

NEMATODES ASSOCIATED WITH EUCALYPTUS SEEDLINGS  
IN PAKISTAN

by

BASHIR HUSSAIN SHAH\*  
M. ISMAIL CHAUDHRY\*

## ABSTRACT

Fifteen genera of plant parasitic nematodes belonging to the Order 'Tylenchida' were found in the soil and roots of 'Eucalyptus' saplings in Silvicultural research plots Pakistan Forest Institute, Peshawar, Forest Plantations at Jallo, Chichawatni and Changa Manga, and in Eucalyptus plantation in Watershed Management Project area at Garhi Habibullah, Distt. Hazara. Nine genera of ectoparasitic nematodes and some free living nematodes were also recovered. The percentage of the plant parasitic nematodes of the genera, 'Rotylenchus', 'Paratylenchus', 'Pratylenchus', 'Helicotylenchus' and 'Tylenchorhynchus' was quite high amongst the parasitic nematodes extracted from the soil and root samples of Eucalyptus saplings at Chichawatni and Garhi Habibullah. Other plant parasitic nematodes belonging to the Order 'Tylenchida' infesting Eucalyptus roots at previous places were, 'Ditylenchus', 'Criconemoides', 'Aphelenchus', 'Aphelenchoides', 'Rotylenchulus', 'Psilenchus', 'Tylenchus', 'Paurodontus', 'Boleodorus', and 'Nothotylenchus'. The ectoparasitic genera belonging to the Order 'Dorylaimida' were, 'Longidorus', 'Nygolaimus', 'Labronema', 'Dorylaimus', 'Discolaimus', 'Discolaimium', 'Tylencholaimus', 'Aulolaimus' and 'Carcharolaimus' and many free living nematodes belonging to the Order 'Rhabditida'.

## Introduction

Among the fast growing forest tree species *Eucalyptus* is outstanding and is being planted more or less in all the irrigated forest plantations throughout Pakistan. Its use in paper pulp industry is well known. Keeping in view its importance and to have a healthy growth of this tree species, it is necessary to study its pests and diseases and to devise control measures against them. Studies on insect pests and fungal diseases of forest nurseries including *Eucalyptus* nurseries have been carried out in Pakistan and in other countries

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\*The authors are Systematic Entomologist and Forest Entomologist respectively at the Pakistan Forest Institute, Peshawar.

of the world. Very little attention is paid to the hidden enemies, like the plant parasitic nematodes which are possibly injurious to *Eucalyptus* and other forest nurseries in Pakistan.

A high mortality of the newly planted seedlings of *Eucalyptus* was reported in Chichawatni Plantation, district Sahiwal by the Divisional Forest Officer. The same problem was also reported from Garhi-Habibullah, District Hazara by the Project Director, Watershed Management Project, Kaghan Valley.

Experiments were conducted to check the attack of termites or other insect pests at Chichawatni. Although in the treated plots very little attack of termites occurred, yet there was a heavy mortality of plants. In order to find out the other factors responsible for the death of *Eucalyptus*, at Chichawatni as well as at other localities was taken up. The results of this preliminary study about the number and kinds of nematodes found are given.

### Materials and Methods

In the second week of July, 1967 we visited *Eucalyptus* plantation at Chichawatni, District Sahiwal. In the last week of December, 1967 we surveyed *Eucalyptus* plantation at Jallo Experimental form and Changa Manga forest plantation in Lahore District. *Eucalyptus* plants in the Silvicultural Research plots, Pakistan Forest Institute, Peshawar were surveyed during August, 1967 and *Eucalyptus* plantation in Watershed Project area at Garhi Habibullah, District Hazara during May, 1969. Diseased and healthy plants were selected before taking the soil and root samples. Soil and root samples were taken from the rhizosphere of the selected plants at a depth of about 15-20 cm. The soil and root samples were enclosed in moist muslin cloth and placed in polythene bags and brought to the laboratory for processing. The root samples were washed gently and stained in cotton blue lactophenol (Franklin and Goodey, 1949). The extraction of nematodes from the soil samples was carried out in the laboratory.

