Sri Lanka J. Tea Science Vol.84, Part (1&2); 15-21

DOI: https://doi.org/10.4038/sljts.v84i1-2.7

(Reproduced from the proceedings of the seventh symposium on plantations crop research published in November, 2019)

Symptomological Variations in Tea caused by Six Isolates of the Root Lesion Nematode, *Pratylenchus Loosi* in Sri Lanka

P.G.D.S. Amarasena[1], K.M. Mohotti[1], D.M. Costa[2] and A.K. Prematunga[1]

[1]*Entomology and Nematology Division, Tea Research Institute of Sri Lanka, Talawakelle, Sri Lanka*
[2]*Department of Agricultural Biology, Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka*

**Corresponding author: deepthiamarasenaep@yahoo.com*

ABSTRACT

The root lesion nematode, *Pratylenchus loosi* is the major nematode species causing significant above-ground and below-ground stress symptoms in tea. Unusual spread and damage levels of *P. loosi* were evident in all tea growing regions in the recent past and warranted in-depth studies on symptomology and pathogenicity caused by different *P. loosi* populations. Hence, a series of studies was conducted to assess the pathogenicity and symptomology of different *P. loosi* populations under controlled conditions and in *P. loosi* infested fields in six locations in Sri Lanka *i.e*. Cicilton (Balangoda), Delmar (Ragala), Hapugastenna (Ratnapura), Mahadowa (Passara), Nawalapitiya and Richiland (Deniyaya).

The data revealed six different *P. loosi* populations causing damage to tea with significantly different virulence and pathogenicity levels and causing varying symptoms. Cicilton population seemed highly virulent at soil temperature 24 ^{o}C showing significantly higher reduction in plant height (52.3%) and root weight (42.6%). However, Richiland (Deniyaya) population caused significantly higher reduction (58.67%) in shoot weight. Data also confirmed significantly different virulence and pathogenicity levels of *P. loosi* triggered by changing soil temperatures. Overall, the six isolates of *P. loosi* showed irreversible damage to tea at different magnitudes in different agro ecological regions and elevations. Stunting of plants, root lesions, fall of feeder roots/fragments, flowering and fruiting as triggered through hormonal imbalances and dry matter production were symptoms in tea. These warranted appropriate isolate/location specific nematode management and mitigation strategies in different tea growing regions.

Keywords: *nematode, pathogenicity, Pratylenchus loosi, symptomological expressions*

INTRODUCTION

Tea, [*Camellia sinensis* (L) O. Kunze (Theaceae)], is the major plantation crop in Sri Lanka which brings substantial foreign exchange to the country. Plant parasitic nematodes are considered as one of the key pests limiting establishment, growth and productivity of tea. Amongst, the Root lesion nematode, *Pratylenchus loosi* is the most predominant nematode species causing economic damage to tea cultivation in Sri Lanka (Gnanapragasam and Mohotti, 2005). It was reported that optimum soil temperature range for the survival of *P. loosi* is 18 to 24°C (Sivapalan, 1972, Gnanapragasam and Mohotti, 2005). Stress symptoms in unthrifty and stunted tea such as smaller and yellow foliage, premature flowering and fruiting are caused by abiotic and biotic factors. Amongst, *P. loosi* is the major biotic factor to cause above-ground and below-ground symptoms in tea amounting 4-40% yield loss.

In Sri Lanka, *P. loosi* infestation in tea is first recorded in 1926 and it was first identified as *Angillulina pratensis* (Gadd, 1939). The zoological nomenclature of the root lesion nematode, *P. loosi* has undergone revisions several times over the years and finally confirmed as *Pratylenchus loosi*. Initial understanding on unique damage expressions on tea caused by *P. loosi* is well established over the years. However in recent past, *P. loosi* infestations from different tea growing regions in Sri Lanka exhibited varying magnitudes of damage symptoms irrespective of cultivar, age and weather (Unpublished data). It is also reported that the nematode population densities and their distribution have changed in tea growing regions (Mohotti, 2009 and Mohotti *et al*., 2017). Furthermore, *P. loosi* behaves as a species complex as reported from Sri Lanka, Japan and Iran (Mizukubo, 1998, Mohotti, 1998 and Hajieghrari *et al*., 2007) and severity of damage and symptoms caused by different *P. loosi* isolates vary as reported by Mizukubo (1995). Although data on intraspecific differences in reproductive fitness in the genus *Pratylenchus* is scarce within a *Pratylenchus* species (Griffin, 1991), different biotypes could exist resulting in differences of damage potentials. In the absence of clear evidences different isolates of *P. loosi* in Sri Lanka and their symptomological differences, it warranted in-depth studies on symptomology and pathogenicity caused by different *P. loosi* populations. This study addresses the symptomological variations in tea caused by six isolates of *P. loosi* in Sri Lanka.

