

## PRELIMINARY STUDIES ON THE PREVALENCE AND CONTROLLING EFFICIENCY OF *PASTEURIA PENETRANS*, A PARASITE OF *MELOIDOGYNE* SPP. IN SRI LANKA

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(Received: 16 October 1996; accepted: 06 February 1998)

**Abstract:** Prevalence of *Pasteuria penetrans* in Sri Lanka was investigated and the effects of microenvironmental factors on its prevalence were determined. Naturally-occurring *Pasteuria* isolates were then evaluated for the efficiency of controlling *Meloidogyne* spp.. Randomly collected 804 soil samples were tested for the presence of *Pasteuria* using second stage juveniles of *Meloidogyne*. Controlling efficiency of *Pasteuria* was evaluated on greenhouse-grown tomato (*Lycopersicon esculentum* var. T-146) plants. Out of the tested soil samples, 26% reported *Pasteuria* spores in varying concentrations. Microenvironmental factors such as soil drainage and cropping history showed a significant influence on the prevalence of *Pasteuria* whereas cropping pattern had no significant effect on its prevalence. However, the interaction between the cropping history and cropping pattern also had a significant effect on the prevalence of *Pasteuria*. Under greenhouse conditions, *Pasteuria* isolates showed 48% control of the nematode in tomato (var. T-146) and the root powder prepared from above tomato plants gave  $8 \times 10^5$  spores/g root (dw). Therefore, naturally-occurring *P. penetrans* isolates of the tested areas of Sri Lanka could be used as a potential biocontrol agent of *Meloidogyne* spp..

**Key words:** Biological control, *Meloidogyne* spp., *Pasteuria penetrans*, Root-knot nematodes.

### INTRODUCTION

Root-knot nematodes (*Meloidogyne* spp.) are serious pests of many agriculturally-important crops grown in Sri Lanka.<sup>4,9,10</sup> The pest can be controlled by cultural and chemical methods but these control measures are associated with practical limitations and environmentally-unfavourable effects.

Crop rotation has limited success in the control of root-knot nematodes and fallowing though effective for the control of the pest, would not be an economical option for the farmer. Use of nematicides is associated with many hazardous effects such as destruction of non-target organisms, contamination of ground water and bioaccumulation. Above all, small scale farmers are unable to afford the cost of chemical control of the nematodes. Therefore, biological control could

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be considered as an alternative approach in managing root-knot nematodes and it can be effectively incorporated in the integrated management of the pest.

*Pasteuria penetrans*, has been identified as a promising biocontrol agent of *Meloidogyne* spp. by several workers.<sup>2,11,12</sup> When the bacterium is added to soil in pots, small plots, container-grown crops and polytunnels, significant reduction of root-knot nematodes has been attained.<sup>5,6,7</sup> *Pasteuria* is a soil inhabiting, mycelial and endospore-forming bacterium and has two modes of action against the *Meloidogyne* spp.. It can inhibit the egg production of adult female nematode and the entry of the infective stage of the nematode into host roots. Therefore, a reduction in the nematode population over time can be seen.

Although the root-knot nematodes have been a problem in Sri Lanka for many decades, investigations so far conducted on potential biological control agents are limited. Therefore, the present study was conducted to investigate prevalence of *P. penetrans* in Sri Lanka, determine the effect of microenvironmental factors on its prevalence and evaluate the controlling efficiency of *Meloidogyne* spp. by naturally- occurring *Pasteuria*.

## METHODS AND MATERIALS

### Experiment No. 1: Prevalence of *P. penetrans* and effect of micro-environmental factors

#### Experimental region

Kandy District of Sri Lanka was selected as the region of investigation.

#### Soil sampling

A total of 804 soil samples were randomly collected from Kandy District. These soil samples represented all the Divisional Secretary Divisions (DSD) of Kandy District and at least five soil samples were taken from each Agricultural Instructors (AI) division which are the subdivisions of DSDs. Soil sampling was restricted only to upland areas. Approximately 200g of soil was collected from a depth of 15 cm from the ground level.

