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# NEMATODE PARASITES AND THEIR NATURALLY OCCURRING BIO CONTROL AGENTS IN TEA SOILS OF SRI LANKA

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## ABSTRACT

Owing to ample soil, areal and host conditions provided in the tea ecosystem, several species of nematodes and their biocontrol agents are associated with tea and other plant species in the vicinity. They include: root knot and sedentary nematodes *i.e. Meloidogyne arenaria, Meloidogyne brevicauda (=Heterodera marioni* Goodey), *Meloidogyne incognita, Meloidogyne javanica* and *Heterodera* sp., Pratylenchus species *i.e. Pratylenchus brachyurus, Pratylenchus loosi* (=*Anguillulina pratensis* = *Pratylenchus pratensis* (de Man) Filipjev = *Pratylenchus coffeae*), *Pratylenchus coffeae, Pratylenchus delattrei*, burrowing nematodes *i.e. Radopholus similis* Cobb (=*Tylenchus similis*), spiral nematodes *i.e. Helicotylenchus namus, Helicotylenchus dihystera, Helicotylenchus erythrinae, Helicotylenchus* sp., *Hoplolaimus* sp., *Scutellonema brachyurus*, Pin nematodes *i.e. Paratylenchus curvitatus, Paratylenchus* sp., *Hemicycliophora* sp. *Criconemoides* sp., reniform nematodes *i.e. Rotylenchulus reniformis, Rotylenchulus* sp., dagger nematodes *i.e. Xiphinema* sp., *Xiphinema americanum, Xiphinema radicicola, Trichodorus* sp., *Longidorus* sp., free living nematodes *i.e. Dorylaimus* sp., *Panagrolaimus* sp., *Mononchus* sp., bacterial feeding nematodes *i.e. Rhabditis* sp., *Diplogastor* sp., fungal feeding nematodes *i.e. Aphelenchus* sp., algal feeding nematodes *i.e. Tylenchus* sp. and insect pathogenic nematodes *i.e. Mermis nigrescens* and *Hekarella talawakelae.*

The endosporming bacterium *Pasteuria penterans,* nematode trapping fungi *Arthrobotrys musiformis* Dreshsler, *A. oligospora* Fres., *Arthrobotrys* sp., *Dactylella* sp. and *Monacrosporium* sp., other fungal species *i.e.* Vesicular Arbuscular Mycorrhiza (VAM), *Fusarium* sp, *Paecilomyces* sp. and *Trichoderma* sp. and Micro Arthropod species *i.e.* Tardigrades, Collembolans, Mites and Myriapods were among the nematode antagonists. Their significantly greater incidence index in Sri Lankan tea soils compared to other tea growing countries was attributed to rational use of soil pesticides, good soil management and organic tea cultivation practices *etc.*

In support of the fair record of Sri Lankan tea nemic fauna, exploration of animal, bacterial and fungal feeding free living nematodes and morphological, taxonomical, genetic confirmations of the different nematode species and their isolates are recognized. Harnessing the effective naturally occurring biological control agents of parasitic nematodes for purpose of developing biopesticides is also proposed.

## INTRODUCTION

### Tea

Tea is a beverage crop grown at latitudes from 27°S (Corrientes, Argentina) to 43°N (Georgia, USSR), as well as from mean sea level up to an altitude of 2,300 m. The tea which is grown as a perennial crop requires well drained acid soils with a pH range of 4.5 to 5.5 and reasonably well distributed rainfall, totaling not less than 1000 mm /year. Commercial tea populations are polymorphic in origin, derived from *Camellia sinensis* (L) O. Kuntze., C. *assamica var. assamica* (Masters) Wight, and *C. assamica var. Lasiocalyx* (planch.) Wight or the hybrids of these different varieties.

In order to achieve sustainable yields, quality and economic profits, specific Good Nursery Practices (GNPs), Good Agricultural Practices (GAPs) and Good Manufacturing Practices (GMPs) are recommended by the Tea Research Institute of Sri Lanka. Amongst the field operations, land selection, site selection for nursery, soil rehabilitation, land preparation, soil and moisture conservation practices, choice of healthy, high yielding and quality cultivars suitable for resistance to drought, pests and diseases, soil, crop and ecosystem management, shade management *etc.* are important.