The method used to recover nematodes from the soil samples was an adaptation of the modification of the method of Cobb's sieving and Baerman's funnel technique. The samples were decanted through a sieve with large meshes to remove stones and other large particles of sand, etc., from the soil. The soil from the sieve was poured on-to muslin cloth and placed in Baerman's funnel containing water. After twenty-four hours the nematodes were removed from the base of the funnel with water. The nematodes were killed by gentle heating and fixed in F.A.A. fixing solution. After picking up the nematodes from fixing solution they were put in 2% glycerine solution for slow dehydration. Complete dehydration of nematodes was made in dessicators. Nematodes were mounted in anhydrous glycerine. The rimming of cover slips was made by ZUT. The diagrams of some genera were drawn with the help of camera lucida.



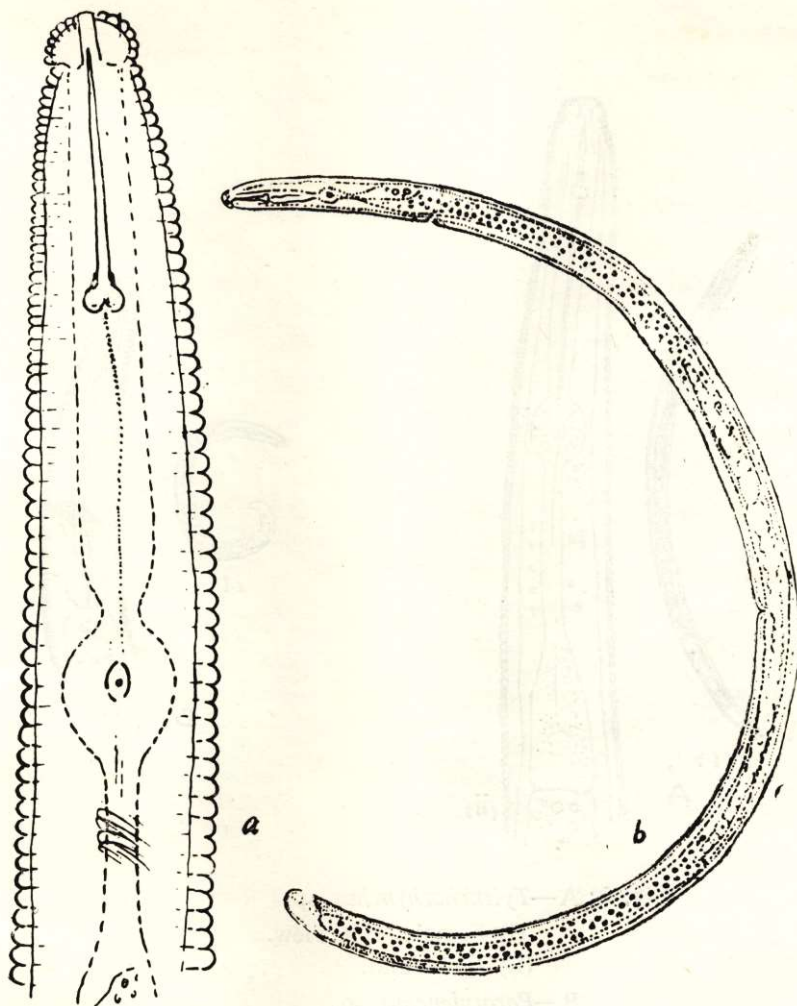


Fig. 1: *Rotylenchus* sp. (a) Head end,  
(b) Female, side view.

