World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(Research Article)

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Diversity of nematode populations on cruciferous and solanaceous crops in Bagmati province of Nepal

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World Journal of Advanced Research and Reviews, 2023, 17(03), 769–775

Publication history: Received on 21 January 2023; revised on 18 March 2023; accepted on 21 March 2023

Article DOI: https://doi.org/10.30574/wjarr.2023.17.3.0360

Abstract

A total of 211 soil samples were collected in different crop fields from 10 districts. Out of 211 samples, 137 samples were diagnosed for different plant parasitic nematodes. The samples were collected from the fields of cruciferous and solanaceous crops. All together twelve different nematodes were identified. The identified genera are *Meloidogyne, Helicotylenchus, Pratylenchus, Tylenchus, Tylenchorhynchus, Hoplolaimus, Belonolaimus, Criconemoides, Aphelenchoides, Hirschmanniella, Longidorus* and *Rotylenchulus*. They found either single or more than one genus in a sample. It means they are existed in both single or combine with two or more genera in a same place or root zone. The population of some of the nematode genera was recorded above the threshold level and some were below the threshold to cause significant yield loss in the crops. In potato, the population of *Helicotylenchus, Tylenchorhynchus, Pratylenchus* and *Tylenchus* were recorded equal to or more than 2 nematodes per gram of soil. Similarly, in tomato, *Helicotylenchus, Meloidogyne, Hoplolaimus,* and *Tylenchorhynchus* were recorded above threshold level. Likewise, the distribution of nematodes population in the fields of cabbage, cauliflower, chilly, capsicum, board leaf mustard, carrot and radish also had above threshold level to reduce the crops yield. Hence, lower nematode population may not show any symptom caused by nematode alone and so the effects may easily be overlooked, but nematodes become a problem when the population level surpasses the damage threshold; at that point the damage due to the pathogen is measurable and also need to manage them.

Keywords: Crops; Genera; Nematodes; Population; Threshold level

1. Introduction

Nematodes are usually microscopic in size and are classified as unsegmented worms and are commonly described as filliform or thread like multicellular invertebrates. At least one nematode species are reported to parasitize every cultivated plant species (Luc *et al.*, 2005). In general, nematodes inhabit all parts of plant and about 95% live in soil and feed in or on plant roots (Baidya *et al.*, 2014) and rest invade leaf, stem tissue (Bird and Bird, 1991) and inflorescence.

In Nepal, more than 50 different plant parasitic nematodes have been reported in different cultivated crops. The first report on plant parasitic nematode (PPN) from Nepal was reported eleven different nematodes. They were *Aphelenchoides besseyi, Criconemoides* sp., *Ditylenchus dipasci, Hemicycliophora gracilis, Heterodera cruciferae, Longidorus elongatus, Tylenchulus semipenetrans, Xiphinema index* and *Xiphinema diversicaudatum* (Bhatta, 1969).

Plant parasitic nematodes have long been known to cause extensive crop losses through reduced yields, shortened productive life, or lowered value of produce. Plant parasitic nematodes are cosmopolitan. The plants infected with root knot nematodes have an unthrifty appearance and often show symptoms of yellowing, rotting, wilting and premature

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shedding of the foliage with severe stunting that result in huge losses to the infected crops (Saifullah *et al*., 1990). It destroys crops and causes economic losses equal to those of' any other plant parasitic species. Plant parasitic nematodes cause an estimated crop yield loss of 14.6% in tropical and sub-tropical climates and losses of 8.8% in developing countries (Juan *et al*., 2013).

In Hemza, a report revealed that root knot nematode had caused 15-30% yield reduction in plastic house (Baidya *et al.,* 2017). Yield can be increased up to 37% when tomato grafted with root stock of *Solanum sisymbriifolium* against root knot nematode (Baidya *et al.,* 2017).

The occurrence and distribution of common plant parasitic nematodes in different vegetable crops a continuous effort to be given for monitoring and identification plant parasitic nematodes which also help to find out the real situation of nematode variability and their population in the field condition. Hence, this study was carried out for updating the nematodes distribution in solanaceous and cruciferous crops in Bagmati province and prioritizes them for their management.

