

Plant-parasitic and Free-living Nematodes infected on *Chrysanthemum* sp. (Linn) Leaves and Cultivated Soil from Kyaing Phaung Village, Kyaing Tong Township

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Abstract

Nematodes including plant-parasitic and free-living were examined, from the *Chrysanthemum* sp. (Linn) leaves and cultivated soil in Kyaing Phaung Village, Kyaing Tong Township, Eastern Shan State from May to December 2018. Samples of soil and leaves were monthly collected from the study site. Nematodes from soil were extracted by Whitehead, 1965 tray methods, and identified according to Hunt, 2002 and Goodey, 1951. Infected leaves were extracted by water embedding practice. The extracted nematodes were examined and identified under microscopes. Eight genera of plant-parasitic nematodes and five genera of free-living nematodes were recorded. Among them, *Rotylenchulus* sp. and *Helicotylenchus* sp. were abundantly found throughout the study period. All of the nematode genera were recorded in September during the study period.

Keywords: *Chrysanthemum*, nematodes, soil and leaf

INTRODUCTION

Chrysanthemums, often called 'mums', are a genus (*Chrysanthemum*) of about 30 species of perennial flowering plants in the family Asteraceae, native to Asia and northeastern Europe. The species of *Chrysanthemum* are white, yellow or pink in the wild species. Cultivated *Chrysanthemums* can be yellow, white, or even bright red. They are an important item in the international cut-flower trade. There is a lesser trade in artificially dwarfed potted plants and they are also very popular with amateur gardeners for garden decoration and for competitive exhibition (CABI, 2007).

Chrysanthemum eelworm, (*Aphelenchoides ritzemabosi*) is a frequent pest. Foliar nematodes are easily confused with leafspot, but fungal leaf spots are most often black, not brown. *Chrysanthemums* are the major host of *Pratylenchus penetrans* and minor host of *Belonolaimus iongicaudatus*, *Hoplotaimus indicus*, *Meloidogyne hapla* (root knot nematode), *Meloidogyne incognita*, *Meloidogyne javanica*. Nematodes are slender, unsegmented roundworms that are barely visible to the unaided eye. They swim up the film of water on the plants, created by spring rains, and enter leaves through the stomata (CABI, 2007).

Most of the nematodes that inhabit the soil can be arranged in three groups; (1) saprophagous species that derive their food directly from decaying organic matter or feed on the microorganisms associated with decay, (2) predaceous species that feed on small animals, including other nematodes, and (3) herbivorous species that feed on plants. The last mentioned group includes species that feed on fungi, algae and other low forms of plant life, as well as those that feed on the higher plants (Christie, 1959).

The nematode infected information regarding with the *Chrysanthemum* spp. were poor in Myanmar. Of this regard, the present study was conducted with the following objectives: to investigate and record the plant-parasitic and free-living nematodes from leaf and cultivated soil of *Chrysanthemum*.

MATERIALS AND METHODS

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Study site and study period

Plant-parasitic and free-living nematodes were examined from the *Chrysanthemum* leaf and cultivated soil in Kyaing Phaung Village, Kyaing Tong Township, Eastern Shan State from May to December 2018.

Samples collection method

Sample collection was done for a total of five months according to the lifespan of host plants. A sampling of soil that is very wet or very dry should be avoided. The soils for the samples were taken at least 15 cm below the surface since the nematodes congregate in the root zone. The individual sample was about 100 ml and a total of five samples were contained at one sampling time. Therefore, one sample consists of soil about 500 ml. After the samples were bulked and thoroughly mixed, a sub-sample of the same weight was taken and analyzed. *Chrysanthemums* leaf samples (the yellowing, brown, and dark spots on the leaf surface) from the study site were selected and were collected.

Nematodes extraction from soil and leaves

Nematodes were extracted from soil samples by whitehead tray method (Whitehead, 1965). The nematodes from infected leaves were extracted by water embedded procedure in which the well-washed leaves were cut off into small pieces and placed in a Petridish, then added with clean water until the cover on the leaves and kept about 24 hours in the laboratory at room temperature. The nematode suspensions from the Petridish were facilitated for examination.