## Nematode Species Encountered in Sri Lanka

Among soil inhabiting animals, various species of nematodes have been reported in Sri Lanka. Majority of them have been recovered from agricultural soils owing to importance as pests and pathogens. The important nematode species reported include species belong to Meloidogyne, Pratylenchus, Helicotylenchs, Hoplolaimus, Radopholus, Scutellonema, Aphelenchoides, Criconemoides, Ditylenchus, Hirschmanniella, Logidorus, Rotylenchus, Trichorodrus, Tylenchulus, Xiphinema and Globodera. As reported by Loss (1953), Hutchinson (1961), Sivapalan (1972, 1978), Gnanapragasam (1995), Lamberti et al. (1987), Lamberti and Ekanayake (1981), Mohotti et al. (1996), Ekanayake and Toida (1997), Mohotti (1998), Premachandra et al. (2007), Vitharana and Amarasinghe (2011), Mohotti unpublished (2012), the soil ecosystems cover tomato, chilli, okra, carrot, snake guard, egg plant, pumpkin, spinach, beans, tea, banana, papaya, paddy, papaw, guava, Centella, strawberry and black pepper etc.

## 3. Nematode Species Encountered in Tea

### *3.1 Soil and Environmental Conditions*

The tea ecosystem provides ample soil, areal and host conditions owing to its perennial nature and reasonably undisturbed crop management practices. The factors in the soil environment therefore, generally facilitate nematode reproduction, survival and establishment in a given specific location. Resultantly, several species of nematodes have been encountered in tea soils in the different tea growing areas in association with tea and other plant species in the vicinity of tea such as weeds, green manure crops, shade tree species, cover crops *etc.*

With respect to pathogenic species, there is a widely varied complex of nematode populations that attack tea due to the wide variability in soil types and climatic conditions under which tea is being cultivated on a commercial scale. The intensity of attack of the respective species and the degree of the induced pathogenicity however vary correspondingly (Gnanapragasam and Mohotti, 2005). A marked periodic fluctuation in population levels is also observed during the year which is correlated to the rainfall pattern and soil temperature. Nematode damage is known to vary with the type of soil (soil texture) as well as the physical condition of the soil. Under poor soil conditions, the rate of replenishment of roots damaged by nematodes is very much curtailed, resulting in the rapid deterioration of the root system, with the consequent restricted uptake of nutrients. Increasing soil acidity has also been observed to aggravate the above condition. The pattern of distribution of nematode infestation in seedling tea fields is highly clustered due to the large genetic variability as compared to that of in genetically uniform high- yielding vegetatively-propagated varieties (Sivapalan, 1972).

### *3.2 Nematode Species*

Several species of plant parasitic nematodes have been encountered in tea soils in the different tea growing areas of the world. However, no positive evidence of pathogenicity has been established in respect of the majority of these nematodes. The species that are either known or suspected to be pathogenic to tea includes *Pratylenchus* spp. *Radopholus similis, Meloidogyne* spp., *Hemicriconemoides kanayaensis, Rotylenchulus reniformis, Helicotylenchus* spp., *Paratylenchus curvitatus, Hoplolaimus* sp., *Rotylenchus* sp. and *Xiphinema* sp. Studies on nematode species non pathogenic to tea, free living and saphrophotic nematode species are scares. Table 1 presents the summary of distribution of the different nematode species pathogenic to tea in the different tea growing areas.

## 4. Nematode Species Encountered in Tea in Sri Lanka

It is worthy to note that the Sri Lankan nematode fauna covers predominantly in the tea ecosystem probably due to much attention given by the Tea Research institute of Sri Lanka compared to that of by the other research institutes. Over 50 nematode species are recorded in Sri Lankan tea soils. However, there have been changes to the reported different nematode species with confirmative identifications with time. *Heterodera marionii* Goodey reported by Light, 1928 has been confirmed as *Meloidogyne brevicauda* by Chitwood (1949). *Anguillulina pratensis* first recorded in 1926 was considered later as *Pratylenchus pratensis* (de Man) Filipjev by Gadd, 1946 and then renamed as *Pratylenchus coffeae* by Loos, 1953 and later confirmed as *Pratylenchus loosi* Loof. The burrowing nematode, earlier named as *Tylenchus similis* was confirmed as *Radopholus similis* (Cobb) by Thorne, 1949. The complete collection of nematode species recorded in the tea rhizophere in Sri Lankan tea soils are presented in Table 2.