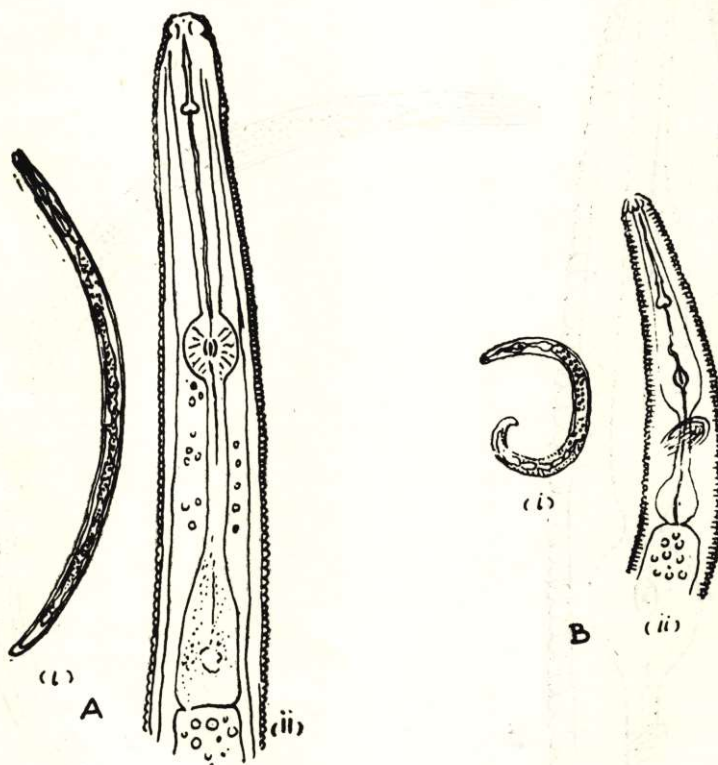


Fig. 2: A—*Tylenchorhynchus* sp.  
 (i) Female, side view.  
 (ii) Head end.  
 B—*Paratylenchus* sp.  
 (i) Female, side view.  
 (ii) Head end.



## Result and Discussion

The survey revealed the presence of twenty-four genera of plant parasitic nematodes besides about six genera of saprophytic nematodes. All the nematodes collected from the five *Eucalyptus* plantations belong to four main groups of nematodes viz., TYLENCHIDA, DORYLAIMIDA, RHABDITIDA and CHROMADORIDA. They were accordingly separated, counted and were found to occur in the proportion at different *Eucalyptus* nurseries in West Pakistan as shown in Table I. The distribution of genera at different localities is shown in Table II.

The most prevalent group of nematodes found in all the nurseries except at Chichawatni and Garhi Habibullah were bacterial feeding forms i.e. non-stylet-bearing nematodes. These nematodes belong to the Order RHABDITIDA. They are generally free-living soil nematodes and do not injure the plant roots directly. This group was represented by genera *Acrobeloides*, (Cobb: 1924) Steiner and Buhner, 1933, *Rhabditis* Dujardin, 1845, *Acrobeles* Linstow, 1877, *Macrolaimus* Maupas, 1900 and *Panagrolaimus* Fuch, 1930 and some other genera which in abundance as compared to other genera.

Another group of free living saprophytic nematodes belonging to Order CHROMADORIDA was recorded from the *Eucalyptus* plantations at Chichawatni, Changa Manga and Jallo. The most common genus among this group was *Plectus* sp. Bastain, 1865.

The nematodes belonging to the Order TYLENCHIDA and DORYLAIMIDA are mostly plant parasitic and they are responsible for injury to the plant roots. The plant parasitic nematodes recovered from *Eucalyptus* nurseries belonging to Order TYLENCHIDA are *Rotylenchus* (Fig. 1) Filipjev, 1934, *Paratylenchus* Micoletzky (Fig 2-B) 1922, *Pratylenchus* Filipjev, 1934, (Fig 3-A) *Tylenchovhynchus* Cobb, 1913, (Fig 2-A) *Helicotylenchus*, Steiner, 1945, (Fig 4-A), *Ditylenchus* Filipjev, 1934, (Fig 3-B), *Criconeimoides* Taylor, 1936, *Aphelenchoides* Fischer, 1894, (Fig 5-A), *Tylenchus* Bastian, 1865, (Fig 4-B), *Psilenchus* deMan, 1921, (Fig 5-B), *Aphelenchus* Bastian, 1865, *Rotylenchulus* Linford and Oliveiray, 1940, *Paurodontus* Thorne, 1941, *Boleodorus* Thorne, 1941, and *Nothotylenchus* Thorne, 1941.

Among the above genera *Paratylenchus* (Pin nematode) was found to be wide spread in *Eucalyptus* plantation at Chichawatni, Changa Manga and Garhi-Habibullah. It constituted one third of the plant parasitic nematodes belonging to Order TYLENCHIDA recovered from Chichawatni. *Paratylenchus* lives in close association with the roots, amongst the root hairs, feeding intermittently by inserting mouth spear into the roots. It may also enter the root tissue. Steiner (1924) reported it from surface lesions on the roots of *Zinnia elegans* and Cobb (1923) from the roots of *Umbellularia californica*. Goodey I, found it in the roots of *Agrostis stolonifera*. Species of *Paratylenchus* have been found browsing on pine apple roots in Oahu, Hawaii. Sutherland (1965) reported *Paratylenchus* amongst the roots of coniferous forest nursery seedlings in Quebec. In India it is reported by Prasad et al (1963) to be associated with banana.