#### Additional information on the sampling site

Microenvironmental factors which could have an influence on the growth and development of *Meloidogyne* and *Pasteuria* were recorded. The factors recorded were, cropping pattern (i.e. continuous or on and off cultivation), cropping history (i.e. for how long the same crop has been in a particular land) and drainage condition.

### Detection of *Pasteuria penetrans* in soil

Second stage juveniles ( $J_2$ s) were used to detect the *Pasteuria* spores in soil.<sup>13,16</sup>  $J_2$ s were initially isolated from the infected roots of several vegetable crop species collected from the fields at Marassana, Thalatuoya and Thannekumbura in Kandy District. After isolation  $J_2$ s<sup>8</sup> were cultured on greenhouse-grown tomato plants (var. T-146).

Detection of *Pasteuria* in soil samples was done on plastic cylinders having 7.5x7.5 cm dimensions. One end of the cylinder was sealed by a piece of muslin and a piece of paper towel was laid over it. Ten grams of soil was spread on the paper towel and approximately 15  $J_2$ s were added on to the soil. Two-day-old  $J_2$ s were used and they were free from pre-encumbered *Pasteuria* spores. These  $J_2$ s were extracted after 24 h<sup>8</sup> and examined under the light microscope (10x10) for spores attached on the cuticle. Soil samples which gave spore encumbered  $J_2$ s were considered as *Pasteuria* positive soils. Average number of spores/ $J_2$  were recorded by examining 5 nematodes per extraction.

### Locating *Pasteuria penetrans* positive sites in maps

Detailed maps of the 17 DSDs of Kandy District, provided by the Divisional Secretariats were used.

### Statistical analysis

Effect of different microenvironmental factors on the prevalence of *Pasteuria* was determined by the chi-square test.

### Experiment No. 2: Determining the efficiency of *P. penetrans* in controlling *Meloidogyne* spp.

#### Experimental layout

A greenhouse experiment was conducted in the Faculty of Agriculture, University of Peradeniya. Four-week old tomato plants (*Lycopersicon esculentum* var. T-146) were used as hosts of *Meloidogyne* and the following treatments were tested.

T<sub>1</sub> = Plants inoculated with  $J_2$ s of *Meloidogyne*

T<sub>2</sub> = Plants inoculated with  $J_2$ s of *Meloidogyne* pre-encumbered with *P. penetrans* spores

Approximately 100  $J_2$  having an average of 4 spores/ $J_2$  were inoculated into the rhizosphere. Spore encumbering was done by adding *Meloidogyne*  $J_2$ s

into randomly selected *Pasteuria* positive soil samples obtained from Experiment No. 1. Each treatment was replicated 10 times and the plants were arranged according to a Completely Randomized Design. Plants were maintained at 28°C for 8 weeks and root systems were observed for the total nematode population. Spore concentration of *P. penetrans* in root powder and the % nematode control by *Pasteuria* were also determined.

### Quantification of nematodes in the soil-root system

Eight weeks after inoculation, second stage juveniles and male nematodes released into the soil were quantified by a soil extraction.<sup>8</sup> The growth stages found in the root system (i.e. eggs, J<sub>2</sub>s, J<sub>3</sub>+J<sub>4</sub>s and adult females) were quantified by staining the roots.<sup>1</sup>

### % control of nematode by *Pasteuria*

Following formula was used for calculation

$$\% \text{ control of nematode} = \frac{P(T_1) - P(T_2)}{P(T_1)} \times 100$$

where;

P(T<sub>1</sub>) = Total nematode population in the soil-root system under T<sub>1</sub> treatment

P(T<sub>2</sub>) = Total nematode population in the soil-root system under T<sub>2</sub> treatment

### Determining the *Pasteuria* spore concentration

Dried root powder was prepared from T<sub>2</sub> treated plants eight weeks after the inoculation of *Meloidogyne*.<sup>15</sup> Dried root powder weighing 0.5g was ground with 50ml water and the homogenate was filtered through a 38 micrometer sieve. The filtrate was observed using a haemocytometer (mag 10x40) to quantify the spore concentration.

