MadrasAgric.J.,2024; https://doi.org/10.29321/MAJ.10.001127

RESEARCH ARTICLE

Unveiling the Host-range Distribution of *Pasteuria penetrans* Against Various Root-knot Nematodes, *Meloidogyne* spp.

Janani Mani*[1], Swarnakumari Narayanan[1], Shanthi Annaiyan[1], Gnanachitra Muthaiyan[2]

[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.

Received: 17 May 2024

Revised: 30 May 2024

Accepted: 11 June 2024

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. The conductance of a host range study is pivotal in understanding the potential efficacy and specificity of biocontrol agents against target pests. The current study aims to explore the host range of *P. penetrans* its effectiveness against three nematode species, *Meloidogyne incognita, Meloidogyne enterolobii. Meloidogyne graminicola* tested its efficacy against various host crops. The outcome of the current study gives the successful attachment of *P. penetrans* against *M. incognita*, whereas *M. enterolobii* were not shown promising results and also *M. graminicola* was identified as a non-host for this bacterium. And also, the results of this study provide valuable insights into the applicability of *P. penetrans* as a biological control agent.

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 (J_2). The life cycle of root-knot nematodes typically involves egg, four juvenile stages (J_1 to J_4) and an adult stage. They are sedentary endoparasites, meaning they feed and reside inside the 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. are 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

*Corresponding author's e-mail: jananimani9830@gmail.com

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 to 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.

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. *Pasteuria 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 under *in vivo* system (Darban *et al* 2005).

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) 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 wasdistributed 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 they 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). This research is aimed at experimenting the different host crops and tested the bacterium's efficacy against root-knot nematode spp. *viz., Meloidogyne incognita, Meloidogyne enterolobii* and *Meloidogyne graminicola*

MATERIALS AND METHODS

Collection of *P. penetrans* endospores

Identification of Native Isolate of *P. penetrans*

The culture inoculum was prepared with J_2 of *M. incognita* attached with *P. penetrans*. From the suspension, once the *P. penetrans* infected adult female of *M. incognita* were observed, the female was crushed in the glass slides to observe the presence of *P. penetrans* endospores. The *P. penetrans* culture were maintained in tomato crop plant under glasshouse condition. The identification of the native isolate of *P. penetrans* was conducted by measuring the diameter of its spores. Specifically, the diameters of 25 spores were measured under a research microscope using an

ocular micrometer. After observation, the culture was inoculated into the maintained pots under glasshouse condition.

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

P. penetrans endospores were collected for spore attachment with *Meloidogyne* spp. The host-range of the native isolate of *P. penetrans* was evaluated by incubating three nematode species in a *P. penetrans* spore suspension. Egg masses of *M. incognita*, *M. enterolobii,* and *M. graminicola* were collected and incubated at room temperature (28±2 °C) in water for hatching. After 3-4 days upon hatching, nematode suspensions containing 200 J_2 of *M. incognita, M. enterolobii*, and *M. graminicola* were transferred to petri dishes (5 cm diameter) in 5 ml of water. Conversely, the endospores of *P. penetrans* were collected and transferred into the nematode suspensions. The J_2 nematodes attached with *P. penetrans* endospores of *Meloidogyne* spp. were inoculated into the respective host plants, (Fig. 1). After 30 days, the plants were uprooted, and the gall formation in roots and spore multiplication were observed. The number of parasitized nematodes and the number of spores per nematode were assessed. The spore-attached juveniles were inoculated into their respective hosts, with tomato being tested 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

Meloidogyne incognita

Observations on root-knot nematode, *Meloidogyne incognita* demonstrated successful attachment of *P. penetrans* spores to the cuticle of second stage juveniles (J_2) resulting (Fig 4). The observation revealed that the spores were attached on J_2 cuticle. The *P. penetrans* encumbered J_2 inoculated plants showed galls. The galls contained J_3 & J_4 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 J_2 of *M. enterolobii*. Hence the J_2's was not inoculated to the host plant.

Meloidogyne graminicola

The observation assured that the inoculated J_2 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.

Observations on *Meloidogyne incognita* demonstrated successful attachment of *P. penetrans* spores to the cuticle of J_2 resulting in gall formation on inoculated plants containing J_3 and J_4 stages of *M. incgonita*. Conversely, *M. enterolobii* J_2 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 the adult females.

The current study with conductance of host-range study of *P. penetrans* against three *Meloidogyne* spp. were also in agreement with the findings of Oostendorp *et al*. (1990) which states that the 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 various 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

Fig. 1. Documentation of host range of *P.penetrans* in *M. incognita* in host crop of Tomato

Fig. 2. Host range study of *P.penetrans* in *M.enterolobii* in host crop of tomato