Preparation for identification

Nematodes are packed out from the suspension by handling needles under the low magnification of stereoscopic microscope. The fine point of the needle is positioned under the nematode which is then gradually worked upwards to the water surface before then being removed by draping over the end of the point, and place in a drop of water on the slide. The nematodes were arranged in the center of the drop and caution was taken not to float. Identified to genus level was done under the compound microscope according to the body shape stylet, oesophagus, vulva position, tail, shape, bursa, and spicules (Hunt, 2002 and Goodey, 1951).



Figure 1. Map of Study Site (source from Google)

A. *Chrysanthemum* farm

B. Infected leaves

Plate 1. Study site and symptom of infected leaves

RESULTS

Occurrence of soil nematodes

In the present study, 13 genera of nematodes belonging to four orders and 11 families were recorded from the *chrysanthemum* leaves and cultivated soil. Eight genera of plant parasitic (harmful) nematodes *Bursaphelenchus* sp., *Aphelenchus* sp., *Aphelenchoides* sp., *Rotylenchulus* sp., *Tylenchus* sp., *Pratylenchus* sp., *Helicotylenchus* sp., and *Tylenchorhynchus* sp. and five genera of free-living (beneficial) nematodes *Cephalobus* sp., *Dorylaimus* sp., *Rhabditis* sp., *Tripyla* sp., and *Ironus* sp. were recorded. Among them, *Rotylenchulus* sp. and *Helicotylenchus* sp. were abundantly found throughout the study period. (Table. 2)

During the study period, all of the recorded species (13 genera) were detected in September followed by August (11 genera), October (11 genera), July (10 genera) and June, (6 genera) were respectively found. (Table.2, Fig.2)

Under the order level, the genus composition percentage was highest in Tylenchida (38.46%) followed by Aphelenchida and Enoplida (23.08% each order) and Rhabditida (15.38%) recorded from the study site. (Table 3. Fig. 3)

Table 1. Systematic classification of recorded soil nematodes of *Chrysanthemum*

Sr. No	Class	Order	Family	Scientific name	Habitat
1.	Phasmidia	Aphelenchida	Parasitaphelenchidae	<i>Bursaphelenchus</i> sp.	Leaf
2.			Aphelenchidae	<i>Aphelenchus</i> sp.	Leaf and Soil
3.			Aphelenchoididae	<i>Aphelenchoides</i> sp.	Leaf and Soil
4.		Tylenchida	Rotylenchulidae	<i>Rotylenchulus</i> sp.	Soil
5.			Tylenchidae	<i>Tylenchus</i> sp.	Soil
6.				<i>Pratylenchus</i> sp.	Soil
7.			Hoplolaimidae	<i>Helicotylenchus</i> sp.	Soil
8.		Belonolaimidae	<i>Tylenchorhynchus</i> sp.	soil	
9.		Rhabditida	Rhabditidae	<i>Rhabditis</i> sp.	Soil
10.			Cephalobidae	<i>Cephalobus</i> sp.	Soil
11.	Aphasmidia	Enoplida	Dorylaimidae	<i>Dorylaimus</i> sp.	Soil
12.			Ironidae	<i>Tripyla</i> sp.	soil
13.				<i>Ironus</i> sp.	soil

Distinctive characters of plant-parasitic nematodes

***Aphelenchus* sp. Bastian, 1865**

The body is cylindrical, and 0.67 to 0.71mm long, usually with slight ventral curvature when killed by heat. Cephalic region bluntly rounded to flattened, not offset from body. The stylet is always present. Basal knobs are usually weakly or absent. Procorpus of oesophagus is cylindrical; median bulb widening abruptly from procorpus, well developed, rectangular to oval in shape, with refractive crescentic valve plates.

In female vulva position is 76.5% of the body length, genital tract monoprodelphic outstretched. The tail is usually bluntly rounded. Juveniles: Oesophageal region, tail, and overall shape of second to fourth stages similar to the female. Males were not found. (Plate 2. A)

***Aphelenchoides* sp. Fischer, 1894**

They are small to long nematodes, usually between 0.4 to 1.2mm in length. Straight to ventrally arcuate with the tail region sharply curled ventrally when killed by heat. Cuticle is finely annulated. Cephalic region usually rounded in form and slightly offset. The stylet is slender, usually with basal knobs or swellings. Procorpus is cylindrical, leading to a well-developed ovoid or spherical median bulb with central valve plates.