MATERIALS AND METHODS

Selection of sampling sites

Sampling sites were selected by reviewing past records maintained by the Nematology Laboratory of the Tea Research Institute, Sri Lanka. Accordingly, six locations evident with moderate to heavy infestations of Pratylenchus loosi representing different elevations were recognized and used for sampling (Table 1).

Experiment 1: Symptomology and pathogenicity of six *Pratylenchus loosi* populations under field conditions

P. loosi populations of the six study locations PL 1 –PL 6 described in Table 1 were used for the study. In each location, three micro plots filled with respective population of P. loosi infested soil were established. Three nursery plants of TRI 2024, the susceptible tea cultivar for P. loosi were used to trap the soil populations of P. loosi. P. loosi populations in soil in each micro plot were assessed at monthly intervals. After 24 months, plants in micro plots were uprooted separately and data were taken on symptomology, growth parameters and *P. loosi* populations in root and soil. Plant height, number of leaves, shoot weight, root weight and total root length were measured as indicators of growth parameters.

Table 1. Details of study locations evident with moderate to heavy infestations of *Pratylenchus loosi* in different elevations

Code No.	Location	Latitude	Altitude	AER	Elevation (m amsl)
PL1	Cicilton Estate, Balangoda	6°43’60” N	80°39’0”E	IM2b	780
PL2	Delmar Estate, Ragala	7°0’0” N	80°52’0”E	IU2	1400
PL3	Hapugastenna Estate, Ratnapuara	6°69’3” N	80°38’6”E	WM1a	810
PL4	Mahadowa Estate, Passara	7°0’0” N	81°10’0”E	IU3c	1280
PL5	Smallholder land, Nawalapitiya	7°3’0” N	80°32’0”E	WM2a	550
PL6	Richiland Estate, Deniyaya	6°19’28” N	80°33’43”E	WM1a	470

Total root length of the tea plants were measured using method ‘intersection method’ described by Tennent (1975). A glass tray was used in this procedure in which 1 cm squares were marked. The equation 1 was used to calculate the total root length of tea plants.

Total root length = 11/14 x number of intersections x grid unit ………. (1)

Soil and root populations of *P. loosi* were assessed as *P. loosi* per 100 g soil and per g root, respectively. Modified Bermann – funnel method and Whiteheadand Hemming method described as standard methods by Southey (1986) were followed for processing and extracting nematodes from soil and roots, respectively.

Data analysis

For statistical analysis of the results, the SAS package was used. All data were subjected to one-way ANOVA and the means were separated using Tukey’s Honestly Significant Difference test ($P \leq 0.05$). Homogeneity of variance was tested and ‘nematode population per gram root’ nematode population densities were $\log_{10}(x+1)$ transformed before analysis. Statistical analysis was conducted using SAS program.

Experiment 2: Symptomology and pathogenicity of six *Pratylenchus loosi* populations under controlled conditions

The *P. loosi* populations (PL 1- PL 6) as described in Table 1 were collected from roots of the original host in respective locations anticipating uniform subpopulations of the original field populations.

Experimental set up

Thermostatically-controlled temperature tanks measuring 2 m x 1 m and 30 cm in depth were used as water baths to warm the soil contained in plastic pots. The temperature of the water in respective tanks was maintained by a thermostatically-controlled immersion heater fitted horizontally, 5 cm from the bottom at the center of the tank. To maintain the temperatures in the tank, warm water was circulated with a pump and maintained the required temperatures within ± 1°C. The tanks were set up in the glass house of Nematology

Experimental Area of the Tea Research Institute of Sri Lanka, Talawakelle.

Plastic pots (18.5 cm in diameter × 16.5 cm height) each were filled with fumigated soil. Uniform, 9 months old healthy TRI 2024 plants were transplanted in the pots and maintained in the glasshouse for 2 weeks prior to transferring into thermostatically controlled temperature tanks. The temperatures of these thermostatically controlled temperature tanks were adjusted to 24± 1°C and transferred the potted plants. After one-month, different *P. loosi* populations were inoculated. For each population, three replicates were used and uninfested controls were maintained for comparison purposes.

The plants were inoculated with different *P. loosi* populations (500 nematodes per plant basis) and maintained for 6 months. Soil temperatures were monitored during the period. After 6 months, plants were uprooted separately and data were recorded on symptomology, growth parameters and *P. loosi* populations in root and soil.

RESULTS AND DISCUSSION

Experiment 1: Symptomology and pathogenicity of six *P. loosi* populations under field conditions

In this study, 24 months after planting, TRI 2024 plants in three micro plots in each location were uprooted separately and data were taken on symptomology, growth parameters and P. loosi populations in roots. Summary of the data obtained is presented in Table 2.

