Prevalence of Nematodes in Rhizosphere of Kiwi Plants (Actinidia delicosa Chev., 1984) in International Centre for Integrated Mountain Development (ICIMOD), Knowledge Park, Godawari, Lalitpur, Nepal

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ABSTRACT

Background: 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.

Method: Altogether 60 samples (30 from 20 cm depth and 30 from 30 cm depth) were examined from the Kiwi plants to detect the nematode distribution in Kiwi plants in ICIMOD, Godawari, Lalitpur, Nepal. Overall, 1510 individuals of 10 nematodes genera (both free living/beneficial and parasitic) belonging to four orders were found to be associated with Kiwi plants, among them the highest report was of order Mononchida (44.04%) which was followed by Rhabditida (33.44%), Dorylaimida (17.88%) and Tylenchida (4.64%). The number of nematodes were higher at 20 cm depth than at 30 cm depth. Iotonchus spp. was highest (32.68%) and the number of Helicotylenchus spp. (3.93%) and Mesorhabditis spp. (3.92) were lowest at 20 cm depth. Similarly, Iotonchus spp. was highest (20.13%) and the number of Discolainus spp. (4.03%) was lowest at 30 cm depth.

Key words: Community analysis, Kiwi, Nematode distribution, Nematodes, Rhizosphere.

INTRODUCTION

Kiwi (Actinidia delicosa) belongs to the order Ericales and family Actinidiaceae, which normally ripens within 25 weeks after the appearance of first flowers (Wilson et al., 2020). The Kiwi fruits are beneficial to the human health because it contains vitamin A, B, C, E and K, copper, potassium, fiber and folate (Beutel, 1990). 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 (Beutel, 1990). Some beneficial soil nematodes increase the plant production by increasing soil fertility, whereas some lower the production by causing harm to the plants (Yeates and Coleman, 1982). Harmful nematodes are more disadvantageous to the crop production and cause massive loss to the farmers (Singha et al., 2015). About 100-157 US$ crop production is lost worldwide per year from the nematodes (Nicol et al., 2011). The crop rotation techniques are being practiced by farmers to lower the crop loss from disease and parasites (Curl, 1963; Nusbaum and Ferris, 1973). 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 (Ingham and Detling, 1984). 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 (Yeates et al., 1993). Likewise, Aphelenchus spp. is one of the fungivorous nematodes, which also turn organic matters into inorganic form (Ingham and Detling, 1984). 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 (Yeates et al., 1993).

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. (Watson et al., 1992). 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

The ICIMOD Knowledge Park at Godawari, on the southern slopes of the Kathmandu Valley, was set up in March 1993, following the generous provision of 30 hectares of land with loamy type soil. Its coordinates are: - Latitude: 27°35'19"N, longitude: 85°23'16"E, altitude: 1540 - 1800 masl and slope gradient: 50 - 600 (North-facing) (Nepal Census, 2001). Study area has 35 Kiwi plants in Knowledge Park at Godawari.

Preliminary survey was done to acquire general information about study area. Interaction with concerned person was done to collect information about Kiwi plants farming in ICIMOD. Total 60 Kiwi plants randomly selected to collect Kiwi plants related soil nematodes. About 20 cm and 30 cm was dug on the side of kiwi plants. Five sub-samples collected randomly from a depth of 15-20 cm (for 20 cm depth) and 25-30 cm (for 30 cm depth) from each rhizosphere of kiwi were mixed to make a composite sample. The soil was kept in plastic bags and tagged and brought to the lab of Central Department of Zoology for further processing. In laboratory, the chunks were smashed and pebbles were removed from soil samples by hand picking method. A 100cc of soil sample from collected sample was processed by Cobb’s sieving (Cobb, 1918) (mesh size: 53µm and 200 mm) and decantation and modified Baermann’s funnel technique (Staniland, 1954) for extraction of nematodes. Total 10 ml of nematode suspension was prepared and 1 ml out of 10 ml suspension was taken into a counting disc for counting of nematodes and result obtained was multiplied by 10. The killing and fixing of nematodes were done by Seinhorst’s process (Seinhorst, 1966) by using 4% formaldehyde heated at 60-70 °C. Dehydration of nematodes was done by keeping them in a desiccator for about 1 week (Seinhorst, 1959). The slides of nematodes were prepared by using glycerin, purified wax and hot plate. 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 (Smart and Nguyen, 1988).

