**Unveiling the host-range distribution of *Pasteuria penetrans* against various root-knot nematode, *Meloidogyne* spp.**

**Janani Mani\*1, Swarnakumari Narayanan1, Shanthi Annaiyan1, Gnanachitra Muthaiyan2**

**Affiliation(s) of the author(s)**

1 Department of Nematology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore, 641 003, Tamil Nadu, India.

2 Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, 641 003, Tamil Nadu, India.

***Corresponding author*-** [**jananimani9830@gmail.com**](mailto:jananimani9830@gmail.com)

**Abstract**

Root-knot nematodes (*Meloidogyne* spp.) are economically significant plant parasites causing extensive damage to various crops globally. Their sedentary endoparasitic nature, facilitated by a stylet, induces the formation of giant cells in plant roots, leading to characteristic symptoms such as galling, stunting, wilting, and reduced yield. To manage these pests, a combination of cultural, biological, and chemical methods is employed. Among biological control agents, *Pasteuria penetrans*, an obligate hyperparasitic bacterium, which shows effectiveness and primarily targets root-knot nematodes and exhibits specificity towards certain species or strains within the *Meloidogyne* genus. While its efficacy is well-studied, this study aims to explore its potential against other nematode pests. Its distribution spans various countries, suggesting its potential for widespread application in nematode management strategies. The conductance and results of a host range study are pivotal in understanding the potential efficacy and specificity of biocontrol agents against target pests. The current study also aims to explore the host range of *P. penetrans* and determine its effectiveness against nematode species or strains. The results of this study provide valuable insights into the applicability of *P. penetrans* as a biological control agent and by employing integrated nematode management strategies aimed at mitigating nematode damage to crops.

**Keywords:** Root-knot nematodes, *Meloidogyne* spp., *Pasteuria penetrans*, host range study, nematode management strategies.

**Introduction**

Root-knot nematodes are a group of plant-parasitic nematodes belonging to the genus *Meloidogyne.* They are among the most economically significant nematode pests worldwide, causing extensive damage to a wide crop including vegetables, fruits, ornamentals and field crops. Root-knot nematodes are microscopic roundworms with a distinctively swollen, lemon shaped- structure in their infective juvenile stage (J2). The life cycle of root-knot nematodes typically involves egg, four juvenile stages (J1 to J4) and an adult stage. They are sedentary endoparasites, meaning they feed and reside plant roots. They penetrate root tissues with their stylet, a piercing- sucking organ and induce the formation of specialized feeding cells called giant cells. These giant cells serve as nutrient sources for the nematodes. Different species and strains of *Meloidogyne* spp. may exhibit preferences for certain host plants.

Symptoms caused by root-knot nematode, *Meloidogyne* spp. leads to characteristic symptoms including root galling, stunting, wilting and reduced yield. Galling is the formation of swellings or knots on infected roots which impairs water and nutrient uptake leading to plant stress and reduced productivity. As a result of nematode feeding, hypertrophy and hyperplasia occurs in root cells leading to the formation of root galls. Based on their widespread distribution and significant impact on agriculture, root-knot nematodes are a major focus of research aimed by developing sustainable and effective management strategies to mitigate their damage to crops.

Management of root-knot nematodes typically involves a combination of cultural, biological and chemical methods. Cultural practices such as crop rotation, use of nematode-resistant cultivars and soil solarization and can help reduce nematode populations. Additionally, nematicides are sometimes employed although their use is often restricted due to environmental concerns. Biological control agents including certain fungi and potential nematode hyperparasitic bacterium like *Pasteuria penetrans* and can also be used. However, the host range of *P. penetrans* against plant-parasitic nematodes is less well-defined and current research is aimed at experimenting the different host crops and tested the bacterium’s efficacy against root-knot nematode spp. and further research is also needed to elucidate its potential broader application in nematode management strategies.

***Pasteuria penetrans***

*Pasteuria penetrans* is a bacterial parasite known for its potential as a biological control agent against various nematode pests, particularly within the genus *Meloidogyne* (root-knot nematodes). The distribution and host range of *P. penetrans* are significant factors in its utilization for nematode management strategies. *P. penetrans* is commonly found in agricultural soils across different regions worldwide. It has been reported in various countries including the United States, Australia, Brazil, India, China, and several European countries. Its prevalence can vary depending on environmental conditions and agricultural practices. *P. penetrans* is a potential biocontrol agent and was studied by several authors (Chen and Dickson 1998). Interaction between an obligate hyperparasitic bacterium, *P. penetrans* and its obligate plant‐parasitic nematode host, *Meloidogyne* spp. was documented by (Davies 2009)*.* Stirling and Wachtel (1980) first developed an *in vivo* mass multiplication system to produce inoculum for experimental purposes but it was not suitable for large scale production. The development of *P. penetrans* in *M. javanica* females was affected by constantly high and fluctuating temperature in an *in vivo* system (Darban *et al* 2005).