**Table 1 Distribution of nematodes suspected to be pathogenic to tea in different geographic regions**

| Nematode species | Argentina | Australia | Africa (East) | Africa (South) | Bangladesh | China | India(North-East) | India(South) | Indonesia | Iran | Japan | Kenya | Korea | Malawi | Malaysia | Sri Lanka | Taiwan | Zimbabwe |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Helicotylenchus dihystera* | | + | | | | | + | | | | + | | | | | + | | |
| *Helicotylenchus erythrinae* | | | | | | + | + | | | | + | | | | | + | + | |
| *Helicotylenchus* sp. | | | + | | + | | + | + | | + | | | | + | | + | | + |
| *Hemicriconemoides kanayaensis* | | | | | | + | | | | | + | | | | | | + | |
| *Hoplolaimus* sp. | | | + | | | | + | | | | | | | + | | + | | |
| *Meloidogyne arenaria* | | | | | | + | | | | + | | | | + | | + | | + |
| Meloidogyne *acrita* | | | | | | + | | | | | | | | | | | | |
| *Meloidogyne brevicauda* | | | | | | | + | + | | | | | | | | + | | |
| *Meloidogyne hapla* | | | | | | | + | | | + | | | | | | | | + |
| *Meloidogyne incognita* | + | + | | | + | + | + | | + | + | + | + | | + | | + | | + |
| *Meloidogyne javanica* | | + | + | | | + | + | + | + | + | | | | + | | + | | + |
| *Meloidogyne thamesi* | | | | | | + | | | | | | | | | | | | |
| *Paratylenchus curvitatus* | | | | | | | + | | | | + | | | + | | + | | |
| *Pratylenchus brachyurus* | | + | | | | | + | | | + | | | | + | | | | |
| *Pratylenchus loosi* | | | | | + | + | + | | | + | + | | + | | | + | | |
| *Radopholus similis* | | | | + | | + | | | + | | | | | | | + | | + |
| *Rotylenchulus reniformis* | | | | | | | + | | | | | | | + | | + | | + |
| *Rotylenchus* sp. | | | + | + | | | + | | | | | | | + | | | | + |
| *Xiphinema* sp. | | | + | + | + | | + | | | | + | | | + | + | + | | + |

**Table 2 Summary of Nematode Species Encountered in Tea Soils in Sri Lanka**

| Nematode group | Nematode species |
|---|---|
| Root Knot and sedentary nematodes | *Meloidogyne arenaria, Meloidogyne brevicauda (=Heterodera marioni* Goodey), *Meloidogyne incognita, Meloidogyne javanica,* |
| Pratylenchus species | *Pratylenchus brachyurus, Pratylenchus loosi (=Anguillulina pratensis = Pratylenchus pratensis (de Man) Filipjev = Pratylenchus coffeae), Pratylenchus coffeae, Pratylenchus delattrei* |
| Burrowing nematodes | *Radopholus similis (Cobb) (Tylenchus similis)* |
| Spiral nematodes | *Helicotylenchus namus, Helicotylenchus dihystera, Helicotylenchus erythrinae, Helicotylenchus sp., Hoplolaimus sp., Scutellonema brachyurus* |
| Pin nematodes | *Paratylenchus curvitatus, Paratylenchus sp., Hemicycliophora sp.* |
| Reniform nematodes | *Rotylenchulus reniformis, Rotylenchulus sp.* |
| Dagger nematodes | *Xiphinema sp., Xiphinema americanum, Xiphinema radicicola, Trichodorus sp., Longidorus sp.* |
| Free living nematodes | *Dorylaimus sp., Panagrolaimus sp., Mononchus sp.* |
| Bacterial feeding nematodes | *Rhabditis sp., Diplogastor sp.* |
| Fungal feeding nematodes | *Aphelenchus sp.* |
| Algal feeding nematodes | *Tylenchus sp.* |
| Insect pathogenic nematodes | *Mermis nigrescens, Hekarella talawakelae* |

Amongst the nematode species recorded in Sri Lankan tea soils, *Pratylenchus loosi. Radopholus similis* and *Meloidogyne brevicauda* are associated with tea causing economic damage.

### 4.1 *Pratylenchus loosi*

The root lesion nematode, *Pratylenchus loosi* is the most serious pest in Sri Lanka. *P. loosi* is widely distributed amongst tea fields at all altitudes. However, severe damage to tea is mostly confined to elevations of 900 to 1,800 m, where crop loss occurs in mature tea, newly planted young fields, as well as in nurseries. The severity of damage to tea depends on the interaction of various factors such as prevailing climatic conditions, type of soil in which the tea is growing, cultural practices and age and vigour of plant. The distribution of *P. loosi* is determined mainly by soil temperature and soil moisture. The highest population is encountered at altitudes with soil temperatures of 18°C to 24°C. While obvious pathogenicity symptoms are observed in this temperature regime, low rate of population build-up is seen at temperatures above and below this range. Damage caused by *P. loosi* was observed to be most severe in clayey ill–drained soils.