Statistical Analysis

Parameters for community analysis of various nematode genera were calculated using formulae by Beals (1960) and Norton (1978).

Frequency (N): Frequency of nematode genus (i.e. the number of samples in which the genus was present).

Absolute Frequency (AF %) = Frequency of the genus \times 100

Relative Frequency (RD %) = \frac{Mean density of the genus}{Sum of mean density of all nematode genera} \times 100

Mean Density (MD) = \frac{Total no specimens of a genera}{Total number of sample counted}

Prominence value (PV) = Density \times Frequency

Population structure of soil inhabiting nematodes of Kiwi plants of ICIMOD, Lalitpur, Nepal.

| Nematodes | N  | AF % | Density | RD  | Total no. of each genus of nematode | PV   | MD    | p-value |
|-----------|----|------|---------|-----|-------------------------------------|------|-------|---------|
| Helicotylenchus spp. | 30 | 50.00 | 1.17 | 4.65 | 70 | 6.41 | 1.17 |
| Iotrichus spp. | 60 | 100.00 | 6.67 | 26.50 | 400 | 51.67 | 6.67 |
| Dorylaimus spp. | 60 | 100.00 | 3.33 | 13.23 | 200 | 25.79 | 3.33 |
| Parahadronchus spp. | 30 | 50.00 | 2.75 | 10.93 | 165 | 11.89 | 2.75 |
| Mononchus spp. | 27 | 45.00 | 1.67 | 6.63 | 100 | 8.68 | 1.67 |
| Rhabditis spp. | 23 | 38.33 | 3.50 | 13.90 | 210 | 16.79 | 3.50 |
| Cephalobus spp. | 24 | 40.00 | 1.58 | 6.28 | 95 | 7.74 | 1.58 |
| Eucephalobus spp. | 24 | 40.00 | 2.00 | 7.95 | 120 | 9.80 | 2.00 |
| Mesorhabditis spp. | 20 | 33.33 | 1.33 | 5.28 | 80 | 5.95 | 1.33 |
| Discolaimus spp. | 15 | 25.00 | 1.16 | 4.65 | 70 | 4.53 | 1.17 |
| Total | 313 | 521.66 | 25.16 | 100.00 | 1510 | 149.25 | 25.17 |

N (Frequency) = No. of samples in which the genus was present; AF% = Frequency of genus x 100 / total no. of sample; MD = Total no. of specimens of a genera / No. of samples; RD % = Mean density of a genus x100 / sum of mean density of all nematode genera and Prominence value (PV) = Density \times Frequency

There was significant difference (\chi^2 > \chi^2_{tab}) between the total number of nematode species in samples studied in ICIMOD Education Park study area (P< 0.05).
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Parahadronchus spp. and Mononchus spp.) 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 (Chhetri and Subedi, 2019) and Machchhegaun (Chhetri, 2019), Kathmandu.

### Order Wise Nematodes Identification

In study, the number of Mononchida order was the highest (44.04%) which was followed by Rhabditida (33.44%), Dorylaimida (17.88%) and Tylenchida (4.64%) (Fig 2).

### Genus Wise Nematodes Identification

The number of Iotonchus spp. was highest (26.49%) followed by Rhabditis spp. (13.91%), Dorylaimus spp. (13.25%), Parahadronchus spp. (10.93%), Eucephalobus spp. (6.29%), Mesonhabditis spp. (5.30%) Helicotylenchus spp. (4.64%) and Discolaimus spp. (4.64%) (Table 1) (Fig 3).

### Depth Wise Nematodes Identified

The number of nematodes were higher at 20 cm depth than at 30 cm depth. Iotonchus spp. was highest (32.68%) and the number of Helicotylenchus spp. (26.49%) and Discolaimus spp. (26.49%) (Table 2). There was significant difference ($\chi^2_{cal} > \chi^2_{tab}$) between the total number of nematode species in samples studied at 20 cm depth of rhizosphere soil of Kiwi plants in ICIMOD Education Park study area (P< 0.05).