TABLE I  
NEMATODES ASSOCIATED WITH EUCALYPTUS IN 5 EUCALYPTUS, PLANTATIONS IN PAKISTAN

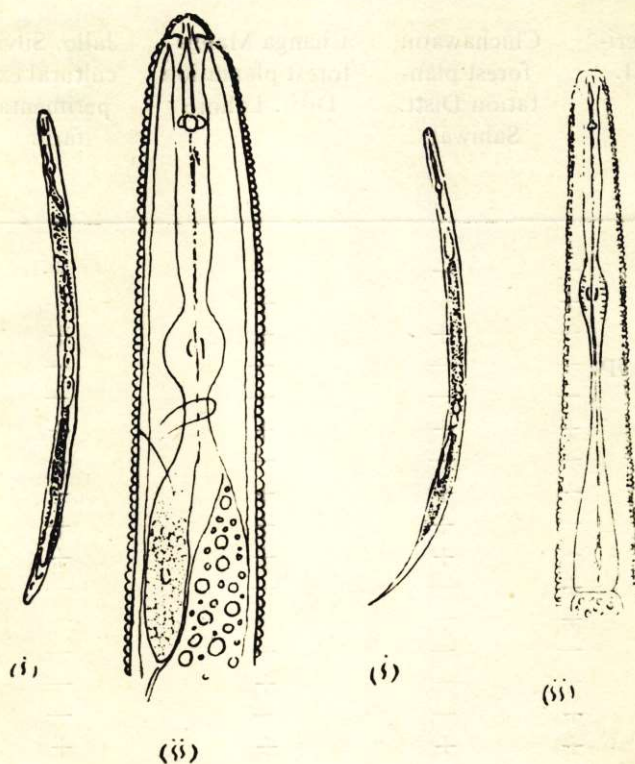
LOCALITY	Total No. of Nematodes recovered	Tylenchida		Dorylaeimida		Rhabditida		Chromadorida	
		No. of specimens	Percent- age	No. of specimens	Percent- age	No. of specimens	Percent- age	No. of specimens	Percent- age
1. Silvicultural farm P.F.I. Peshawar.	280	33	11 %	117	41 %	135	48 %	—	0 %
2. Chichawatni forest plantation Distt: Sahiwal.	720	320	45 %	175	24 %	195	27 %	27	3 %
3. Changa Manga Forest Plantation Distt: Lahore.	336	79	24 %	13	4.5 %	244	73 %	—	0 %
4. Jallo Silvicultural ex- perimental farm. Distt: Lahore	85	20	23.5 %	2	2.3 %	62	73 %	1	1.2 %
5. Watershed Project area, Garhi-Habibullah, Distt: Hazara.	210	141	66.6 %	27	13 %	39	18.5 %	3	1.4 %

TABLE II

## The Distribution of Genera by Sites

Silvicultural Experi- mental farm P.F.I. Peshawar	Chichawatni forest plan- tation Distt. Sahiwal	Changa Manga forest plantation Distt. Lahore	Jallo, Silvi- cultural ex- perimental farm	Watershed project area, Garhi- Habibullah Distt. Hazara
Rotylenchus	—	+	—	+
Paratylenchus	+	+	—	+
Pratylenchus	—	+	+	+
Tylenchorhynchus sp.	—	—	—	+
Aphelenchoides	—	—	—	+
Helicotylenchus	+	+	+	+
Rotylenchus	—	—	+	—
Tylenchus	+	+	+	+
Aphelenchus	—	+	+	+
Ditylenchus	—	+	+	+
Psilenchus	+	—	—	+
Criconemoides	+	—	—	—
Nothotylenchus	—	—	—	+
Paurodontus	—	—	+	+
Boleodorus	+	—	—	—
Longidorus	+	+	+	—
Nygolaimus	+	—	+	—
Doryllium	+	+	—	—
Labronema	+	+	—	—
Tylencholaimus	+	+	+	—
Aulolaimus	+	—	+	—
Dorylaimus	+	—	+	+
Carchaerolaimus	—	+	+	—
Acrobeles	+	+	+	+
Acrobeloides	+	+	+	—
Macrolaimus	+	—	+	+
Panagrolaimus	+	—	—	—
Mononchus	—	+	—	—
Plectus	—	+	+	—





A

B

Fig. 3: A—*Pratylenchus* sp.

(i) Female, side view.

