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DISTRIBUTION OF PLANT PARASITIC NEMATODES ASSOCIATED WITH TREE CROP NURSERIES

P.G.Kavitha, P.S.Devanand K.Kumaran and K.K.Suresh

Department of Tree Breeding, Forest College and Research Institute, Mettupalayam

ABSTRACT: A study was carried out for plant parasitic nematodes occurring on three economical tree crops such as *Melia dubia*, *Bixa orellana*, and *Pongamia pinnata*. In the survey seedlings of each tree species were selected randomly from the nurseries. Soil and root samples were collected from the seedlings. Soil samples were processed by Cobb's sieving and decanting method and recovery of nematodes by Modified Baermann's funnel method. Nematode community analysis was done in terms of density, frequency and prominence value of nematode. Results of the study revealed that the highest relative density of the Dorylaimid genera, *Longidorus* in *Pongamia pinnata* (56.26). Similarly the nematodes found in *Melia dubia* were *Longidorus* with relative density of about 58.27. In *Bixa orellana,* among several genera, *Pratylenchus* was in very large numbers with relative density of 48.97. The community analysis done in the *Pongamia pinnata* seedlings showed that the indices of *Longidorus* were higher (Frequency, density and prominence value - 22.8, 56.98 and 228.00) respectively when compared to the genera *Helicotylenchus. Melia dubia* in tree nurery crops showed that the indices of the nematodes of *Longidorus* were highest (Frequency, density and prominence value 21.6, 58.27 and 216.00 respectively). *Bixa orellana* nursery showed that the indices for the nematode, *Pratylenchus* were highest (Frequency, density and prominence value - 44.4, 48.97 and 444.00 respectively).
Key words: Community, Forest trees, Nurseries, Nematodes, *Melia dubia*, *Bixa orellana*, and *Pongamia pinnata*

*Corresponding author: Kavitha Govindasamy. Department of Tree Breeding, Forest College and Research Institute, Mettupalayam, India Email: kavitha_nem@yahoo.com

INTRODUCTION

The significance of forest resources in maintaining the well being of mankind has come in to limelight in the recent times. In realization of the present context there has been a paradigm shift towards a forestation and reforestation, especially with native tree species that has high ecological, social and economic values. This requires the production of quality seedlings in mass quantities. The seedlings should be healthy and free from any pests and diseases to ensure a very negligible casualty in the field upon which the success of a forestation programme depends. Many studies have been conducted on the nursery and field pests and diseases that occur on forest crops and their management as well. However, there exists a large lacuna of negligence of parasitic nematodes that affect the forest trees in India except for a very few and scattered studies.

Since nematodes cause serious damage to citrus, peaches, walnuts, cherries, almonds, and many other horticultural important trees, it is not surprising that they also damage forest trees. Yet nematode diseases of forest trees remain virtually unknown. The presence of plant-parasitic nematodes and their importance as pest of agricultural, medicinal and ornamental crops have long been recognized. Disregard of rhizosphere ecology by forest scientists probably accounts for past failures to recognize nematodes as important soil and site factors in forestry. Forest soils are one of the largest reservoirs of nematode fauna.

Damages plant parasitic nematodes are usually on root and above ground plant parts either by sucking cell sap, modifying the cells for their development and multiplication, or through inter and intracellular movement in plant system. These parasites are recognized as causal agents for heavy loss in forest nurseries, plantations and natural forest stands by way of loss of transplanted seedling which lead to the problem of replanting often. The evidences of Pathogenicity are lacking for majority of nematodes recorded as host of forest plantations and natural forest stands.

The seeding of acacia infested by *Tylenchorhychus claytoni* showing intensive browning of leaves, seedling of yellow poplar, (*Liriodendron tulipifera*) and eucalyptus spp, by *Pratylenchus brachyurus* showing root lesions and fissures could not get good price in the market. Similarly, *Ficus elastica* in the open fields in southern Florida caused discoloured lesions in the intraveinal leaf tissue due to *Aphelenchoides* spp infestation and rendered the plants unsaleable by making their distinct foliage unattractive [1].