### Statistical analysis

The effect of *Pasteuria* in the reduction of *Meloidogyne* population was tested by 't' test.

## RESULTS

### Experiment No.1: Prevalence of *Pasteuria penetrans* and the effect of microenvironmental factors

Two hundred and seven (207) soil samples out of 804 (i.e. 26%) collected from Kandy District reported *P. penetrans* in varying concentrations (Figures 1 and 2).

Among the tested microenvironmental factors, cropping history and soil drainage showed a significant influence on the prevalence of *Pasteuria* ( $p=0.05$ ). Out of the total *Pasteuria* positive locations, 87% were obtained from well-drained soils. Locations having longer cropping histories showed high % prevalence of the biocontrol agent. *Pasteuria* were reported from 74% sampling sites which had more than 30 year cropping histories. In contrast, *Pasteuria* positive soils were reported only from 9% sampling sites which had a cropping history of less than 15 years. Cropping pattern in the sampling location did not show a significant effect on the prevalence of the biocontrol agent. However, the interaction between cropping history and cropping pattern had a significant effect on the prevalence of *Pasteuria*.

In the present study, high concentrations of *Pasteuria* were reported from locations under tea or mixed cropping cultivations, which had longer cropping histories (more than 30 years) and well-drained soils.

### Experiment No. 2: Efficiency of *Pasteuria penetrans* in controlling *Meloidogyne* spp.

Numbers of different growth stages of *Meloidogyne* spp. found in  $T_1$  and  $T_2$  treatments are shown in Figure 3. No male nematodes were found in the tested samples.

Total nematode population of *Meloidogyne* spp. in  $T_2$  treatment was significantly lower than that of  $T_1$  treatment. Significantly low number of eggs were found in *Pasteuria* treated root systems compared to the plants which were not treated with *Pasteuria* ( $p=0.05$ ). *P. penetrans* found from different locations of Kandy District gave 48% control of the *Meloidogyne* spp. in greenhouse-grown tomato (var. T-146) plants. Root powder prepared from  $T_2$  plants had an average spore concentration of  $8 \times 10^5$  spores/g root.