Results of the micro plot experiments carried out under field conditions in six locations showed that all *P. loosi* populations studied (PL 1 – PL 6) resulted symptomological expressions at varying levels (Table 2). Tea plant growth was reduced significantly due to infestation of *P. loosi* populations. It showed that PL 5 population exhibited significant reduction in shoot and root weights compared to other populations. The results also showed that PL 6 population resulted in significantly higher number of fruits and flowers and reduction of total root length as symptoms of nematode damage. On the other hand, PL 2 Delmar population showed significantly higher root fragments in infested soil.

Data on mean number of *P. loosi* invaded to the root system of tea plants revealed significant variations among study populations (Figure 1). The highest root population was evident in PL 4 population which was significantly different compared to that of other five study populations. This data corroborates with the data on population densities in the existing field with TRI 2023 and other mixed cultivars.

However, other symptomological expressions such as number of fruits and flowers in PL 4 were not significant ($P \leq 0.05$) with other populations. Significantly lowest plant height and number of leaves were observed in PL 4. Lowest shoot weight and root weight of tea plants were also observed in location PL5, while in PL6 the lowest total root length was observed which was significantly different.

It appeared that all six populations have reproduced at different rates and have built up their root populations differently. It also appeared that higher root *P. loosi* always have not related to damage expressions of symptoms and/or pathogenicity as per the results of symptomological data.

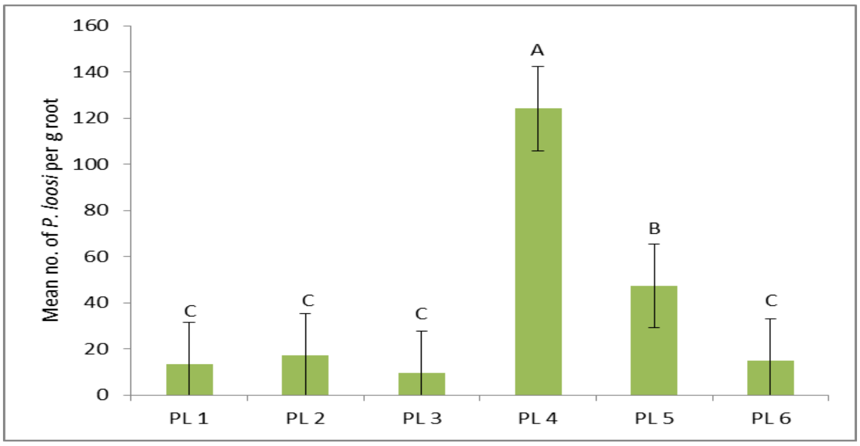

Figure. 1. Mean number of *P. loosi* invaded to tea plants 24 months after planting in respective location.

Experiment 2: Symptomology and pathogenicity of six *Pratylenchus loosi* populations under controlled conditions

In addition to the population build up in tea roots, the growth parameters were assessed as indications of damage by the different *P. loosi* populations. The results of the symptomological and pathogenicity studies of different *P. loosi* populations at soil temperatures of 24± 1°C are presented in Table 3.

Results showed that plant height, shoot and root weights, total root length and girth of TRI 2024 plants were declined by all the six *P. loosi* populations examined as compared to the un-infested control plants. Amongst, significant (P $\leq$ 0.05) differences were observed in plant height, shoot weight, root weight and total root length among the populations examined.

Results of the glass house experiments carried out under soil temperature 24± 1 °C (Table 3) showed that all *P. loosi* populations studied (PL 1 – PL 6) resulted a reduction in plant height, shoot weight, root weight and total root length compared to un-infested control. It was evident that even with a less population of PL 1, a significantly higher reduction in plant height indicating severe stunting as a symptom and total root length as an indication of massive root damage by nematodes was seen as compared to un-infested control. Therefore, PL 1 seems to be recognized as a virulent pathotype of *P. loosi* at its optimal soil temperature of 24 °C.

Table 3. Growth parameters of TRI 2024 plants inoculated with six *Pratylenchus loosi* populations, six months after inoculation at soil temperature 24° C

***P. loosi* Population**	**Mean Plant Height**		**Mean Shoot Weight**		**Mean Root Weight**		**Mean Total Root Length**	
	(cm)	**% change**	**(g)**	**% change**	**(g)**	**% change**	**(cm)**	**% change**
PL 1	34.3^{c}	(-52.3)	28.8^{c}	(-41.94)	18.66^{b}	(-43.23)	5205.99^{c}	(-66.80)
PL 2	36.2^{c}	(-49.8)	25.1^{c}	(-49.40)	22.82^{ab}	(-30.56)	7787.80^{b}	(-50.33)
PL 3	48.9^{bc}	(-32.1)	21.0^{c}	(-57.66)	18.87^{b}	(-42.60)	5780.27^{c}	(-63.14)
PL 4	61.9^{b}	(-14.1)	32.7^{b}	(-34.07)	22.25^{ab}	(-32.30)	6624.85^{b}	(-57.75)
PL 5	60.5^{ab}	(-16.0)	37.3^{b}	(-24.80)	26.50^{ab}	(-19.37)	10223.02^{ab}	(-34.80)
PL 6	60.1^{b}	(-16.5)	20.5^{c}	(-58.67)	19.42^{b}	(-40.91)	6556.95^{b}	(-58.18)
Un-infested Control	72.0a	(0.0)	49.6^{a}	(0.0)	32.87^{a}	(0.0)	15680.31^{a}	(0.0)