Plant parasitic nematodes like *Aphelenchoides, Helicotylenchus, Hemicriconemoides, Hoplolaimus, Longidorus, Paralongidorus, Pratylenchus, Xiphinema, Rotylenchulus, Rotylenchus, Siddiqia, Psilenchus, Trichodorus* and *Tylenchorhynchus* have been reported to cause disease symptoms on tree species like *Populus ciliata, P. alba, Bombax ceiba, Tectona grandis, Pterocarpus santalinus, Cedrus deodara, Quercus leucotrichophora, Emblica officinalis, Shorea robusta, Barringtonia acutangula, Strychnos nux-vomica, Cassia fistula, Syzygium cumini, Terminalia alata* etc. from various parts of India by several workers like Swart [2], Koenning, [3], Knight [4] etc. Thus, it is evident that plant parasitic nematodes have a wider distribution and has a definite negative bearing on forest productivity.

Considering the importance and the lack of in depth knowledge of nematode pests in the forest tree crops, the present study was conducted to assess the nematode incidence by community analysis in nurseries of tree crops *viz*., *Pongamia pinnata, Melia dubia, and Bixa orellana.*

MATERIALS AND METHODS

Survey of plant parasitic nematodes in *Pongamia pinnata Melia tubia, and Bixa orellana* in tree nursery crops

Collection of soil samples

A detailed survey was conducted in the nursery during December 2015 in the Forest College Research Institute, Mettupalayam to study the association of plant parasitic nematodes and document their community structure in the seedlings of tree crops *viz*., *Melia dubia, Bixa orellana and Pongamia pinnata.* In this survey at least ten seedlings from each group of tree were selected. Soil and root samples of each seedling were collected. Thus a total of thirty soil samples were collected for assessing nematode population. During collection of soil and root samples, care was taken to have soil sample with moisture content of 60 per cent field capacity for better recovery of nematodes. Collected samples were stored in polythene bags of size 15cm x 10cm and tied with a rubber hand and labeled properly with a luggage label showing the location and recorded symptoms found on the tree. In the laboratory, soil & root samples were stored at 10° to 15°C in a refrigerator for further processing.

Description of the surveyed areas

Mettupalayam

It is located in Coimbatore district of Tamil Nadu just on the foothills of Nilgiris, situated 11.71° .N latitude and 76.5.3° E longitude and receives 1100 mm rainfall annually a majority being from South West Monsoon. The annual temperature varies from (14 to 32°C) m.msl.

Processing soil samples by Cobb's (1998) Sieving and Decanting methods

This method takes advantage of the difference in size and specific gravity between nematodes and other soil components. The equipments required for processing are, plastic basins of two to three litter capacity, a stirring stick about 45 cm long and 2.5 cm wide and test sieves of 20,100 and 350 for soil analysis. The sieves are about 20 cm in diameter. The coarse sieve had 20 meshes to remove large stones and plant debris and a fine sieve of 100 to 350 mesh sieves were used for the recovery of nematodes. Plastic beakers of capacity of 500 ml were used. The step-by- step procedure for processing of soil samples are as follows (Plate 1).

Two hundred g of soil sample was placed in a plastic basin and two to three liters of water was added. It was stirred well with a wooden stick until all the clods were broken up. Then, the soil suspension was allowed to remain still for a period of 30 seconds to one minute. The purpose of stirring was to separate the nematodes from the soil particles and then suspend them in water. The soil suspension was poured through a 20-mesh sieve and collected in the second basin, leaving the heavy pebbles and root debris retained in the 20-mesh sieve itself.