In the female, vulva post-median, usually at between 60-75% of the body length, Genital tract monoprodelphic, typically outstretched but may reflex. Post uterine sac usually present. The tail is conoid with a variable terminus which may be bluntly or finely rounded, with a ventral projection. One or more mucrons present. (Plate 2. B)

***Bursaphelenchus* sp. Fuchs, 1937**

The body is small to long (about 0.4-0.5mm) and slender nematodes. The cuticle is finely annulated. Cephalic region relatively high usually offset. Stylet well developed, procorpus cylindrical leading to a strong median bulb which is usually ovoid. Oesophageal glands are forming a dorsally overlapping lobe. Excretory pore usually in the vicinity of the nerve ring but, exceptionally, may be anterior to the median bulb.

Females tend to die ventrally arcuate on heat relaxation. Vulva, posterior is usually 70-80% of the body length. Vulva lips are slightly protuberant. The vagina is at right angles to the body axis. Genital tract monoprodelphic, outstretched. Post-uterine sac is rarely less than a body width long. Tail usually medium conoid, but may be longer and more attenuate. Terminus rounded. (Plate 2. C)

***Rotylenchulus* sp. Linford & Oliveira, 1940**

Small sized about 1mm or less long. Immature female found free in the soil, vermiform and dying C-shaped when head-relaxed. Head region rounded to conoid and continuous with body contour. The head skeleton is medium development, stylet moderately strong with a rounded basal knob.

Vulva usually posteriorly located (58-72%), genital tracts didelphic, each with a double flexure. The tail is conoid with a rounded terminus. Male is vermiform, free-living in soil. Head skeleton, stylet, and oesophagus reduced, but still conspicuous. Tail pointed, spicules curved, bursa not reaching the tail tip. Juveniles free-living in soil and resembling immature females in general respects but tail more rounded terminally. (Plate 2. D)

***Tylenchus* sp. Bastian, 1865**

Body length is small to medium-sized (0.4-1.3mm), ventrally curve upon relaxation. The cephalic region is continuous, annulated; framework with light or no sclerotization. The stylet is small with posterior sloping basal knobs. The median oesophageal bulb is oval, muscular anterior to middle of oesophagus; basal bulb pyriform.

In the female, the vulva is a transverse slit, usually at 60-70% of body length. The anterior ovary is outstretched. The posterior uterine branch is short and rudimentary. Spermatheca round to oval, offset. Tail elongate and filiform, ventrally arcuate regularly tapering to a pointed or minutely rounded terminus. Male is not found. (Plate 2. E)

***Pratylenchus* sp. Filipjev, 1936**

They are small nematodes (less than 1mm long) dying slightly curved ventrally on the application of gentle heat. The head region is low, flattened, usually appearing as a flat, and black cap under the stereomicroscope. Lip region divided into 2, 3, or 4 annules and continuous with the body contour; strongly cuticularized. Stylet knobs are rounded or anteriorly concave. Median bulb is well developed; oesophageal gland lobes overlapping the intestine ventrally.

In the female: vulva is well posterior, usually at 70-80% of body length; the genital system with a single anteriorly directed tract. Tail sub-cylindrical conoid with a broad to narrowly rounded, terminus. (Plate 2. F)

***Helicotylenchus* sp. Steiner, 1945**

Body length is small to medium-sized nematodes (0.4-1.2mm) long usually dying in a spiral (rarely C-shaped) on heat relaxation. The labial region is conoid-rounded rarely truncate, sclerotization moderate. Stylet is well developed, usually 3-4 times the lip region diameter in length and with rounded or cup-shaped knobs.

