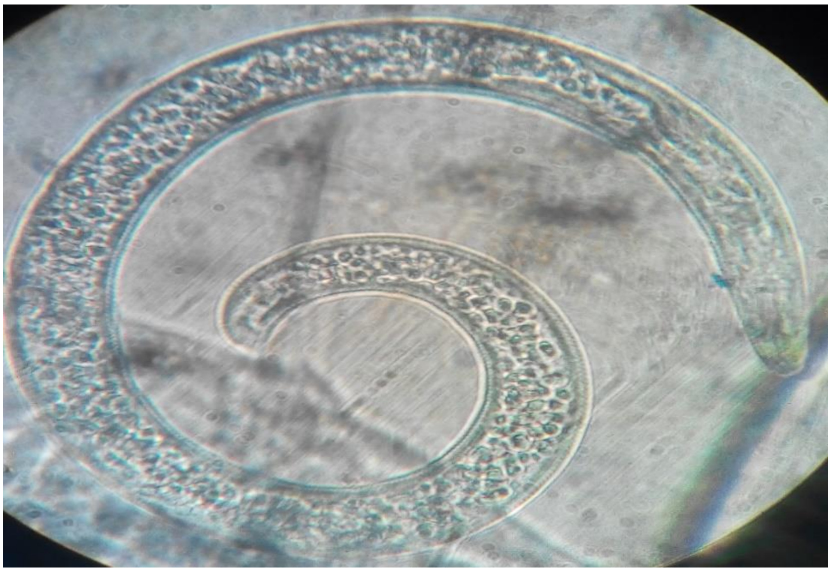

Figure 3: Entire body of *Helicotylenchus* sp. through 10×100X magnification.

Figure 4: Anterior region of *Mononchus* sp. through 10×40X magnification.

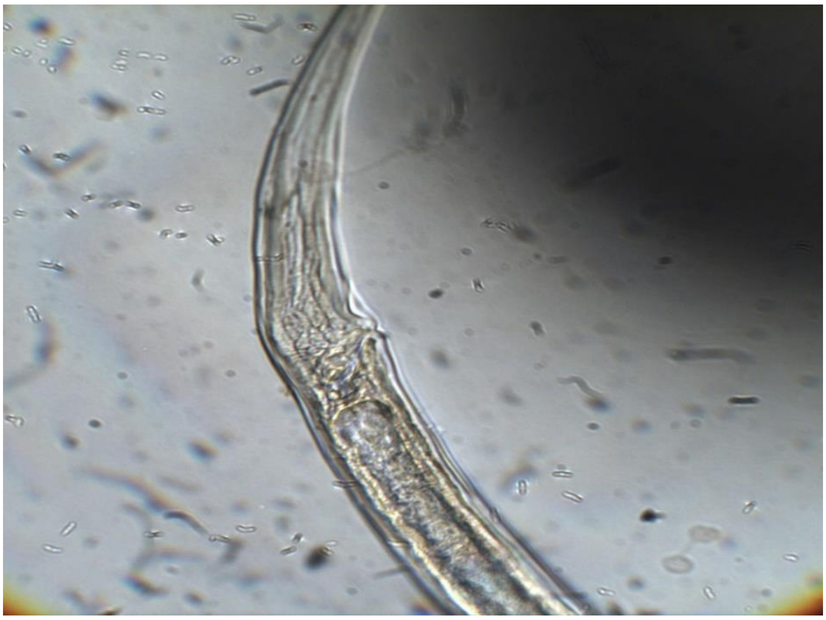

Figure 5: Posterior region of *Mononchus* sp. through 10×40X magnification.

Figure 6: Anterior region of *Iotonchus* sp. through 10×40X magnification.

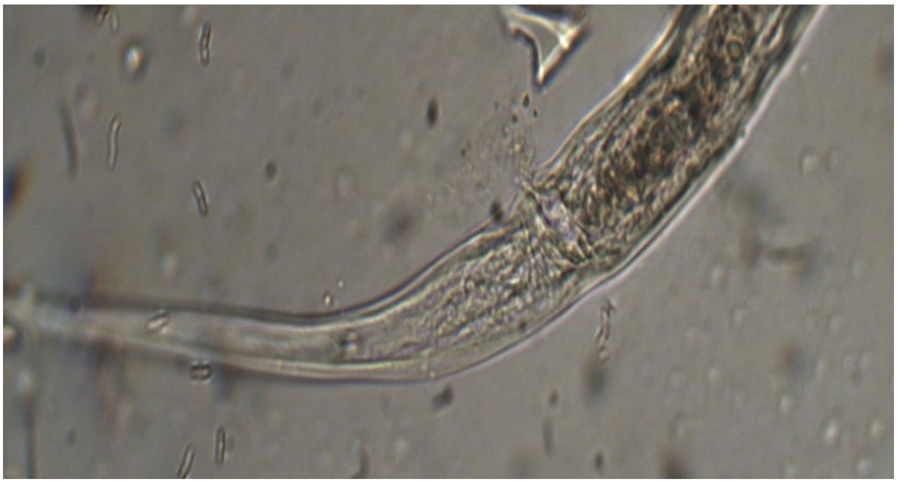

Figure 7: Posterior region of *Iotonchus* sp. through 10×40X magnification.