Again a liter of water was added, stirred well and passed through 100 mesh sieve and soil suspension was received in another basin. The process of adding water and stirring followed / passing through 200 and 350 mesh sieves was repeated. The nematode soil suspensions caught from 100, 200 and 350 mesh sieves were transferred to a 100 ml beaker and the nematodes were washed into a cup or bowl with about 25 ml of water separately and pooled together in a separate beaker. The material collected at the end of the procedure was further processed through modified Baermann's funnel method.

Recovery of nematodes by Modified Baermann's funnel method (Schindler, 1961)

A molded sieve of wire gauge over which one to two layers of paper tissue was trimmed down to the edges of gauge. The soil suspension collected from the fine sieve was filtered through the above wire gauge placed in a Petri dish with sufficient water to wet the Soil samples .The setup was allowed to remain for 48 hours without any disturbance (Plate 1). After 48 hours, the nematodes which were collected in the petri plates were poured into a 50 ml beaker and the number of nematodes was counted using stereoscopic binocular microscope.

Processing root samples

The root sample more thoroughly washed in running tap water finely chopped and thoroughly mixed. Five grams of these composite samples were stained in acid fuchsine and lacto phenol method, and the nematode population in root was estimated through maceration by using a kitchen blender. Nematodes collected from soil samples were killed in hot water and later fixed in 4% formaldehyde solution. Nematode population was estimated by using a stereoscope microscope. Plant parasitic nematodes were identified up to genus/species level by using standard monograph.

Community analysis of parasitic nematodes recorded in the survey

Community analysis plant parasitic nematodes collected under different locations was done in order to find out their Absolute Frequency (AF), Relative Frequency (RF), Absolute density (AD), Relative Density (RD) and Prominence Value (PV) by following the methods of Norton (1978).

Absolute frequency: Absolute frequency is a measure of distribution uniformity or rate of occurrence.

$$\textbf{Absolute frequency} = \frac{\text{Number of samples containing species}}{\text{Number of samples collected}} \times 100$$

Relative frequency: When absolute frequency is an independent calculation for rate of occurrence for each species, relative frequency gives the total frequency of all species on the basis of 100 per cent, which was calculated as

$$\textbf{Relative frequency} = \frac{\text{Absolute frequency of species}}{\text{Sum of frequency of all species}} \times 100$$

Absolute density: Absolute density was based on the number or density per unit volume or Weight of soil. Mean population per unit volume of soil was taken into consideration.

Relative density: Relative density is a measure in per cent to determine the number of species in a sample in relation to total number of nematodes of all the species encountered and was arrived as follows:

$$\textbf{Relative density} = \frac{\text{Number of individuals of species in a sample}}{\text{Total number of all individuals in a sample}} \times 100$$

Prominence value: The relation of population density and frequency was worked out and expressed as prominence value i.e.

Prominence value = Density x √Frequency

RESULTS

Three major economically important trees such as *Pongamia pinnata*, *Melia dubia* and *Bixa orellana* were analyzed for infestation of plant parasitic nematodes during the period from December, 2015 to February, 2016 in the nurseries of Forest College and Research Institute, Mettupalayam.

Frequency, density and prominence value of plant parasitic nematodes in nurseries *Pongamia pinnata, Melia dubia* and *Bixa orellana*

The survey for plant parasitic nematodes conducted in *Pongamia pinnata* nursery of FC & RI revealed that a broad range of plant parasitic nematodes were associated with tree seedlings. The samples contained ectoparasitic nematodes *viz*., *Helicotylenchus, and Longidorus* which were ectoparasitic in nature. The community analysis done in the *Pongamia pinnata* nursery showed that the indices *viz*., Frequency, density and prominence value 22.8, 56.98, 100, 50 and 228.00 respectively were highest when compared to the genera *Helicotylenchus* (Table :1, Fig. 1 and Plate 2)

In *Melia dubia* seedling we conducted plant parasitic nematode survey nursery in which we revealed broad range of plant parasitic nematode associated with tree nematode genus. The samples contained ectoparasitic nematodes *viz*., *Tylenchorhynchus, Longidorus* and *Xiphinema*. The community analysis done in the *Melia dubia* nurery crops showed that the indices of the nematode, *Longidorus* was highest with Frequency, density and prominence value 21.6, 58.27, 100, 41.66 and 216.00 respectively (Table 2, Fig 2). Among the two Dorylaimid genera namely *Longidorus and Xiphinema*, *Xiphinema* recorded the lower values of Frequency, density and prominence values *viz*., 24.64, 33.33 and 100.62.