In the female, the vulva is posterior (60-70%) of the body length, both genital tracts usually fully developed, posterior branch rarely reduced. The tail is short, usually dorsally convex-conoid or hemispherical. A terminal projection or mucron may be present. (Plate 2. G)

***Tylenchorhynchus* sp. Cobb, 1913**

It is small nematodes (0.7-1 mm long), dying more or less straight or slightly curved ventrally on the application of gentle heat. No marked sexual dimorphism in form of the anterior region. Labial region rounded, continuous with body contour. The stylet is slender, 15-30µm long, with rounded, backwardly sloping, knobs. Oesophagus is equally developed; median bulb fusiform, moderately developed; oesophageal glands are elongated and overlapping to the intestine ventrally.

In the female, the vulva median (50-59%) of body length with two equally developed genital tracts; one directed anteriorly, one posteriorly. Spermatheca rounded. The tail is about (37-48 µm) long, conoid with a blunt tip. The Tails of males are not found. (Plate 2. H)



A. *Aphelenchus* sp. (100X)



B. *Aphelenchoides* sp. (100X)



C. *Bursaphelenchus* sp. (100X)



D. *Rotylenchulus* sp. (100X)

E. *Tylenchus* sp. (100X)F. *Pratylenchus* sp. (100X)G. *Helicotylenchus* sp. (100X)H. *Tylenchorhynchus* sp. (100X)

Plate 2. Recorded genera of plant- parasitic nematodes

Distinctive characters of free-living nematodes

Rhabditis sp. Dujardin, 1845

The body is 0.45 to 0.7mm long, the cuticle is smooth or striated. Head usually distinct but sometimes not conspicuously offset; lips generally present, originally six in number, each with three papillae; labial or interlabial bristles sometimes present. the stoma is tubular. Oesophagus is made up of corpus, isthmus, and posterior bulb, latter containing valve apparatus.

In the female, the vulva is in majority of species almost equatorial in position; gonads paired, opposed and reflexed; some species, however, with single prodlphic gonad and posterior vulva. The tail is blunt or tapering. Male not found. (Plate 3. A)

Cephalabuss sp. Bastain, 1865

The body is usually between 0.6 to 1mm long. Lips are low, rounded, obscurely duplex, median lip asymmetrical. The oesophagus is cylindrical except in the basal fifth where it tapers to the isthmus from which it is spread only by a break in the musculature.

Female gonad is single, prodelphic, ovary with double flexure in posterior part and spermatheca at the anterior bend. Vulva position is situated at 60-63% of the body length. The female tail is generally uniformly conoid to the blunt terminus. (Plate 3. B)

***Dorylaimus* sp. Dujardin, 1845**

It is usually large species, body is over 1 mm and up to 7 mm long; the cuticle always thick. Head narrower than the body, not, or only slightly, offset; lips moderately developed, bearing an inner circle of 6 papillae. Spear strong and well developed; 'guiding ring' usually appearing double. Amphids broad, shield-shaped with slit-like apertures, located close to the base of lateral lips. In the female, the vulva is well developed usually at 44% of the body length, longitudinal in one species but no data available about the others: gonads paired.

In the male, testes are paired, outstretched from the proximal end of vas deferens. The tail is short, broad, and rounded. It is free-living nematodes. (Plate 3. C)

***Tripyla* sp. Bastian, 1865**

The body is usually between 1.3 to 2.05 mm long. The cuticle is transverse striations. Head not offset, composed of 3 large, flatly rounded lips, stoma simple, only recognizable by the hyaline lining, a tooth present in the dorsal wall. Oesophagus almost cylindrical but slightly swollen behind.

In the female, the vulva almost equatorial; gonad paired, opposed, and reflexed. In the male, testes paired and opposed but emptying into a common vas deferens. A pair of ejaculatory glands is one on either side. Spicules paired, caudal glands present. (Plate .3 D)

***Ironus* sp. Basaian, 1865**

The body is slender, cuticle without striations, setae, or lateral alae. Head offset by shallow constriction with 3 moveable lips each with a small apical papilla and 4 submedian setae. Amphid is cup-like at the level of head setae, apertures slit-like. Stoma long, tubular, with 3 anterior eversible hook-like teeth. Oesophagus is almost cylindrical but a little wider behind than in front.