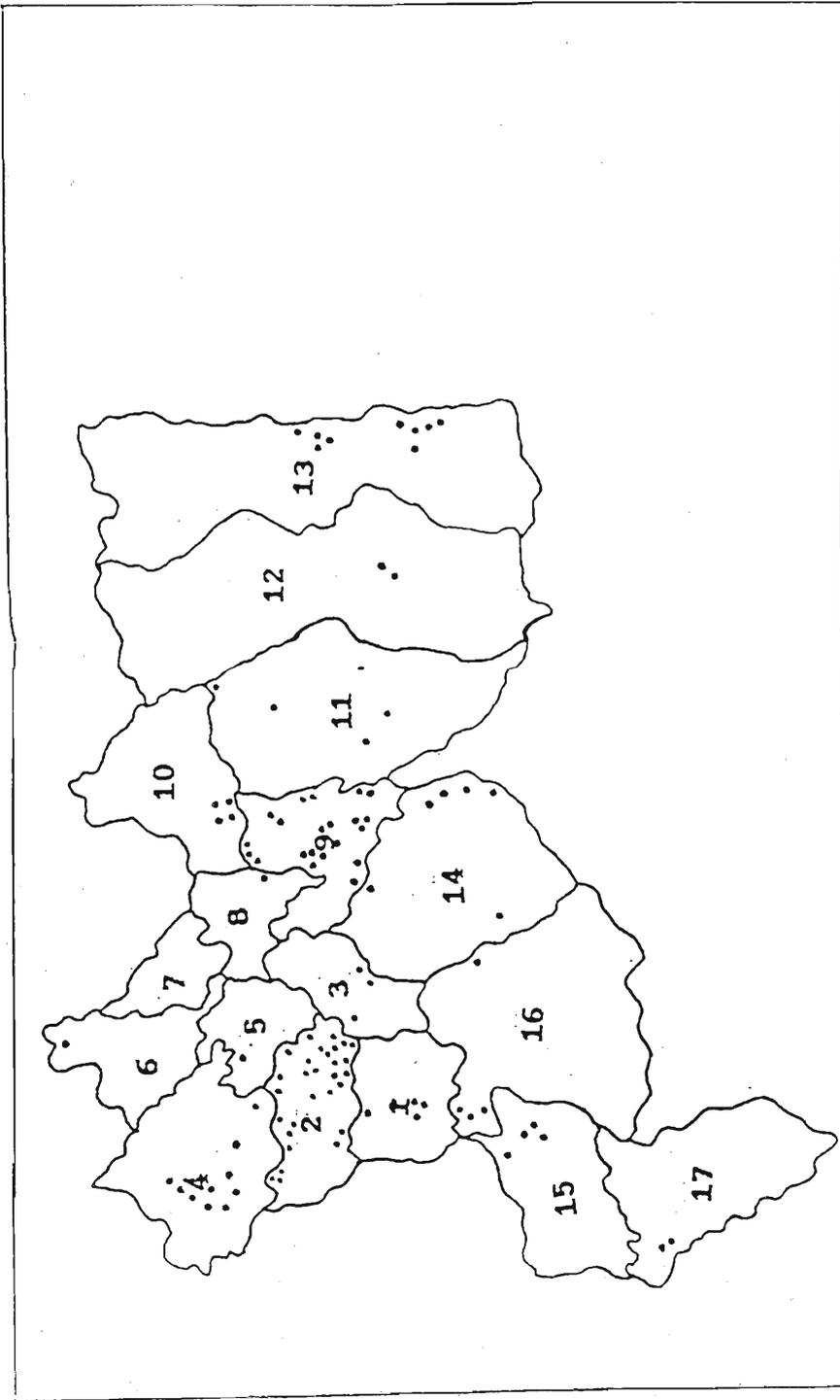


Figure 1: *Pasteuria penetrans* positive locations in Kandy District, Sri Lanka.