(ii) Head end, magnified.

B—*Ditylenchus* sp.

(i) Female, side view.

(ii) Head end, magnified.



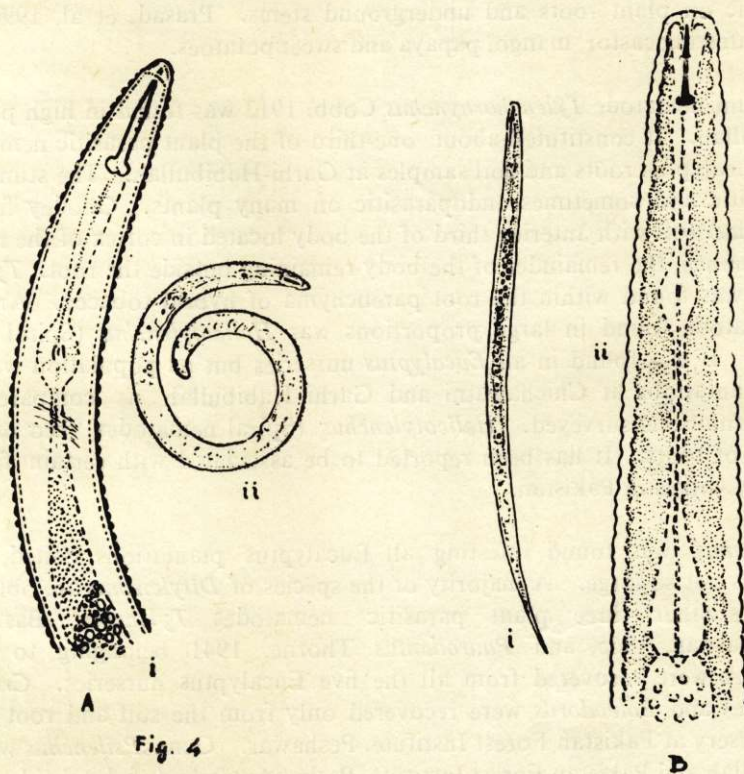


Fig. 4

Fig. 4: A—*Helicotylenchus* sp.

(i) Head end.

(ii) Female, side view.

B—*Tylenchus* sp.

(i) Female, side view.

(ii) Head end.



The second group of parasitic nematodes found in large numbers infesting all the *Eucalyptus* plantations was *Pratylenchus* Filipjev, (1934). All species of *Pratylenchus* (Root lesion nematodes) are root parasites, except two species which have been found in mahogany wood and potato tubers. *Pratylenchus* was reported to be infesting coniferous nurseries in Quebec by Jack R. Sutherland in 1965. *Rotylenchus* Filipjev, 1934, is the 3rd plant parasitic nematodes which was found in high population at Chichawatni and Garhi-Habibullah. All the species of *Rotylenchus* are wholly or intermittently parasitic on plant roots and underground stems. Prasad et al, 1964 (Fig 4) was found in high population at Chichawatni and Garhi Habibullah. All the species of *Rotylenchus* are wholly or intermittently parasitic on plant roots and underground stems. Prasad, et al, 19964 reported *Rotylenchus* infesting castor, mango, papaya and sweet potatoes.

The stunt nematode *Tylenchorhynchus* Cobb, 1913 was found in high population at Garhi-Habibullah. It constituted about one-third of the plant parasitic nematodes recovered from *Eucalyptus* roots and soil samples at Garhi-Habibullah. The stunt nematodes are ectoparasitic and sometimes endoparasitic on many plants. Goodey found *Tylenchorhynchus macrurus* with anterior third of the body located in cortex of the roots of oats and *Lolium perenne*, the remainder of the body remaining outside the root. *Tylenchorhynchus claytoni* was found within the root parenchyma of hybrid tobacco. Another plant parasitic nematode found in large proportions was *Helicotylenchus* (Spiral nematodes) Steiner, 1945. It was found in all *Eucalyptus* nurseries but its population was higher in *Eucalyptus* plantations at Chichawatni and Garhi Habibullah as compared to other *Eucalyptus* plantations surveyed. *Helicotylenchus* (Spiral nematodes) feed on parenchymatous tissue of roots. It has been reported to be associated with certain fruit trees in India and with Citrus in Pakistan.