Figure 8: Anterior region of *Rhabditis* sp. through 10×40X magnification.

Figure 9: Posterior region of *Rhabditis* sp. through 10×40X magnification.

Figure 10: Anterior region of *Dorylaimus* sp.

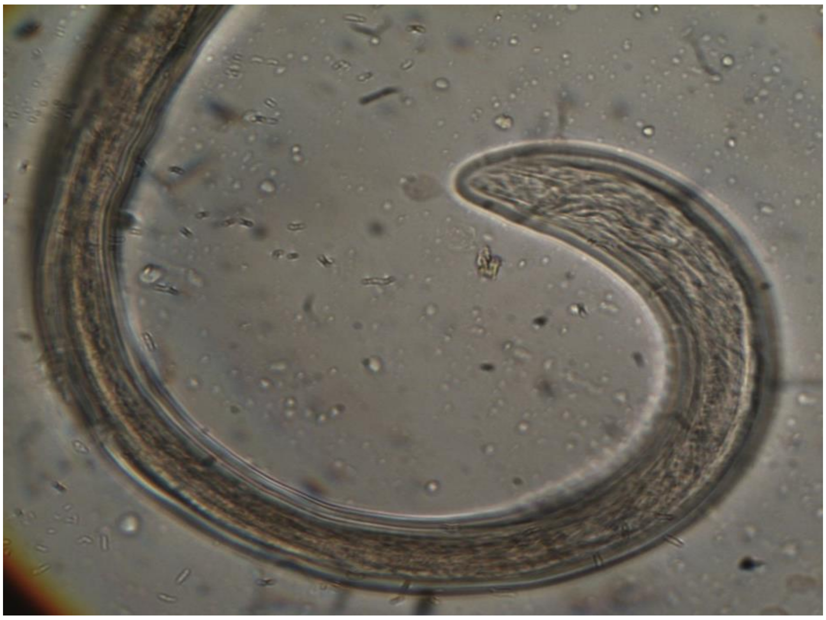

Figure 11: Posterior region of *Dorylaimus* sp. through 10×40X magnification.

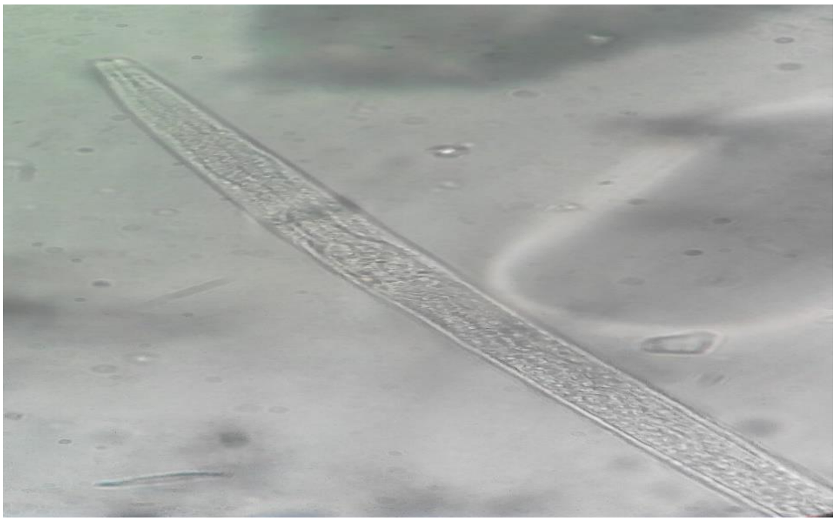

Figure 12: Anterior region of *Cephalobus* sp. through 10×40X magnification.

Figure 13: Posterior region of *Cephalobus* sp. through 10×40X magnification.