2. Material and methods

The samples were collected from 13 different crops (Tomato, potato, lady finger, chilly, capsicum, coriander, Broad leaf mustard, radish, carrot, cress, brocauli, cabagge and cauliflower) from 10 districts (Kathmandu, Dhading, Kavrepalanchok, Lalitpur, Chitwan, Ramechhap, Bhaktapur, Sindhuli, Nuwakot and Dolkha) of the Bagmati province from 2018-2020 AD. Approximately, 200 g soil samples from a15-20 cm deep soil layer, close to the plant roots by means of soil auger. In total of 211 soil samples were collected using the soil auger from four to five locations of each field in a systematic or zigzag pattern and mixed them as composite sample. Immediately after collection, samples in the plastic bag were sealed to prevent moisture loss.The sample was labeled with name of crop, location and date of collection. The sample were handled gently and kept out of the sun, preferably in a cooler area.

Nematodes were extracted following the method described by Hooper *et al.* (2005). The equipment needed for the extraction of nematodes included a plastic sieve with coarse mesh, a plastic tray or extraction plate and paper napkin, beakers to wash the extraction into, wash bottle, labels, and weighing scale.

The composited soil was gently mixed before withdrawing a sub-sample. One hundred grams of soil were weighed. A paper napkin was placed in the plastic sieve placed on a plastic plate. A coarse plastic mesh sieve was placed on a plastic tray and its bottom and sides covered with a layer of a serviette tissue paper. One hundred grams of finely crumbled soil was spread out over the serviette paper. Clean water was carefully added down the sides of the collecting tray until the soil layer was wet. The set up were left overnight for 24 hours. After 24 hours, the sieve was lifted to drain the water into the extraction plate. The soil sample was disposed off from the sieve. The water from the plate was poured into a labeled beaker. Water in a wash bottle was used to rinse the plate and poured into the beaker. The total volume of the water maintained about 300 ml. The set up was left to settle for about an hour. Around 10 ml solution from the stock sample was pipette out mixing uniformly and kept in the Petri plates for extraction. Manual fishing was done using nematode fishing needle and collected them in the cavity blocks. The nematodes numbers and species/genera were diagnosed using a compound stereo-microscope and compound microscope.

3. Results

Out of 211 samples, 137 samples had presence of plant parasitic nematodes i.e., 35 percent of total samples and rest 65 percent had only saprophytes nematodes (Fig. 1). From 137 samples, twelve different genera of plant parasitic nematodes were identified. They are *Meloidogyne, Rotylenchus, Tylenchus, Hoplolaimus, Belonolaimus, Criconemoides, Helicotylenchus, Tylenchorhynchus, Pratylenchus, Hirschmanniella, Aphelenchoides* and *Longidorus.* Among them, the most commonly detected genera were *Helicotylenchus* (28%) followed by *Tylenchorhynchus* (19%)*, Meloidogyne* (10%) and *Pratylenchus* (10%) as shown in figure 2*.* They found either single or combination with two to more than two genera. Around 57 percent sample had the presence of single genus. Similarly, 31% sample had two different types of nematode (Figure 3). Ten different types of nematodes in solanaceous and nine in cruciferous crops were recorded. Out of 10, six species were at level of damage in cruciferous crops whereas four species in solanaceous crops at medium to high level of population distributed. Population of both ecto-parasite (*Helicotylenchus, Tylenchorhynchus*, and *Belonolaimus*) and endo-parasites (*Meloidogyne, Pratylenchus* and *Hoplolaimus*) were recorded at moderate to high level to cause yield loss in crops (Table 1 and 2).

In solanaceous crops, ten different nematodes *Tylenchorhynchus* sp., *T. vulgaris*, *T. acutus*; *Helicotylenchus* sp., *H. indicus*; *Pratylenchus* sp. *P. coffeae, P. penetrans*; *Tylenchus* sp.; *Meloidogyne* sp., *M. javanica*, *M. incognita, Criconemoides ornatus; Aphelenchoides* sp.; *Belonolaimus* sp.; *Hoplolaimus* sp. and *Rotylenchus* sp. were recorded. Among these nematodes, *T. vulgaris*, *M. javanica*, *Helicotylenchus* sp. and *Hoplolaimus* sp. were recorded medium to high population density to cause the yield loss in solanaceous crops.