***P. penetrans* host-range and distribution**

*Pasteuria penetrans* primarily targets root-knot nematodes (*Meloidogyne* spp.), which are economically important pests affecting a wide range of crops including vegetables, fruits, ornamentals, and field crops. (Tzortzakakis *et al,* 1997) studied and reported the potential use of *Pasteuria penetrans* as a biocontrol agent of root‐knot nematodes, *Meloidogyne* spp. Within the *Meloidogyne* genus, *P. penetrans* exhibits specificity towards certain species or strains. While *P. penetrans* is most commonly associated with *Meloidogyne* spp., there have been reports of its parasitic activity against other nematode species as well. However, its efficacy and host range specificity are predominantly studied within the context of root-knot nematodes. Additionally, ongoing research aims to explore its potential against other nematode pests, which could further expand its utility in integrated pest management strategies. The bacteria were found to be widespread in South Australian vineyards naturally suppressing *M. incognita.* Stirling *et al.* (1982) and Mani (1996) reported that the hyper-parasite bacterium was distributed in Madurai, Dharmapuri and Dindigul districts of Tamil Nadu.

Cho *et al,* (2005) conducted the comparative inoculation methods by testing different *Meloidogyne* spp. against various host plants for production of *Pasteuria penetrans*. The bacterium is associated with soil and aquatic nematodes originating from many different biotypes. It has a wide range of hosts. There are about 150 species belonging to 50 nematode genera have been reported as host members of the *Pasteuria* group but there appeared to be a degree of specificity among populations and isolates of the parasite.

Brown and Smart Jr (1985) experimented the root penetration by *M. javanica* juveniles infected with *B. penetrans.* The host-range of each isolate is specific and limited but some isolates have a broad spectrum (Sayre and Starr 1985, Gowen and Ahmed 1990, Walia, Bansal *et al.* (1990).

**Materials and Methods**

**Collection of *P. penetrans* endospores**

**Identification of native isolate of *P. penetrans***

Identification of native isolate of *P. penetrans* was done by measuring the diameter of the spores. The diameter of 25 spores was measured under a research microscope using an ocular micrometer.

**Host-range study using native isolate of *P. penetrans* under glass house conditions**

The host-range of native isolate of *P. penetrans* was determined by incubating different nematode species in the *P. penetrans* spore suspension. Egg mass of *M. incognita*, *M. enterolobii,**M. graminicola* were collected and incubated at room temperature (28±2 °C) in water for hatching.*P. penetrans* endospores were collected as above said method for spore attachment. Nematode suspensions containing 200 J2 of *M. incognita*, *M. enterolobii*, *M. graminicola* were transferred to Petridish (5cm dia) in 5ml of water. The J2 attached with *P. penetrans* endospores of *Meloidogyne* spp*.* was inoculated into the host plant Tomato (Fig 1). After 30 days, the plants were uprooted and observed the gall formation in root and spore multiplication. The number of nematodes parasitized and the number of spores / nematodes were assessed. The spore attached juveniles were inoculated to their respective host. The host crops tested were tomato for *M. enterolobii* (Fig 2) and *M. incognita*, and paddy for *M. graminicola* (Fig 3).

**Results and Discussion**

**Documentation of host range of *P. penetrans* native isolate (Cbe PpM2) under glasshouse condition**

The research findings on the interaction between *P. penetrans* and various species of root-knot nematodes provide valuable insights into the host-parasite relationship and the potential applicability of *P. penetrans* as a biological control agent. Observations on *Meloidogyne incognita* demonstrated successful attachment of *P. penetrans* spores to the cuticle of J2 resulting in gall formation on inoculated plants containing J3 and J4 stages of *M. incognita*. Conversely, *M. enterolobii* J2 showed incomplete attachment of *P. penetrans* endospores indicating a limited infection. Similarly, *M. graminicola* was identified as a non-host for *P. penetrans* as gall formation occurred without the presence of endospores inside adult females.