Means in the same column followed by the same letter do not differ significantly according to Tukey's test ($P \leq 0.05$)
(% change compared with the un-infested control plants)

CONCLUSION

In general, all six populations have reproduced at different rates and built up their root populations. However, higher root density of *P. loosi* always do not relate to damage and expression of symptoms and/or pathogenicity. The results of symptomological data showed that populations PL 1- PL 6 behaved in a different manner when causing symptomological expressions under actual field condition, where weather and other soil parameters (except tea cultivar) were uncontrollable.

Data revealed that population PL 1 to be a highly virulent at soil temperature 24 °C showing significantly higher (p<0.05) reduction in plant height (52.3%) and root weight (43.6%). In contrast, the shoot weight showed a significantly (p<0.05) higher reduction (58.67%) in PL 6 even with the *P. loosi* invasion in root was low.

Overall, *P. loosi* seemed to cause irreversible damage to tea at different magnitudes irrespective of the isolate, evident in different Agro-ecological regions and elevations. They included, stunting of plants, root lesions, fall of feeder roots/fragments, flowering and fruiting as triggered through hormonal imbalances and dry matter production in tea. Nevertheless, the loss of yield, impacts on field establishment and field survival under unthrifty conditions are considerable.

Hence, the proof of the presence of different *P. loosi* populations causing different damage symptoms warrants population/isolate or location specific nematode management approaches and strict adherence to Integrated Nematode Management (INM) strategies in all tea growing regions.

REFERENCES

Gadd, C.H. (1939). A destructive root disease of tea caused by the nematode, *Anguillulina pratensis*. Tea Quarterly **12**, 131-139.

Gnanapragasam, N. C. and Mohotti, K. M. (2005). Nematode Parasites of Tea. (In) Luc, M., Sikora, R. A. and Bridge, J. (Eds). *Plant Parasitic Nematodes in Sub Tropical and Tropical Agriculture*. 581-593.

Griffin, G.D. (1991). Differential pathogenicity of four *Pratylenchus neglectus* populations on alfalfa. Journal of Nematology **25,** 380-385.

Hajieghrari, B., Torabi-Giglou, M. and Waeyenberge, L. (2007). Comparative d2/d3 LSU–rDNA sequence study of some Iranian *Pratylenchus loosi* populations. *African Journal of Biotechnology*. **6** (21), 2458-2466.

Mizukubo, T. (1995). Evidence for *Pratylenchus coffeae* races in differential reproduction on fifteen cultivars (Nematoda: Pratylenchidae*). Japanese Journal of Nematology* **25**, 85-93.

Mizukubo, T. (1998). Morphometric and molecular differentiation of *Pratylenchus loosi* (Nematode: Pratylenchidae) species complex from Japan and Sri Lanka. In: *Proceedings of the 24th International Nematology Symposium of European Society of Nematologists*. pp.72.

Mohotti, K.M. (2009). Marked shift in damaged caused by parasitic nematode species in tea with long term climate change in Sri Lanka. (In) Abstracts of 1st National Conference, 10-11 September 2009, Kandy, Sri Lanka. pp.30.

Mohotti, K.M. (1998). Non-chemical approaches for management of root lesion nematode, *Pratylenchus loosi* Loof, 1960 in tea (*Camellia sinensis* (L.) O. Kuntz) with special reference to use of endospore-forming bacterium. *Pasteuria penetrans*. PhD Thesis, University of Reading, 289 p.

Mohotti, K.M., Prematunga, A.K., Amarasena, P.G.D.S., Navaratne, N. and Herath, U.B. (2017). Attributes of nematode infestations to bush debilitation, unusual yield decline and death of tea in low grown areas. In: *Proceedings of the 234th Experiments and Extension Forum, Tea Research Institute of Sri Lanka.* pp.45-56.

Sivapalan, P. (1972). Nematode pest of tea. (In) Economic Nematology. J.M. Webster (Eds). Academic Press, New York. pp.285-310.

Southey, J.F. (1986). Laboratory methods for work with plant and soil nematodes. Technical Bulletin. No. 2, Ministry of Agriculture, Fisheries and Food. United Kingdom, 202 pp.

Tennent, D. (1975). A test of a modified line intersects method of estimating root length. *Journal of Ecology* **63,** 995-1001.