In the female, the vulva equatorial; gonads paired, opposed, and reflexed. In the male, spicules are paired, arcuate, strong, with a central strengthening piece. The tail is tapering to a fine point in both sexes. (Plate 3. E)



A. *Rhabditis* sp. (100X)

B. *Cephalobus* sp. (100X)C. *Dorylaimus* sp. (100X)D. *Tripyla* sp. (100X)E. *Ironus* sp. (100X)

Plate 3. Recorded genera of free-living nematodes

Table 2. Monthly occurrence of soil nematodes during study period

Sr. No	Nematodes	June	July	August	September	October
1.	<i>Aphelenchus</i> sp.	√	√	√	√	√
2.	<i>Aphelenchoides</i> sp.	X	√	√	√	√
3.	<i>Bursaphelenchus</i> sp.	X	X	√	√	√
4.	<i>Rotylenchulus</i> sp.	√	√	√	√	√
5.	<i>Tylenchus</i> sp.	√	√	X	√	√
6.	<i>Pratylenchus</i> sp.	X	√	√	√	X
7.	<i>Helicotylenchus</i> sp.	√	√	√	√	√
8.	<i>Tylenchorhynchus</i> sp.	X	X	√	√	√
9.	<i>Rhabditis</i> sp.	√	X	X	√	√
10.	<i>Cephalobus</i> sp.	X	√	√	√	X
11.	<i>Dorylaimus</i> sp.	√	√	√	√	√
12.	<i>Tripyla</i> sp.	X	√	√	√	√
13.	<i>Ironus</i> sp.	X	√	√	√	√
	Total	6	10	11	13	11

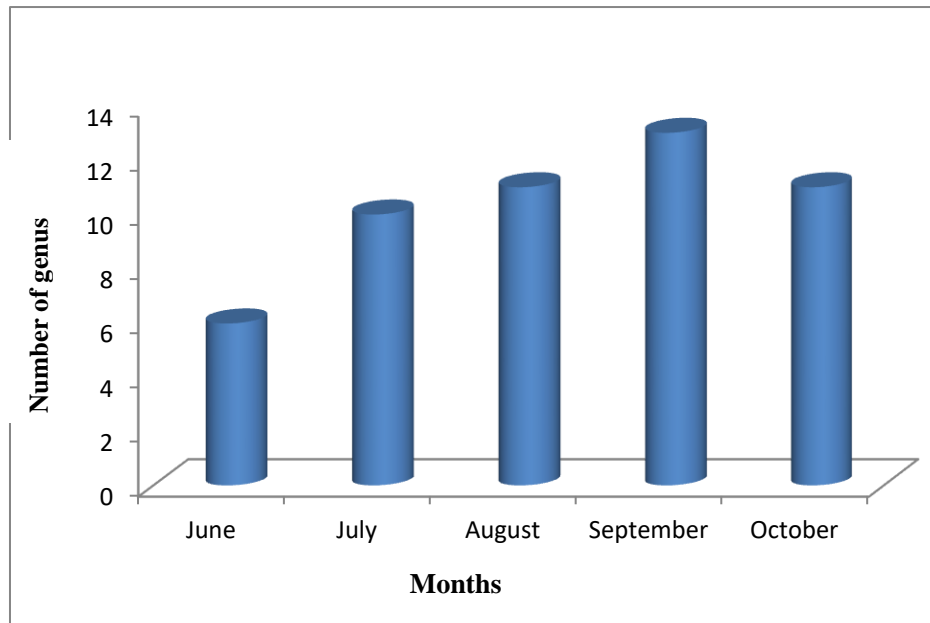


Figure 2. Monthly recorded number of soil nematodes genera from the study site

Table 3. Genus composition percentage of recorded orders

Sr. No	Order	No. of Family	No. of Genus	% of Genus composition
1.	Aphelenchida	3	3	23.08
2.	Tylenchida	4	5	38.46
3.	Rhabditida	2	2	15.38
4.	Enoplida	2	3	23.08
	Total	11	13	100%