### Table 2: Nematodes identified at 20 cm depth of rhizosphere soil of Kiwi plant.

| Nematodes            | Frequency | No. of each genus of nematode |
|----------------------|-----------|-------------------------------|
| Helicotylenchus spp. | 15        | 30                            |
| Iotonchus spp.       | 30        | 250                           |
| Dorylaimus spp.      | 30        | 90                            |
| Parahadronchus spp.  | 15        | 65                            |
| Mononchus spp.       | 14        | 60                            |
| Rhabditis spp.       | 13        | 100                           |
| Cephalobus spp.      | 12        | 50                            |
| Eucephalobus spp.    | 12        | 50                            |
| MesoRhabditis spp.   | 10        | 30                            |
| Discolaimus spp.     | 5         | 40                            |
| **Total**            | **765**   |                               |

There was significant difference ($\chi^2_{cal} > \chi^2_{tab}$) between the total number of nematode species in samples studied at 20 cm depth of rhizosphere soil of Kiwi plants in ICIMOD Education Park study area (P< 0.05).

### Table 3: Nematodes identified at 30 cm depth of rhizosphere soil of Kiwi plant.

| Nematodes            | Frequency | No. of each genus of nematode |
|----------------------|-----------|-------------------------------|
| Helicotylenchus spp. | 15        | 40                            |
| Iotonchus spp.       | 30        | 150                           |
| Dorylaimus spp.      | 30        | 110                           |
| Parahadronchus spp.  | 15        | 100                           |
| Mononchus spp.       | 13        | 40                            |
| Rhabditis spp.       | 10        | 110                           |
| Cephalobus spp.      | 12        | 45                            |
| Eucephalobus spp.    | 12        | 70                            |
| MesoRhabditis spp.   | 10        | 50                            |
| Discolaimus spp.     | 10        | 30                            |
| **Total**            | **745**   |                               |

There was significant difference ($\chi^2_{cal} > \chi^2_{tab}$) between the total number of nematode species in samples studied at 30 cm depth of rhizosphere soil of Kiwi plants in ICIMOD Education Park study area (P< 0.05).

### Table 4: Plant parasitic and plant non-parasitic nematodes at various depths of rhizosphere.

| Nematodes            | Plant Parasitic | Plant Non-Parasitic | P-value |
|----------------------|-----------------|---------------------|---------|
| 20 cm depth          |                 |                     |         |
| Helicotylenchus spp. | 15              | 30                  |         |
| Iotonchus spp.       | 30              | 250                 |         |
| Dorylaimus spp.      | 30              | 90                  |         |
| Parahadronchus spp.  | 15              | 65                  |         |
| Mononchus spp.       | 14              | 60                  |         |
| Rhabditis spp.       | 13              | 100                 | 2.2×10^{-16} |
| Cephalobus spp.      | 12              | 50                  |         |
| Eucephalobus spp.    | 12              | 50                  |         |
| MesoRhabditis spp.   | 10              | 30                  |         |
| Discolaimus spp.     | 5               | 40                  |         |
| **Total**            | **765**         |                     |         |

There was significant difference ($\chi^2_{cal} > \chi^2_{tab}$) between the total number of nematode species in samples studied at 20 cm depth of rhizosphere soil of Kiwi plants in ICIMOD Education Park study area (P< 0.05).

### Genus Wise Nematodes Identification

The number of Iotonchus spp. was highest (26.49%) followed by Rhabditis spp. (13.91%), Dorylaimus spp. (13.25%), Parahadronchus spp. (10.93%), Eucephalobus spp. (7.95%), Mononchus spp. (6.62%), Cephalobus spp. (6.29%), Mesonhabditis spp. (5.30%) Helicotylenchus spp. (4.64%) and Discolaimus spp. (4.64%) (Table 4) (Fig 3).

### Depth Wise Nematodes Identified

The number of nematodes were higher at 20 cm depth than at 30 cm depth. Iotonchus spp. was highest (32.68%) and the number of Helicotylenchus spp. (26.49%) and Discolaimus spp. (26.49%)
Mesorhabditis spp. (3.92) were lowest at 20 cm depth. Similarly, Iotochus spp. was highest (20.13%) and the number of Discolaimus spp. (4.03%) was lowest at 30 cm depth (Table 2) (Table 3) (Table 4).