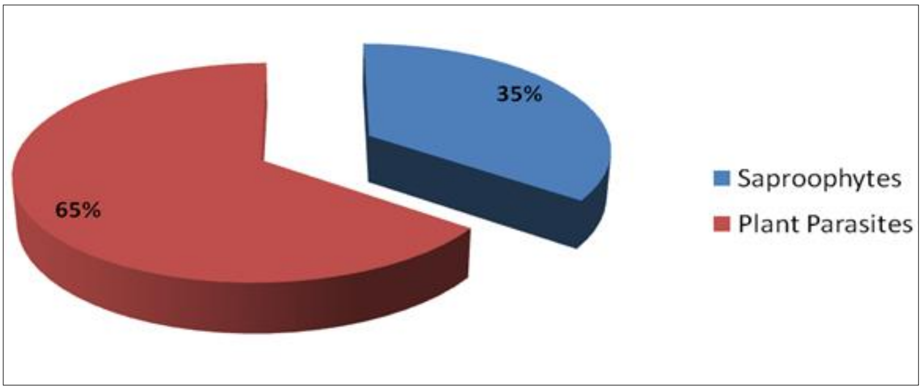

Figure 1 Distribution of Plant parasitic nematodes in the samples

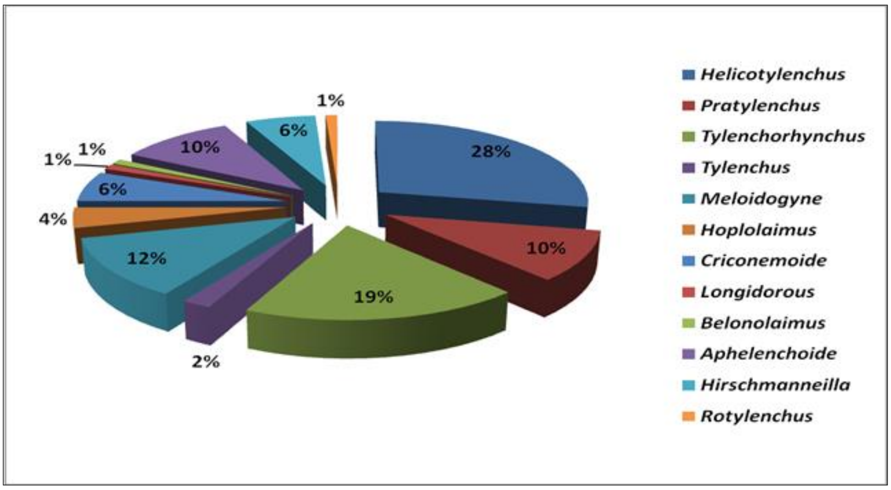

Figure 2 Frequency patterns of nematode genera in vegetable fields

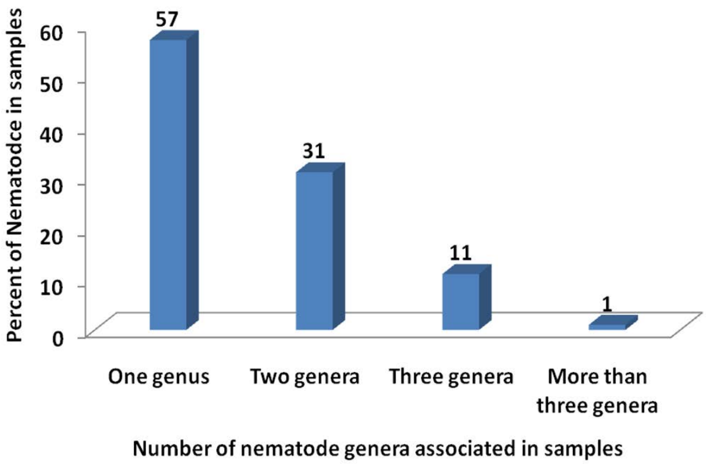

Figure 3 Existing patterns of nematode genera

Similarly, in crucifers, *Helicotylenchus* sp., *H. dihystera*; *Belonolaimus* sp., *B. longicaudatus*; *Longidorus* sp., *L. elongatus*; *Tylenchorhynchus brassicae, T. acutus*; *Pratylenchus brachyurus, P. penetrans, P. crenatus*; *Hoplolaimus indicus*; *Criconemoides oranatus*; *Meloidogyne* sp. *M. incognita*; and *Hirschmanniella* sp.. Among these nine different types of nematodes, *Tylenchorhynchus, Pratylenchus* sp., *Meloidogyne* sp. *Belonolaimus* sp., *Hoplolaimus* sp., and *Helicotylenchus* sp. were found medium to high population density in cruciferous crops (Table 1 and 2).