***Meloidogyne incognita***

Observations on root-knot nematode, *Meloidogyne incognita* demonstrated successful attachment of *P. penetrans* spores to the cuticle of second stage juveniles (J2) resulting (Fig 4). The observation revealed that the spores were attached on J2 cuticle. The *P. penetrans* encumbered J2 inoculated plants showed galls. The galls contained J3 & J4 stages of *M. incognita* (Fig 5) with two cell and single cell stage of *P. penetrans* endospores.

***Meloidogyne enterolobii***

The *P. penetrans* endospores were not completely attached with the cuticle of the nematode, only endospore was nearer to the cuticle portion of J2 of *M. enterolobii*. Hence the J2’s was not inoculated to the host plant.

***Meloidogyne graminicola***

The observation assured that the inoculated J2 were penetrated and gall formation was recorded. The gall contains only healthy adult females. It did not have any endospores present inside the female. Hence, it proved to be a non-host for this bacterium.

Host range study of *P. penetrans* conducted by Oostendorp *et al*. (1990) states that isolates of *P. penetrans.* P- 20 survived without loss of its ability to attach to its host nematode in dry, moist and wet soil and in soil wetted and dried repeatedly for 6 weeks. Five isolates were tested in spore-infested soil, (P- 104, P-122, B-3) attached to two or more nematode species, where B-8 attached only to *M. hapla* and B-1 did not attach to any one of the nematodes tested. Different isolates showed attachment to different species of root knot nematode. Isolate P-100 attached in high numbers to *M. arenaria* when spores were extracted from females of this nematode when extracted from *M. javanica* females, fewer spores attached to *M. arenaria* than to *M. javanica* or *M. incognita*. Two species of the genus *Pasteuria, P. thornei* Starr and Sayre and *P. penetrans* (Thorne) Starr and Sayre, were described based on host range and morphological differences. As the genus is likely to contain more than two species parasitic nematodes, Starr and Sayre proposed the term *P. penetrans* for all non-speciated nematode parasitic isolates.

Kutywayo and Been (2006) conducted a glasshouse experiment to investigate the host status of six important weeds in intensive agricultural cropping systems to *Meloidogyne chitwoodi* and *P. penetrans*. *Seneico vulgaris* and *Echinochloa crusgalli, S. nigrum* were hosts of *P. penetrans* with multiplication factors of 1.6, 1.82 and 4.29 respectively. The results indicate that the call attention to the possibility of weeds acting as a carrier and point sources of possible high population densities of plant-parasitic nematodes. It emphasizes the importance of adequate weed control in an integrated programme for management of *M. chitwoodi* and *P. penetrans* and the possible failure of the successful use of non-host crops and fallow in crop rotations when weed control is inadequate.

Davies *et al.* (1988) conducted a test to determine whether *P. penetrans* spores would attach to 17 species of nematodes. All susceptible individuals had spores attached to their cuticles after 24hr of gentle agitation in suspension containing 105 spores/ ml. spores of *P. penetrans* from six populations of *Meloidogyne* only adhered to species of *Meloidogyne* and they adhered to greatest numbers to the species from which they have been originally isolated. Invasion of Tomato roots was reduced by up to 86% rather than using healthy juveniles, second stage juveniles bearing15 or more spores were added to soil at high densities (1000 or 3000 /plant); at low densities (500/plant) invasion was not significantly affected. The numbers of second-generation of *M. incognita* were reduced by 82-93% when juveniles encumbered with 1-15 spores were added to soil instead of those bearing no spores. *P. penetrans* populations differed in their aggressiveness and when juveniles encumbered with the same number of spores from two populations were added to the soil there were differences in the numbers of females that became infected.

Overall, these studies contribute to our understanding of the host range and behavior of *P. penetrans* and underscore its potential as a potential biological control agent against root-knot nematodes, while also highlighting the importance of considering different host status in nematode management strategies.

**Conclusion**

The study on host range *P. penetrans* native isolate (Cbe PpM2) highlight the intricate dynamics between *P. penetrans* and various species of root-knot nematodes, shedding light on their host-parasite relationship. These findings underscore the importance of understanding the specificity of *P. penetrans* interactions with different nematode species as well as the variability in infection success across nematode populations. Additionally, this research contributes to the broader understanding of host-parasite relationships in agricultural ecosystems by paving the way for more targeted and effective approaches to nematode control that harness the potential of beneficial bacterium like *P. penetrans*. Further investigations into the factors influencing the effectiveness of *P. penetrans* such as strain variability and environmental conditions are essential for optimizing its use in integrated nematode management approaches. Hence the present study gave a preliminary result on the distribution of host range of *P. penetrans* against *Meloidogyne* spp.

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