The survey for nematodes conducted in *Bixa orellana* nursery of FC & RI revealed that broad range of plant parasitic nematode associated with tree nematode genus. The samples contained ectoparasitic nematodes *viz*., *Longidorus* and *Xiphinema*, semi endo parasitic nematode, *Rotylenchulus reniformis* and endoparasitic nematode *Pratylenchus*. The community analysis done in the *Bixa orellana* nursery showed that the indices for the nematode, *Pratylenchus* was highest (Frequency, density and prominence value - 44.4, 48.97, 100, 27.77 and 444.00 respectively) (Table 3, Fig 3) when compared to other three nematodes, *Longidorus*, *Xiphinema* and *Rotylenchus reniformis.*

Table 1: Frequency, Density and Prominence value of plant parasitic nematodes occurring in tree nursery crop of *Pongamia pinnata.*

Nematodes	Density		Frequency		Prominence value
	Absolute	Relative	Absolute	Relative	
Longidorus	22.8	56.26	100	50	228.00
Helicotylenchus	18.4	45.48	100	50	184.00

Table 2: Frequency, Density and Prominence value of plant parasitic nematodes occurring in tree nursery crop of *Melia dubia.*

Nematodes	Density		Frequency		Prominence value
	Absolute	Relative	Absolute	Relative	
Xiphinema	11.25	24.64	80	33.33	100.62
Tylenchorhynchus	11.25	36.66	60	25	87.142
Longidorus	21.6	58.27	100	41.66	216.00

Table 3: Frequency, Density and Prominence value of plant parasitic nematodes occurring in tree nursery crop of *Bixa orellana.*

Nematodes	Density		Frequency		Prominence value
	Absolute	Relative	Absolute	Relative	
Pratylenchus	44.4	48.97	100	27.77	444.00
Xiphinema	12.6	13.35	100	27.77	126.00
Rotylenchulus	17	38.47	60	16.66	137.68
Longidorus	23	23.21	100	27.77	230.00