Table 1 Distribution and population density of plant parasitic nematodes in different vegetables of cruciferous crops

S.N.	Crops	Nematodes species	No. of samples	Population density / g soil	Level of threshold
1.	Cress	*Longidorus* sp.	4	1	Low
		Tylenchorhynchus sp.		1	Low
		Hoplolaimus sp.		1	Low
2	Cauliflower	*Hoplolaimus* sp.	26	4	High
		Belonolaimus longicaudatus		2	Medium
		Meloidogyne sp.		1	Low
		Tylenchorhynchus sp.		2	Medium
		Hirschmanniella sp.		1	Low
		Helicotylenchus sp. *H dihystera, H. indicus*		5	High
		Criconemoides sp., *C. annulatus*		1	Low
3	Cabbage	*Pratylenchus* sp. *P. penetrans*	4	1	Low
		Helicotylenchus indicus		2	Medium
4	Broad leaf mustard	*Helicotylenchus* sp., *H. dihystera*	31	5	High
		Belonolaimus sp.		1	Low
		Longidorus sp., *L. elongatus*		1	Low
		Tylenchorhynchus brassicae, T. acutus		5	High
		Pratylenchus brachyurus, P. penetrans, P. crenatus		3	High
		Hoplolaimus indicus		1	Low
		Criconemoides oranatus		1	Low
		Meloidogyne sp.		2	Medium
5	Carrot	*Pratylenchus* sp.	1	2	Medium
6	Brocauli	*Tylenchorhynchus* sp.	2	1	Low
7	Radish	*Tylenchorhynchus* sp., *T. vulgaris*	6	2	Medium
		Longidorus elongatus		1	Low
		Helicotylenchus dihystera, H. indicus		1	Low
		Belonolaimus sp.		1	Low
		Meloidogyne sp., *M. incognita*		1	Low

		Hoplolaimus sp.		1	Low

Table 2 Distribution and population density of plant parasitic nematodes in different vegetables of solanaceous crops

S.N.	Crops	Nematodes species	No. of samples	Population density/g soil	Level of threshold
1.	Tomato	*Helicotylenchus* sp., *H. vulgaris, H. indicus*	28	2	Medium
		Meloidogyne incognita, M. javanica		3	High
		Tylenchorhynchus sp., *T. acutus*		1	Low
		Hoplolaimus sp.		2	Medium
		Rotylenchus sp.		1	Low
		Criconemoides sp.		1	Low
2.	Potato	*Tylenchorhynchus* sp.	12	2	Medium
		Helicotylenchus sp.		4	High
		Pratylenchus sp., *P. coffeae, P. penetrans*		1	Low
		Tylenchus sp.		1	Low
		Meloidogyne sp.		1	Low
		Criconemoides ornatus		1	Low
3.	Chilly	*Meloidogyne javanica*	18	2	Medium
		Tylenchorhynchus sp.		2	Medium
		Helicotylenchus sp.		1	Low
		Pratylenchus sp., *P. coffeae*		1	Low
		Belonolaimus sp.		1	Low
		Hoplolaimus sp.		1	Low
4.	Lady finger	*Meloidogyne* sp.	2	1	Low
		Helicotylenchus sp.		1	Low
5.	Capsicum	*Aphelenchoides* sp.	3	1	Low
		Tylenchorhynchus vulgaris		2	Medium

4. Discussion

According to their feeding strategy, plant parasitic nematodes are broadly classified into three major types; ecto-parasitic, endo-parasitic and semi-endoparasitic nematodes. These all three types of nematodes were detected from the samples. The ecto-parasitic type lives outside the plant, feeding on roots with the ability to move about 3 feet to find a host, depending on the soil and species. Ecto-parasitic nematodes such as *Tylenchorhynchus, Helicotylenchus, Hoplolaimus, Longidorus, Rotylenchus, Belonolaimus* etc. have a rather long and powerful stylet with the help of which they can penetrate the root tissue. Endo-parasitic types penetrate the root, then enter and live inside it and they are either sedentary or migratory. Some endo-parasitic nematodes such as root-knot nematode (*Meloidogyne*) can develop their feeding sites inside the root and become sedentary whereas other endo-parasitic nematodes of genera *Pratylenchus, Radopholus* and *Hirschmanniella* migrate in the roots and form root lesion by burrowing the tissue and again invading other healthy root tissue (Luc *et al*., 2005).