*Ditylenchus* was found infesting all *Eucalyptus* plantations visited, however its population was not so large. A majority of the species of *Ditylenchus* are obligate plant parasites. The other three plant parasitic nematodes *Tylenchus*, Bastian, 1865, *Aphelenchus* Bastian, 1865, and *Paurodontus* Thorne, 1941 belonging to the Order TYLENCHIDA were recovered from all the five *Eucalyptus* nurseries. *Criconeimoides* (ring nematode) and *Boleodorus* were recovered only from the soil and root samples of *Eucalyptus* nursery at Pakistan Forest Institute, Peshawar. Genus *Psilenchus* was found at Garhi-Habibullah and Pakistan Forest Institute, Peshawar while *Aphelenchoides* and *Nothotylenchus* were found in the soil at Garhi-Habibullah. *Criconeimoides* (ring nematodes) are ectoparasitic on many plants. Only two specimens of this genus were recorded.

Amongst the collection of ectoparasitic nematodes belonging to the Order, DORYLAIMIDA, genus *Longidorus* Micoletzky, 1922 was observed to be widely spread, infesting all the four *Eucalyptus* nurseries, except at Garhi-Habibullah. *Longidorus* is ectoparasitic and is always associated with the plant roots. Prasad (1964) found it in association with Citrus, grape, guava and tobacco in India. *Dorylaimus* Dujardin, 1845 was most abundant in *Eucalyptus* nursery at Peshawar. *Tylencholaimus* deMan, 1876, *Nygolaimus* Loos, 1949, *Doryllium* Cobb, 1920, *Discolaimus* Cobb, 1930, *Labronema* Thorne,



1939, *Discolaimium* Thorne, 1939 *Aulolaimodes* Micoletzky, 1914 and *Carcharolaimus* Thorne, 1939 were the other nematodes belonging to the Order DORYLAIMIDA which were found associated with all the *Eucalyptus* nurseries. Some species of *Labronema*, *Nygolaimus*, *Dorylaimus*, *Discolaimus* and *Discolaimium* are predatory in habit, feeding on small nematodes and other organisms.

Besides the above, nematodes of the genus *Mononchus* Bastian, 1865 were also found in *Eucalyptus* plantation at Chichawatni. Species of this genus are carnivorous, feeding on Protozoa, nematodes, rotifers, tardigrades and small oligochaetes. Members of this genus thus play a part in maintaining the microbiological balance in the soil.

It may be seen from Table 1 that the population of parasitic nematodes belonging to the Order TYLENCHIDA was the largest in *Eucalyptus* plantations at Garhi-Habibullah and Chichawatni as compared to their populations in the other three plantations. The percentage of the plant parasitic nematodes belonging to the Order TYLENCHIDA in *Eucalyptus* nurseries at Garhi-Habibullah, Chichawatni, Jallo, Changa-Manga and at Pakistan Forest Institute, Peshawar was 66%, 45%, 25.5%, 24%, 11%, respectively. The most injurious forms like *Helicotylenchus* (spiral nematodes), *Rotylenchus*, *Pratylenchus* (root lesion nematodes), *Paratylenchus* (pin nematodes) and *Tylenchorhynchus* (stunt nematode) were found in large numbers infesting *Eucalyptus* roots at Chichawatni and Garhi-Habibullah.

As a matter of fact there was no trouble with *Eucalyptus* at Changa Manga and Pakistan Forest Institute, Peshawar. The problem of death and decay of the *Eucalyptus* plant was more serious in Watershed Project area Garhi-Habibullah and at Chichawatni forest plantation in Block No. 2 compartment No. 4, compartment No. 49 B and in Block No. 5 compartment No. 142 A, at these places it was observed that a majority of the *Eucalyptus* plants die within a month or two after planting. It is suspected that when the *Eucalyptus* nursery seedlings are planted in these compartments, the plant parasitic nematodes attack the root hairs and roots of *Eucalyptus* at a stage when the seedlings are not fully established. So these seedlings cannot survive the injury done by these pathogenic nematodes and start decaying. First indication of injury in such infestations is reduction in terminal growth, followed by general appearance of reduced vigour, accompanied by yellowing and dying of the leaves and twigs. When plants become weak other pathogenic organisms like termites may attack and hasten the death of the plants. Many pathogenic fungi also invade the plant roots through the injuries made by parasitic nematodes and thereby bring about the death of the plants.

However, further research is needed to establish whether the death of *Eucalyptus* seedlings is primarily due to nematodes or due to some other factors.