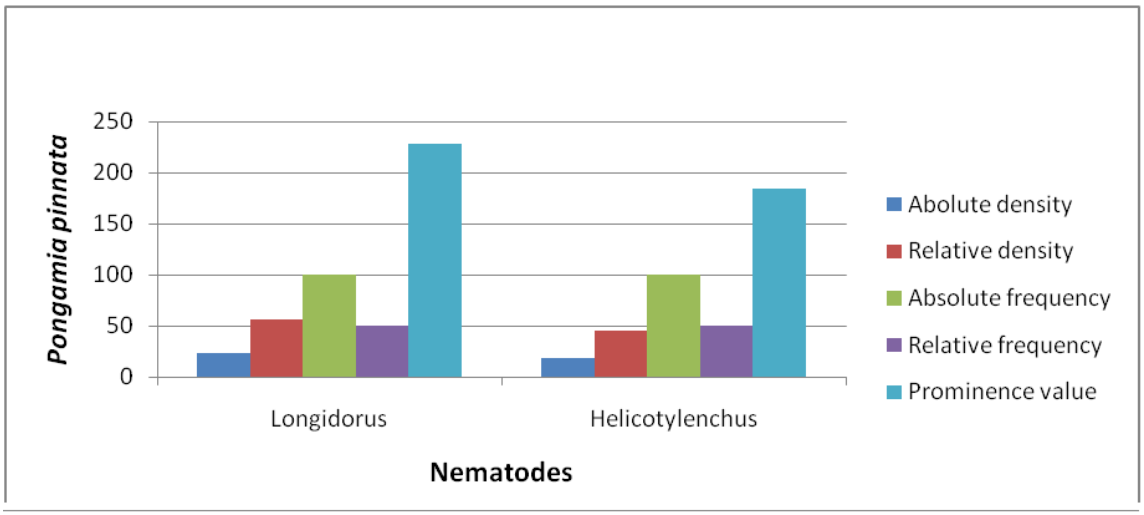

Fig-1: Frequency, density and prominence value of plant parasitic nematodes in nursery of *Pongamia pinnata*

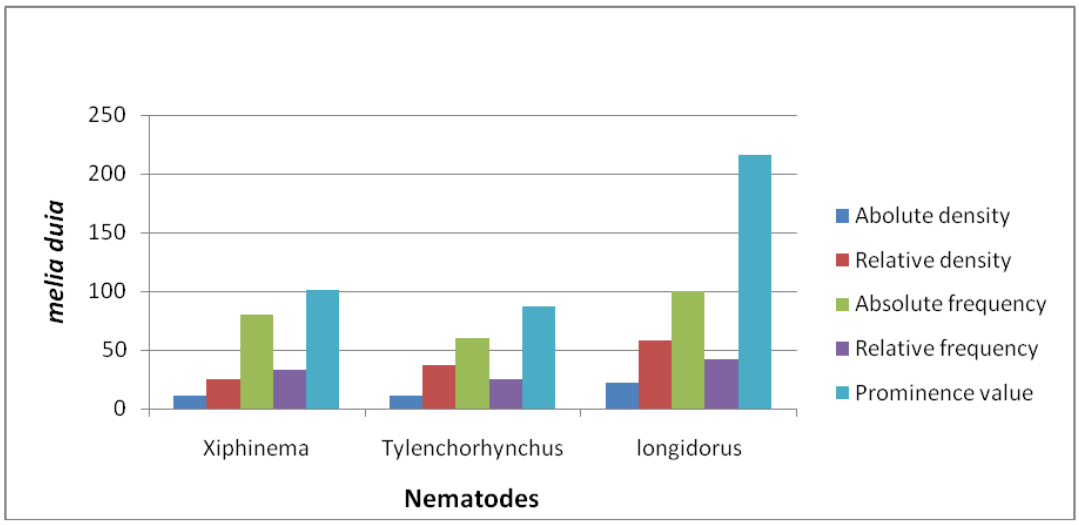

Fig-2: Frequency, density and prominence value of plant parasitic nematodes in nursery of *Melia dubia*