The presence of shade trees and green manure crops amongst tea fields, which form part of the normal cropping pattern, also influences the distribution pattern and the intensity of build-up of this species of nematode.

### 4.2 *Radopholus similis*

The burrowing nematode, *Radopholus similis* in Sri Lanka was first reported in 1968, when infestations were observed in young tea fields at an elevation range of 500 to 1,000 m. The nematode is widely distributed in the tea areas at lower altitudes as well, up to 200m. On susceptible tea cultivars (young and mature) and under favourable climatic conditions, *R. similis* occurs even at very low altitudes of 50m. This is also a pest of tea in Java, Indonesia. Even though this species has been reported from tea in China, Zimbabwe and S. Africa, no detailed work has yet been carried out.

*R. similis* appeared to be quite sensitive to cold temperatures and has a poor survival rate in tea at elevations above 1000 m. At lower elevations *R. similis* has been observed in the rhizosphere along with *P. loosi*. In semi-dry areas *R. similis* occurs concomitantly with *R. reniformis* as well. *R. similis* in the tea areas appears to favour uniformly distributed high rainfall. In very wet or dry soil, the population was found to decline. Soil type and texture were also found to have significant influence on the reproductive rate and population build- up of this pest. Rapid build up of *R. similis* populations was revealed in sandy soil, followed by gravely or loamy soil at 25°±1°C. Damage to tea was also found to be significantly more in gravely, sandy, and loamy soil. There was hardly any build- up in clayey soil.

Host plant species grown and in the vicinity of tea fields significantly govern the distribution pattern and the level of build-up of this nematode species. Banana, coconut, arecanut, anthurium and gautemala grass were found to promote *R. similis* population build up while Vetiver and African love grass seemed to suppress the populations.

### 4.3 *Meloidogyne brevicauda*

This root-knot nematode species is the only *Meloidogyne* species that attacks mature tea. *M. brevicauda* needs cooler soil temperature for the build-up of populations. So far, *M. brevicauda* has been recorded only in tea, only in three plantations bordering the same jungle at an altitude of 1,500 to 2,000 m in Sri Lanka, Darjeeling in N. E. India and Nilgiris, Wynaad and Karnataka Districts in South India. Successful parasitism of tea plants was observed only at 12°C, whilst no parasitism was found to occur at higher temperatures. With increasing soil temperatures towards 18°C, *M. brevicauda* seemed suppressed as well as appeared to concomitantly associated with *P. loosi*.

### *4.4 Control of Nematode Parasites in Tea*

Nematodes are microscopic and considered as hidden enemies of crops as once they are introduced to any field, it is difficult to eradicate. Hence, nematode management should commence from nursery by adopting adequate hygienic to ensure that only healthy vigorously growing nematode-free plants are transferred to the field.

For reducing nematode populations below the economic damage threshold to help avert reduction in crop productivity, an integrated management strategy should be advocated in young and mature tea. The most useful resources of nematode management in tea include; exclusion of lands with parasitic nematode history, soil rehabilitation using grasses, planting of nematode tolerant and resistance cultivars, use of nematode free healthy tea planting materials, rational use of environmentally friendly agrochemicals with short soil persistence, proper soil management to maintain soil pH within the range of 4.5 to 5.0, incorporation of soil organic matter, cultivation of soils by regular forking, use of trap crops and botanicals, the use of biological control agents, Good Agricultural Practices to ensure plant health.

### 4 *5 Conservation of Beneficial Nematode Species in Tea*

Free living and saprophytic nematode species proved to assist in organic matter decomposition, enriching soil biological properties, act as natural enemies to soil borne pest and diseases including parasitic nematodes as well as serve as environmental indicators. Therefore, Good Agricultural Practices aiming at sustainable soil and crop management will help enumerate naturally occurring free living and saprophytic nematode species. Amongst, soil rehabilitation using grasses, rational use of environmentally friendly agrochemicals with short soil persistence, proper soil management, incorporation of soil organic matter, cultivation of soils by regular forking, organic tea cultivation practices *etc.* help conserve and increase populations of such nematode species.



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## 5. Nematode Biological Control Agents in Tea Soils

Until recently, very little information was available with regard to control of plant parasitic nematodes in tea by biological agents except for sporozoan endoparasites, predatory nematodes and phycomycete fungus *etc.*

Various soil organic amendments included in the integrated nematode management proved to increase natural predators and parasites of nematodes pathogenic to tea. Mohotti (1998) revealed several microbial antagonists of plant pathogenic nematodes to be present in Sri Lankan tea soils which are significantly greater as compared to other tea growing countries. Good Agricultural Practices with special reference to soil management and organic tea cultivation practices were seen predominantly beneficial in achieving greater incidence indexes of the soil organisms similar to natural forest soils. The records of naturally occurring nematode antagonists encountered in Sri Lanka are presented in Table 3.