Among the identified nematodes in the kiwi plants the free-living species such as Iotochus spp., Rhabditis spp., Dorylaimus spp., Eucephalobus spp., Parahdronchus spp., Cephalobus spp., Mononchus spp., Mesorhabditis spp. and Discolaimus spp. were more dominant at 20 cm depth than 30 cm depth of rhizosphere. It is probably due to the regular supplying of organic matters at less depth (10-20 cm) in the rhizosphere of the kiwi plants in study area. This assumption can be supported by the occurrence of free-living nematodes Iotochus spp. and Rhabditis spp. in Central Horticulture Centre, Kirtipur and Machchhegaun, Kathmandu (Chhetri, 2017).

**Trophic Group Wise Nematodes**

Overall predatory nematodes (Mononchus spp., Parahdronchus spp., Iotochus spp., Discolaimus spp. and Dorylaimus spp.) comprised more than half of the soil nematode community (61.92%) in study area, but bacterivores (Rhabditis spp., Mesorhabditis spp., Cephalobus spp. and Eucephalobus spp.) (33.44%) and herbivores (Helicotylenchus spp.) (4.64%) were also represented (Fig 2). 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 (El-Borai and Fahiem, 2005).

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, Eudiplagaster larva, Sphaerularia spp. and Mermis spp., etc. (Chhetri, 2019). Among the identified nematodes in the Kiwi plants the free-living species such as Iotochus 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 Iotochus spp. and Rhabditis spp. in Central Horticulture Centre, Kirtipur and Machchhegaun, Kathmandu (Chhetri, 2019) Kathmandu and established grasslands (Freckman, 1982; Reyes and Domingo, 1991; Èerevková, 2011). The occurrence of predatory nematodes (Mononchus spp., Parahdronchus spp., Iotochus 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 (El-Borai and Fahiem, 2005).

The identified herbivorous nematode (Helicotylenchus spp.) in the Kiwi plants was less dominant, probably due to the presence of free-living nematodes like Iotochus 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 (Wallace, 1973; Chhetri, 2019; Chhetri and Subedi, 2019). 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) (FAO, 2005).

The predatory nematodes such as Mononchus spp., Parahdronchus spp., Iotochus 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 Dorylaimus spp.,) comprised more than half of the soil nematode community (61.92%) in study area, but bacterivores (Rhabditis spp., Mesorhabditis spp., Cephalobus spp. and Eucephalobus spp.) (33.44%) and herbivores (Helicotylenchus spp.) (4.64%) were also represented (Fig 2). 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 (El-Borai and Fahiem, 2005).

![Fig 2: Orders of soil nematodes.](image)

![Fig 3: Trophic group of soil nematodes.](image)
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 (McSorley and Frederick, 1999; Djigal, 2004). 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, Kathmandu, Nepal (Chhetri, 2019; Chhetri and Subedi, 2019) and litter present in the soil (Xue, 2013).

**Community Analysis of Identified Nematodes**

Total 10 genus of plant nematodes were encountered in the rhizospheres of Kiwi plants. Iotolochus and Dorylaimus genera were the most frequently encountered species with the absolute frequencies of 100% each and least frequent was Discolaimus (25%). Genus Iotolochus had the highest mean density of 400 nematodes and lowest mean density was possessed by Helicotylenchus and Discolaimus (each 70) per 250 ml soil. In this study, genus Iotolochus had the highest (51.67) and Discolaimus had lowest (4.53) prominence value.

Looking at the different parameters used in this study, genera Helicotylenchus, with its lowest value in all parameters, seems to be the least important nematode population in Kiwi plant and could not be the reason for decline of the plantation in the area. This is in contrary with the supposition of Norton (1978); Samathanam and Chawla (1982) and Keshari et al. (2018), that importance value (IV) of a nematode need not be confined to considerations based on frequency, density and biomass only but should be broad based to include facts reflecting its pathogenie abilities. Therefore, IV as applied now may not be sufficient to provide all informations about pathogenic potential of a parasite and needs consideration for either modification of the present formula or incorporation of a new one, for the analysis of community structure of nematodes.

**CONCLUSION**

The presence of predatory nematodes and bacteriovore nematodes almost in equal number and also high number of herbivorous nematode species in ICIMOD 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.

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