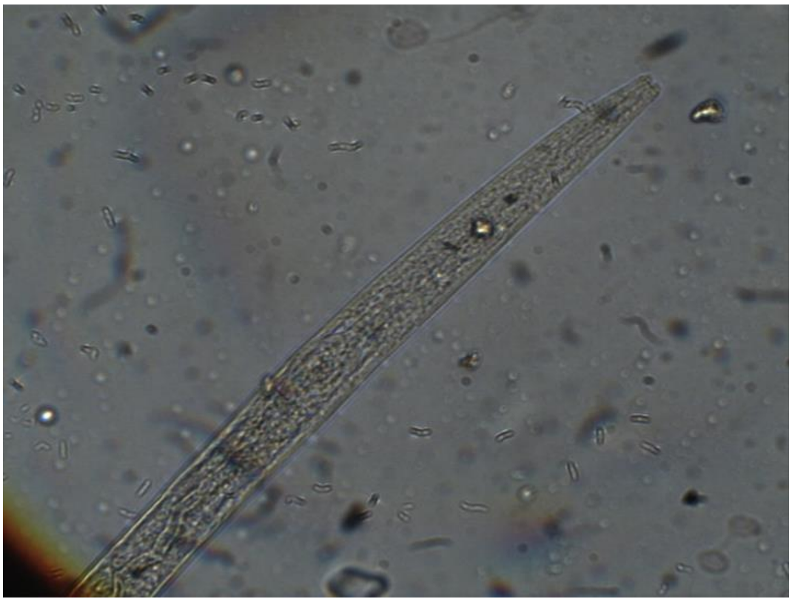

Figure 14: Anterior region of *Eucephalobus* sp. through 10×40X magnification.

Figure 15: Posterior region of *Eucephalobus* sp. through 10×40X magnification.

Figure 16: Entire body of *Discolaimus* sp. through 10×40X magnification.

Figure 17: Posterior region of *Discolaimus* sp. through 10×40X magnification.

Figure 18: Anterior region of *Parahadronchus* sp. through 10×40X magnification.

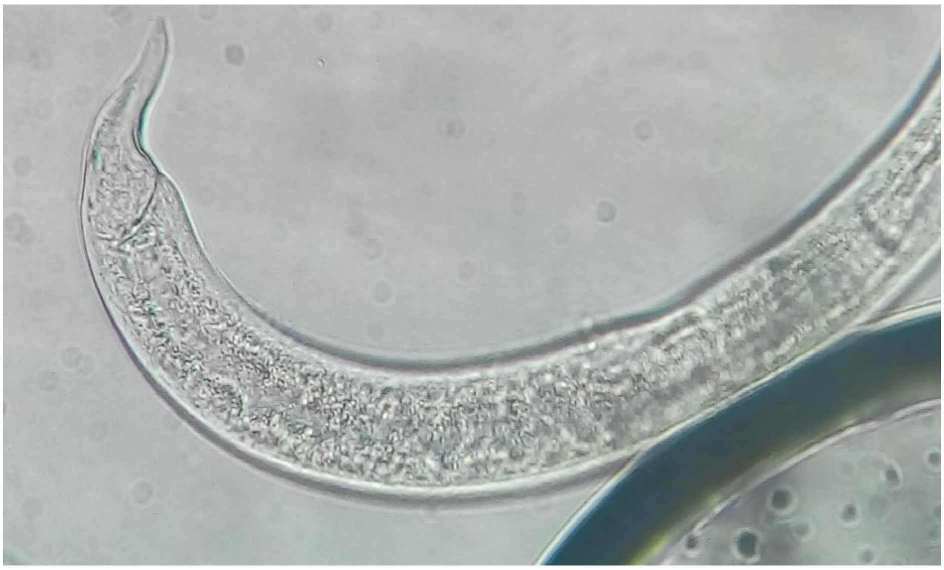

Figure 19: Posterior region of *Parahadronchus* sp. through 10×40X magnification.

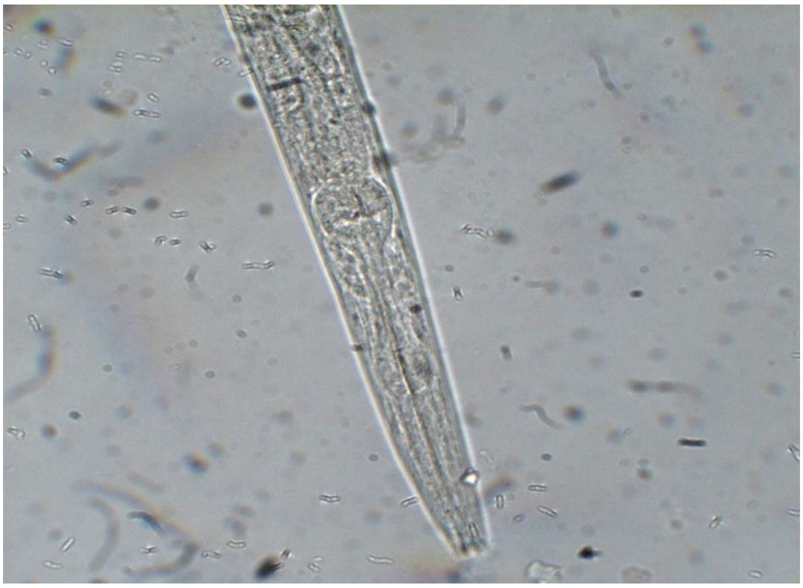

Figure 20: Anterior region of *Mesorhabditis* sp. through 10×40X magnification.

Figure 21: Posterior region of *Mesorhabditis* sp. through 10×40X magnification..