**Table 3: Records of naturally occurring nematode antagonists in tea soils.**

| Bio control group | Organism | Reference |
|---|---|---|
| Bacteria | *Pasteuria penetrans group* | Mohotti (1998) |
| Nematophagous Fungi | *Fusarium* sp. | Mohotti (1998) |
| | *Paecilomyces* sp. | Mohotti (1998) |
| | *Trichoderma* sp. | Mohotti (1998) |
| | *Verticillium* sp. | CABI-Herbarium records, Mohotti (1998) |
| Nematode Trapping Fungi | *Arthrobotrys musiformis* Drechsler | Mohotti (1998) |
| | *Arthrobotrys oligospora* Fres. | Mohotti (1998) |
| | *Arthrobotrys robusta* Duddington | CABI-Herbarium records |
| | *Arthrobotrys* sp. | Mohotti (1998) |
| | *Dactylella* sp. | Mohotti (1998) |
| | *Monacrosporium* sp. | Mohotti (1998) |
| Micro Arthropods | Tardigrades (Water bears) | Hutchinson and Streu (1960) |
| | Collembolans (Spring tails) | Mohotti (2002) |
| | Mites (Acari) | Gadd & Loos 1946, Mohotti (2002) |
| | Myriapods | Mohotti (2002) |
| Nematodes | *Mononchus* sp., *Diplogastor* sp. and *Dorylaimus* sp. | Gadd & Loos, 1946 |
| Miscellaneous | VAM (Vesicular Arbuscular Mycorrhiza) | Balasuriya *et al.* (1991) |
| | Protozoan | Gadd and Loos, 1946 |

Nevertheless, the frequency of occurrence of *Pasteuria penterans* to be relatively low compared to other species in the Sri Lankan soils. Detailed studies indicated host cuticles of *P. loosi, R. similis, M. brevicauda, Pratylenchus* sp. and *Rhabditis* sp. being encumbered with the endospores of *Pasteuria penetrans* Mohotti (1998). The most common nematode trapping fungi encountered were *Arthrobotrys musiformis* Dreshsler, *A. oligospora* Fres., *Arthrobotrys* sp., *Dactylella* sp. and *Monacrosporium* sp. *Fusarium* sp, *Paecilomyces* sp. and *Trichoderma* sp. were also recovered.

## CONCLUSIONS AND RECOMMENDATIONS

Though a fair record on nemic fauna in tea soils in Sri Lanka is available, there is yet a need to explore additional information on free living nematodes responsible as animal, bacterial and fungal feeding and specifically species that are responsible in biological control of parasitic nematode species and environmental indicators. Overall, morphological, taxonomical, genetic confirmations of the different nematode species and their isolates are recognized as gaps in research. Harnessing the effective naturally occurring biological control agents of parasitic nematodes for purpose of developing biopesticides to control parasitic nematodes and insects is another area to reap the benefits in the tea ecosystem.

## ACKNOWLEDGEMENT

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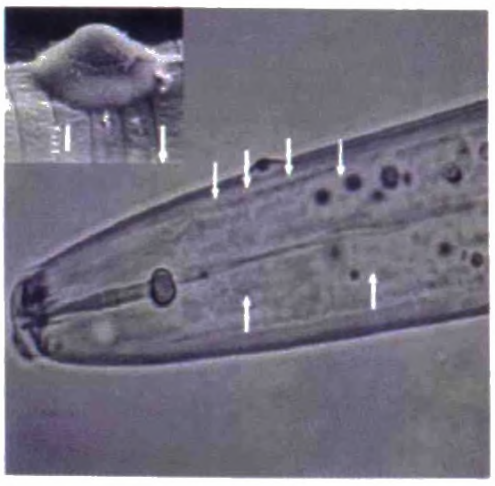
**Plate 1: Common nematode species encountered in tea in Sri Lanka.**

a. Root Lesion Nematode (*Pratylenchusloosi*)

b. Burrowing Nematode (*Radopholussimilis*)



c. Endospores of *Pasteuriapenetrans*, a bacterial nematode parasite

d. Nematode trapping fungi, *Arthrobotrys musiformis* Dreshsler, a fungal pathogen of nematodes