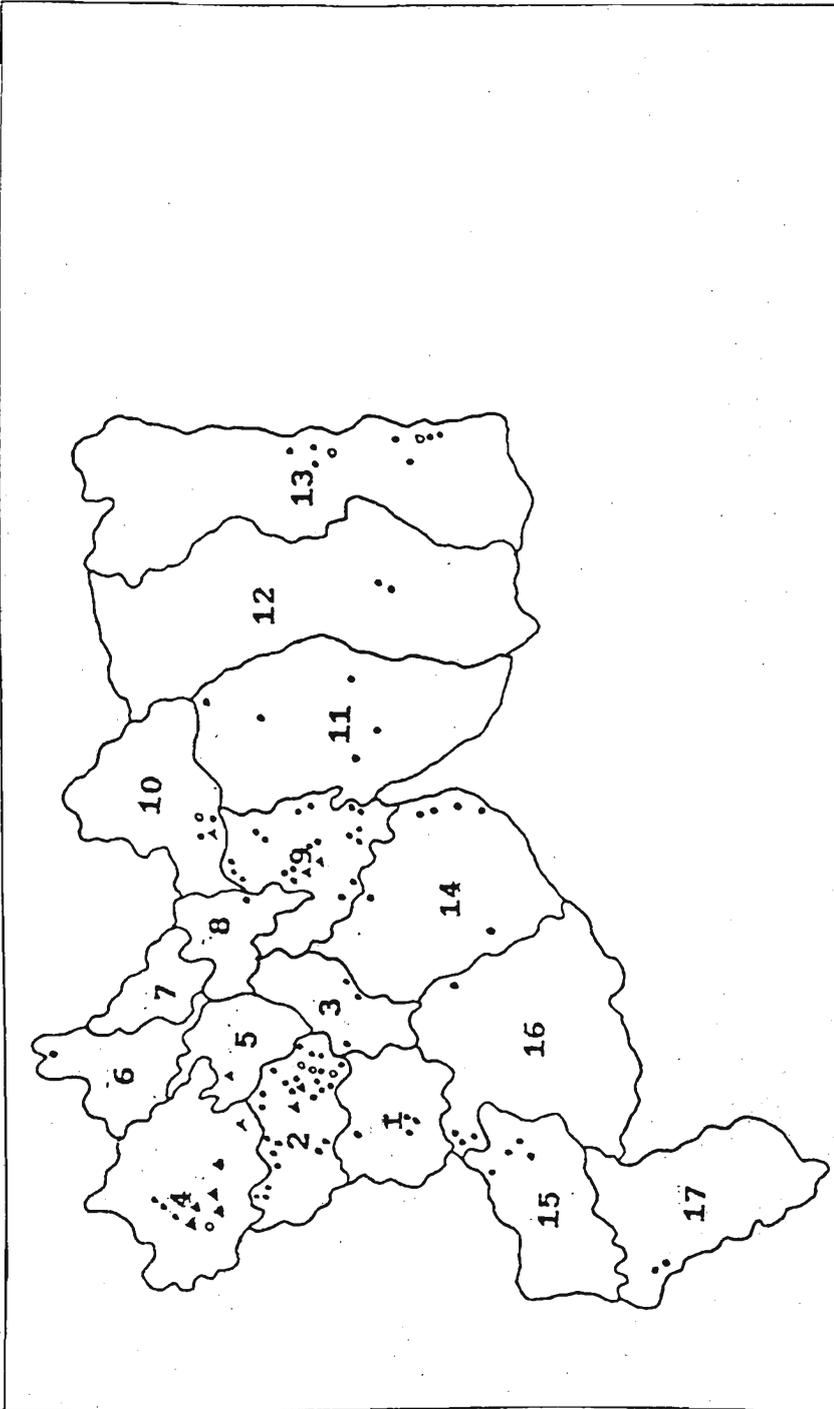


Figure 2: Different spore concentrations of *P. penetrans* found from the *Pasteuria* positive locations in Kandy District, Sri Lanka.