The genera *Helicotylenchus, Tylenchorhynchus, Pratylenchus* and *Meloidogyne* were commonly recorded than the others genera (Baidya, 2013). Nematodes can reduce crop yield significantly when their population establish two or more than two number per gram of soil (Baidya *et al.,* 2014). The result showed that the presence of high population of nematodes is also one of the possible biotic factors to reduce yields in both solanaceous and cruciferous vegetables at Bagmati province. In potato, the population of *Helicotylenchus, Tylenchorhynchus, Pratylenchus* and *Tylenchus* were recorded equal to or more than 2 nematodes per gram of soil. Similarly, in tomato, *Helicotylenchus, Meloidogyne, Hoplolaimus*, and *Tylenchorhynchus* were recorded above threshold level. Likewise, the distribution of nematodes population in the fields of cabbage, cauliflower, chilly, capsicum, broad leaf mustard, carrot and radish also had above threshold level to reduce the crops yield. Hence, lower nematode population may not show any symptom caused by nematode alone and so the effects may easily be overlooked, but nematodes become a problem when the population level surpasses the damage threshold; at that point the damage due to the pathogen is measurable and also need to manage them.

The crops or field infested by high density of nematode population then there are need to applied soil amendment to manage the nematode for reducing the yield loss. The plant parasitic nematode can be managed through integrated approaches. In tomato, the application of mustard cake 60g/1500g soil, the lowest gall index was observed and followed by the mixture of cow dung and urine @ 300g/1500g soil against root knot nematode (Baidya *et al*., 2008). Similarly, root knot nematode infected field, tomato yield increased over control with grafted plant with using *Solanum sisymbriifolium*, 2% formaldehyde @ 600ml/m^2, application of mustard cake @ 250g/m^2, biogas slurry @2kg/m^2 by 37%, 30%, 24%, 17% than control (Baidya *et al*., 2013).

The current report indicated that the nematode might be widespread than previously known. Spiral nematode (*Helicotylenchus* spp.), Root knot nematode *(Meloidogyne* spp.) and Stung nematode (*Tylenchorhynchus* sp.) were observed in all the crops, therefore indicated its wide spread as it is the cosmopolitan pest of vegetables distributed worldwide and infesting more than 2500 kinds of host plants (Siddiqui, 1986). Estimation vegetable crop losses in the tropics (Sasser, 1979) ranged from 24 and 38% on tomato.

More than 55 percent samples showed the presence of single genus and one third percent of total samples occupied the combination of two genera and rest 12 percent contained more than two genera. High percentage of existing of single nematode may be due to their competition behavior nature on feeding sties and pushing one another within intra and inter species. Nematodes are various and sometimes numerous nematodes species can survive in the same root zone. When there are two or more nematode species present, their co-existence in the same location is possible, but never long-term stable and they tend to push out one another. The strength of competing is not exactly the same between the competitors and those who have more strength; they can survive and less competitive ones may either vanish, even extinct, or try to find another feeding site. The competition level may be inter specific or intra specific or both, depending on the population density with respect to the feeding area (Schoener, 1983). Competition for feeding sites is one of the factors that limit nematode establishment and reproduction in the plant roots (Duncan and Ferris, 1983). There are three possibilities of competitive interactions among plant-parasitic nematodes: Preemptive competition occurs when a unit of space is occupied by one species, thereby preventing another species from entering that space. Nematode species may inhibit other nematode species through competition for feeding sites (Duncan and Ferris, 1983). Chemical competition is another possibility that a species produces a toxin or allele-chemical that suppresses another species. Likewise, Consumptive competition occurs when some essential resource, usually food, is consumed by one species, reducing or depleting the quantity available to the competing species. Inhibition of root growth and disruption of root tissues in a host plant may be indications of consumptive competition (Sikora *et al*., 1979).

5. Conclusion

Both ecto and endo parasitic nematodes are found in moderate to high level of population density to cause crop yield loss. Genera *Tylenchorhynchus*, *Pratylenchus*, *Meloidogyne*. *Belonolaimus*, *Hoplolaimus*, and *Helicotylenchus* in cruciferous and genera *Tylenchorhynchus*, *Meloidogyne*, *Helicotylenchus* and *Hoplolaimus* are recorded medium to high population density in solanaceous crops to reduce crop yield. In high population of nematodes, they can cause both quantitative and qualitative yield loss. Nematode may also one of the hidden biotic factors to reduce crop yield in the fields. Hence, management of nematode in vegetable fields is necessary step to increase production by reducing the losses due to their damage.

Compliance with ethical standards

Acknowledgments

Authors are very grateful to financial and technical assistance of Nepal Agricultural Research Council (NARC) management. We also thank to staff of National Plant Pathology Research Centre (NPPRC) for their support on samples collection and laboratory works. We are also acknowledged to the farmers of respective locations for providing soil samples collection from their fields.

Disclosure of conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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