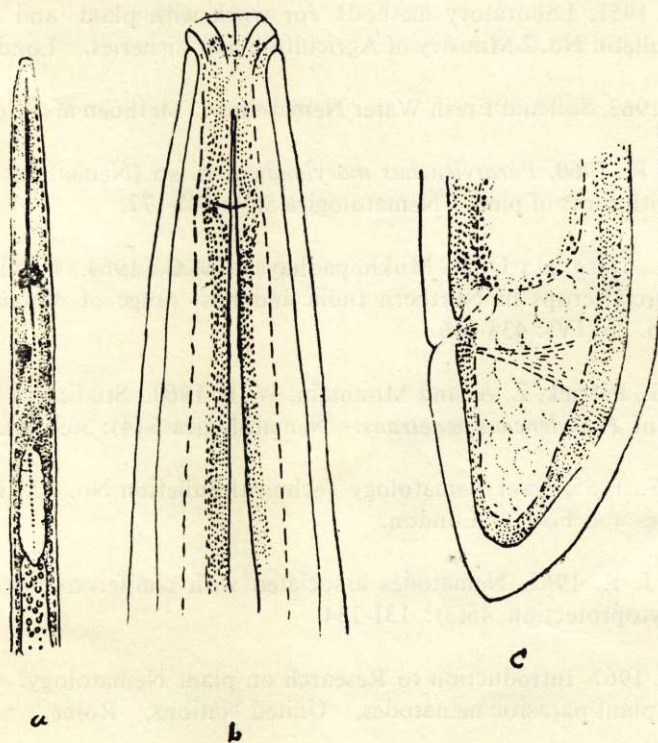


Fig. 6: *Longidorus* sp. (a) Anterior region, side view. (b) Head end, side view. (c) Tail end, side view.



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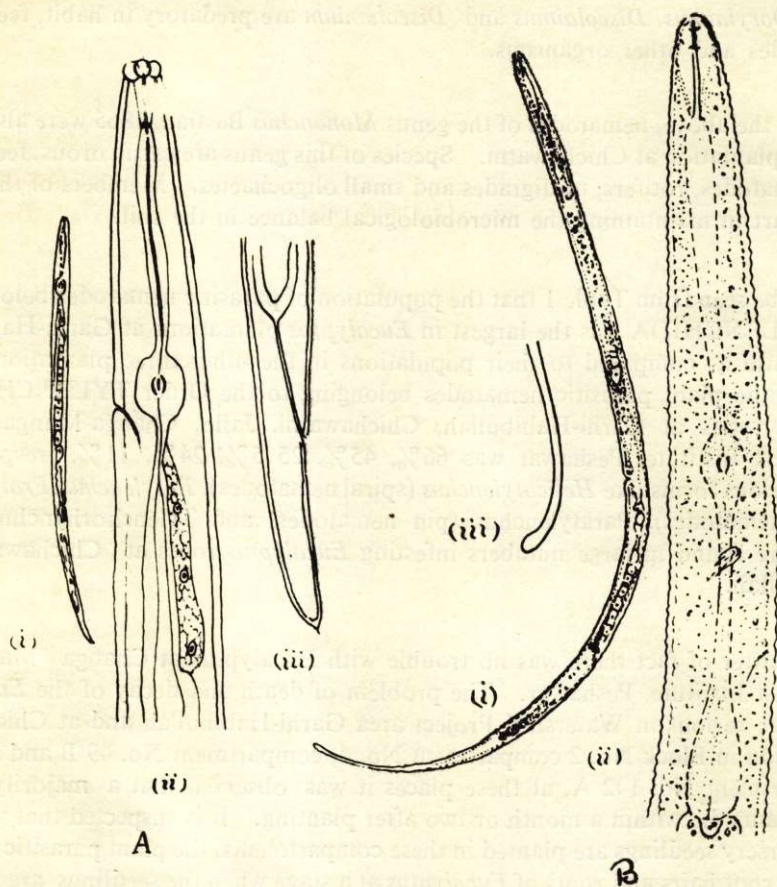


Fig. 5: A—*Aphelenchoides* sp.

- (i) Female, side view.
- (ii) Head end, magnified.
- (iii) Tail end magnified.

B—*Psilenchus* sp.

- (i) Female, side view.
- (ii) Head end, magnified.
- (iii) Tail end magnified.