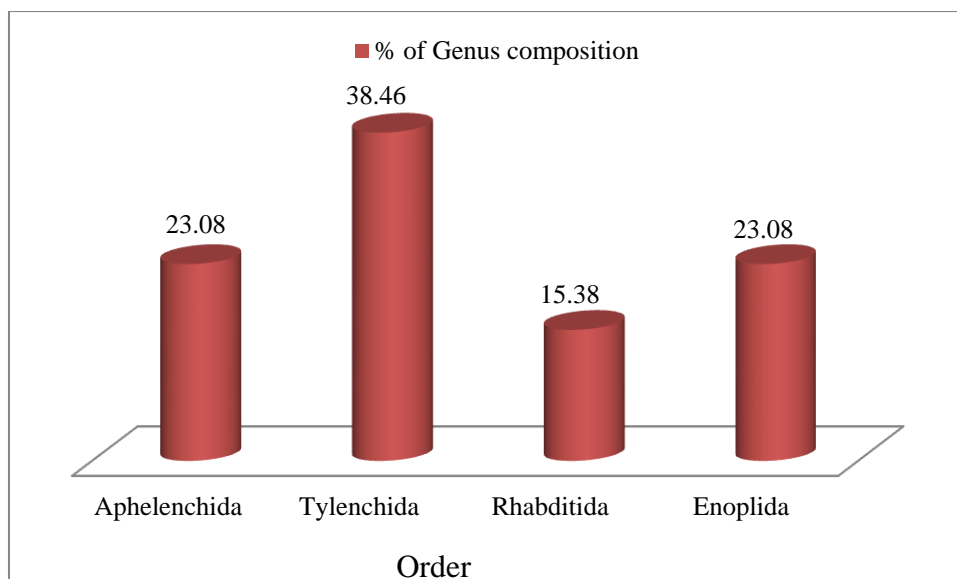


Figure 3. Genus composition percentage belonging to recorded orders

DISCUSSION

In the present study, eight genera of plant-parasitic nematodes were recorded from the *Chrysanthemum* leaves and cultivated soil. Among them, *Aphelenchus* sp. and *Aphelenchoides* sp. were recorded from *Chrysanthemum* leaves and soil of Kyaing Tong Township. *Bursaphelenchus* sp. was found on only leaves of *Chrysanthemum*.

According to CABI, 2007, *Chrysanthemum* leaf nematode is the *Aphelenchoides ritzemabosi*. It is a major pest. The conspicuous characters of it are the tail terminal, which has two to four-minute mucron, thus giving it a point brush-like appearance. This tail terminus is dissimilar to the recent finding of *Aphelenchoides* sp. tail, which has one-minute mucron.

Although *Aphelenchus* sp. and *Aphelenchoides* sp. were recorded not only from *Chrysanthemum* leaves but also from the soil, actually they were not leaf nematode.

This finding suggested that as they were mycetophagous, they swim up from the soil to leaf for their food such as fungi. CABI, 2007 was in line with this suggestion. *Chrysanthemums* are subjected to two vascular wilt diseases caused by *Fusarium* and numerous fungal diseases are recorded from it.

CABI, 1993 also assisted the present suggestion that *Aphelenchus* sp. is primarily mycetophagous and commonly in soil as well as in leaf sheaths, plant crowns, and the cortex of same roots, especially if they are infected with fungi. They swim up the film of water on the plants, created by spring rains and enter leaves through the stoma ([http//](http://))

Wingfield, 1983, further provided that, *Bursaphelenchus* in common with most of the bud and leaf nematodes of the genus *Aphelenchoides* can be either mycetophagous or phytoparasitic.

Plant-parasitic nematodes, *Pratylenchus* sp., *Rotylenchulus* sp., *Tylenchus* sp., *Tylenchorhynchus* sp. and *Helicotylenchus* sp. were also recorded from the soil. CABI, 2007 provided this finding that *Chrysanthemum* is the major host of *Pratylenchus penetrans* and a minor host of other nematodes.

CONCLUSION

In the present study, eight genera of plant-parasitic nematodes belonging to two orders, seven families were recorded from the study site from June to October in 2018. Among them, three genera were recorded from *Chrysanthemum* leaves and others were from the soil. Five free-living genera were also recorded from the soil.

In a recent study, *Chrysanthemum* leaves and soil from the Kyaing Tong Township were infected with fungal diseases, and two kinds of nematodes, such as *Bursaphelenchus* sp. and *Pratylenchus* sp. were major infections to *Chrysanthemum* plants.

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