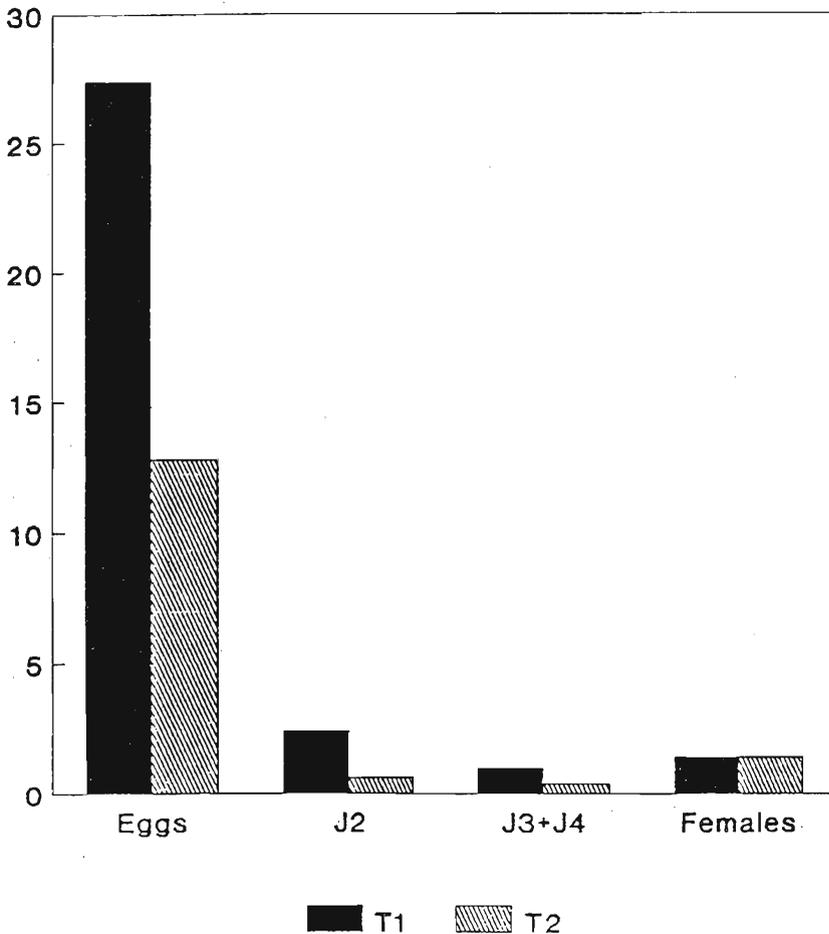


Figure 3: Mean number of different developmental stages of *Meloidogyne* spp/g root (fw) found in tomato (var. T-146) plant-root systems, 8 weeks after inoculation. T<sub>1</sub> = plants inoculated with J<sub>2</sub>s of *Meloidogyne* spp. T<sub>2</sub> = Plants inoculated with *Pasteuria* spore encumbered J<sub>2</sub>s of *Meloidogyne* spp.

## DISCUSSION

### Prevalence of *Pasteuria penetrans* and effect of microenvironmental factors

*P. penetrans* is prevalent in many locations of Kandy District, Sri Lanka in varying concentrations. Most of the sampling sites reported adequate concentrations of spores to perform a successful infection of *Meloidogyne*.<sup>3</sup>

### Effect of microenvironmental factors

Well-drained soils and longer cropping histories provide favourable growth and developmental conditions for the nematode and its obligate parasite, *Pasteuria*.<sup>14</sup> Aerobic conditions in well-drained soils are conducive for the growth and development of the nematode and *Pasteuria*. Cropping histories of more than 30 years provide continuous availability of the hosts for the survival of both organisms. Similar observations have been reported from vineyards which had more than 25 year-cropping histories and it had been difficult to find *Pasteuria* in locations having less than 10 year cropping durations.<sup>16</sup> In the present study also, high concentrations of *Pasteuria* were reported from perennial cropping systems having well-drained soils.

Since *Pasteuria* spores can survive in the soil for several years without losing their viability<sup>15</sup> the effect of cropping pattern may not have an effect on its prevalence. Ability to infect a wide range of hosts by *Meloidogyne* would be the reason for the interaction between the cropping pattern and cropping history on the prevalence of *Pasteuria*.

### Controlling efficiency

*Pasteuria* isolates of different locations of Kandy District, showed a 48% control of the *Meloidogyne* spp. in tomato (var. T-146) under greenhouse conditions. However, the field performances of these isolates of *Pasteuria* could vary from the greenhouse condition.

### Spore concentration

*P. penetrans* can reduce fecundity of *Meloidogyne* at concentrations as low as  $1 \times 10^4$  spores/g soil and the number of root-knot nematodes invading roots is substantially reduced as the spore concentration approaches  $1 \times 10^5$  spores/g soil.<sup>5,17</sup> Since the spore concentration of the root powder obtained from the present study was  $1 \times 10^5$  spores/g root (dry weight) an effective control of the nematode could be obtained.

### Potential use in an Integrated Management Programme

Present study revealed the presence of *P. penetrans*, in many locations of the Kandy District. Further indepth studies are essential to investigate the field performance of the biological control agent under local environmental conditions. *P. penetrans* can be introduced and once introduced, the biocontrol agent will get augmented in the fields provided the availability of the host plants.<sup>14</sup> Therefore, microenvironmental factors which favour the gradual increase of the biocontrol agent can be promoted to enhance the naturally-inhabiting populations

of *Pasteuria*. Future investigations have to be focused on the determination of ecological factors which are conducive for the biological control by *Pasteuria*. It will be helpful to develop an integrated management programme especially for small-scale vegetable cultivations which are being heavily infested with *Meloidogyne* spp. at present.

### Acknowledgement

We thank staff of the Department of Agriculture and the Divisional Secretariats in Kandy District for their assistance.

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