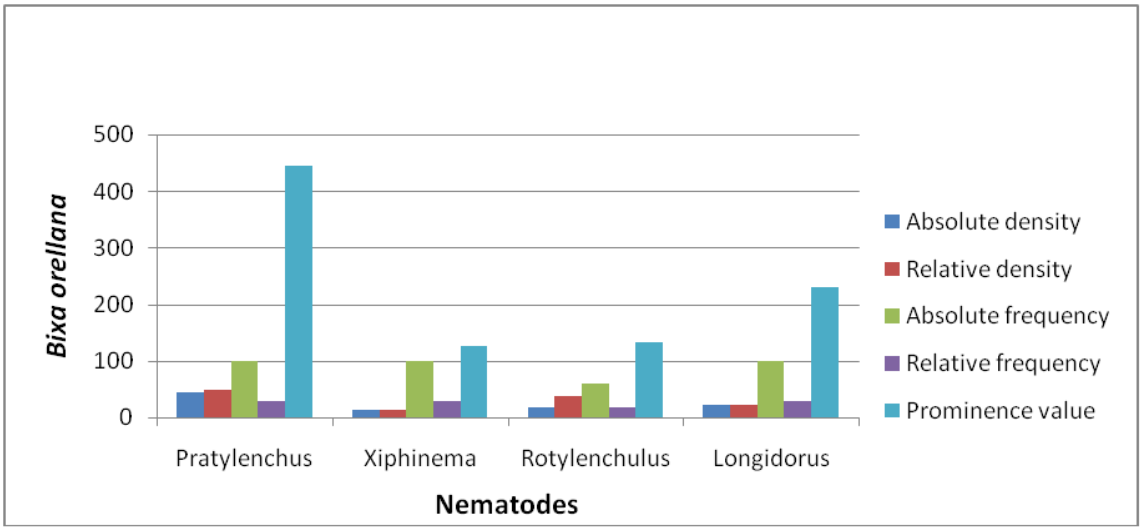

Fig-3: Frequency, density and prominence value of plant parasitic nematodes in nursery of *Bixa orellana*

Plate-1. Collection of Soil & Root samples and their processing for plant parasitic Nematodes

Plate-2. *Helicotylenchus* spp.

SUMMARY AND DISCUSSION

A widespread occurrence of *Longidorus* and *Helicotylenchus* was recorded in most of the areas surveyed and recovery of higher number in Pungam and *Melia* suggests that the nematode role in limiting banana production. The survey results showed that the infested seedlings exhibited stunted growth and yellowing leaves accompanied by necrotic root system. Earlier report on the wide spread occur of *Helicotylenchus, M. incognita, R. similis* and *P. coffeae*in banana plantations [9, 10, 11, 12, 4, 3] and on oil palm [6] supports the present findings

Survey of plant parasitic nematodes associated in tree nursery crops *viz*., *Pongamia pinnata, Melia dubia* and *Bixa orellana* revealed invariably presence of *Longidorus spp, Pratylenchus spp, Xiphinema* spp, *Tylenchulus spp, Helicotylenchus spp, Rotylenchulus reniform.* So far no study was done on the infestation of plant parasitic nematodes in the nursery seedlings of tree crops. Hence this project would provide an insight on nematode problem and their level of occurrence in the tree seedlings as seedlings are the primary source of infestation.

The study conducted in *Pongamia pinnata* revealed that the infestation of *Longidorus* is higher which has the potential of damaging in the seedling stage. Since these nematodes are ectoparasitic in nature, the damage and yield loss caused will be more when compared to other nematode. The infestation of *Helicotylenchus* showed the potential of damaging *Pongamia pinnnata.* Since this nematodes is both endoparasitic & ectoparasitic nematodes in nature, the damage and yield loss caused will be more when compare to other nematode.

The survey conducted in *Melia dubia* nursery showed the infestation of several ectoparasitic (*Longidorus* and *Xiphinema*) and semiendoparasitic nematode (*Rotylenchulus reniform)*. The occurrence of *Rotylenchulus reniform* should be considered for management.

The occurrence of *Pratylenchus* in *Bixa orellana* caused lesions in the roots. Due to its migratory endoparasitic nature, this nematode has greater potential to Hence this nematode can cause damage to the roots of the *Bixa orellana* seedlings. The infestation of *Longidorus* is also higher in *Bixa orellana* which is an ectoparasitic nematode. Owing to the medicinal values of this tree crop, even a small damage caused by nematodes will cause greater yield loss.

It is obvious from the above study that plant parasitic nematodes *viz*., *Tylenchorhynchus*, *Helicotylenchus***,** *Rotylenchulus reniformis, Xiphinenma*, *Pratylenchus, Longidorus,* are found to occur in the tree crop seedlings *viz*.,*Pongamia pinnata, Melia dubia, Bixa orellana* . Although the infestation is not so severe the nematodes must be contained in the nursery in order to control their spread to the field. Suitable management strategies using biocontrol agents *viz*., *Pseudomonas fluorescens* or chemical Carbofuran 3G and physical method like soil solarization can be used singly or integrated approach can be followed to manage these nematodes and produce nematode free seedlings of tree crops.

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