Fig. 3. Host range study of *P.penetrans in M. graminicola* in host crop of rice

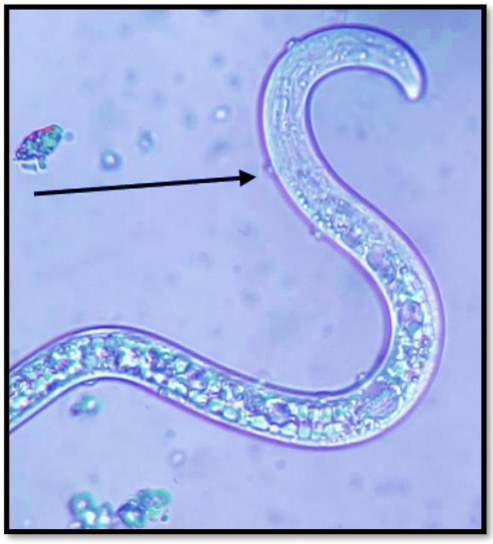

Fig. 4. *P.penetrans* endospores attached to J_2 of *M. incognita*

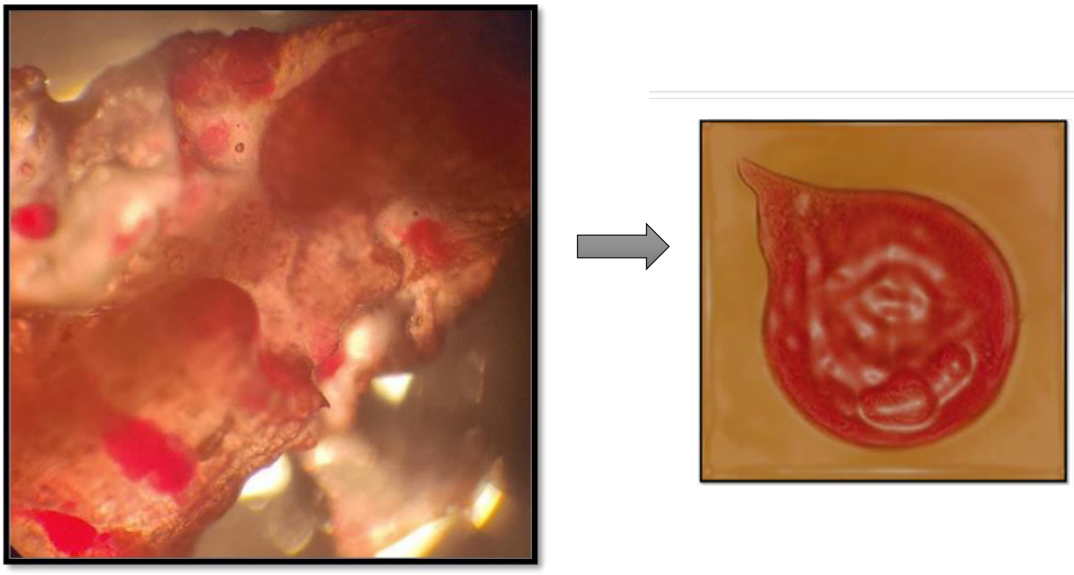

Fig. 5. Penetration of adult female in infested portion

M. javanica females, fewer spores attached to *M. arenaria* than to *M. javanica* or *M. incognita*.

The findings of Kutywayo and Been (2006) also showcased the host-range study of *P. penetrans* against different weed crops by conducting a glasshouse experiment and investigated 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 there is a possibility of weeds acting as a carrier and point sources of possible highpopulation densities of plant-parasitic nematodes. It emphasizes the importance of adequate weedcontrol 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) also attempted a study to determine whether *P. penetrans* spores would attach to 17 species of nematodes. The observation revealed that all susceptible individuals had spores attached to their cuticles after 24h of gentle agitation in suspension containing 10^5 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.

Thus, the present study explores the host-range status of *P. penetrans* with various root-knot nematode spp. which aids in better understanding of the host range and behavior of *P. penetrans* by underscoring 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, by 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. Meanwhile, future research should focus on exploring factors such as strain variability and environmental conditions that influence the effectiveness of *P. penetrans*, which are crucial for optimizing its use in integrated nematode management strategies. The present study provides initial findings on the host range distribution of *P. penetrans* against *Meloidogyne* spp.

REFERENCES

Brown, S. M., & Smart Jr, G. C. (1985). Root penetration by *Meloidogyne incognita* juveniles infected with *Bacillus penetrans*. *Journal of Nematology*, *17*(2), 123.

Chen, Z. X., & Dickson, D. (1998). Review of *Pasteuria penetrans*: biology, ecology, and biological control potential. *Journal of nematology*, *30*(3), 313.

Cho, M. R., Dickson, D. W., & Hewlett, T. E. (2005). Comparison of inoculation methods, *Meloidogyne* spp. and different host plants for production of *Pasteuria penetrans*. *Journal of Asia-Pacific Entomology*, *8*(3), 297-300 doi.org/10.1016/S1226-8615(08)60249-6 .

Darban, D. A., Gowen, S. R., Pembroke, B., & Mahar, A. N. (2005). Development of *Pasteuria penetrans* in *Meloidogyne javanica* females as affected by constantly high vs fluctuating temperature in an *in-vivo* system. *Journal of Zhejiang University-SCIENCE B*, *6*(3), 155-157 doi:10.1631/jzus.2005.B0155.

Davies, K. G. (2009). Understanding the Interaction Between an Obligate Hyperparasitic Bacterium, *Pasteuria penetrans* and its Obligate Plant-Parasitic Nematode Host, *Meloidogyne* spp. *Advances in parasitology*, *68*, 211-245 doi.org/10.1016/S0065-308X(08)00609-X.

Davies, K. G., Kerry, B. R., & Flynn, C. A. (1988). Observations on the pathogenicity of

Pasteuria penetrans, a parasite of root-knot nematodes. *Annals of Applied Biology*, *112*(3), 491-501 doi.org/10.1111/j.1744-7348.1988.tb02086.

Gowen, SR, and R Ahmed. "*Pasteuria penetrans* for Control of Pathogenic Nematodes." *Aspects of Applied Biology*, no. 24 (1990): 25-32.

Kutywayo, V., & Been, T. H. (2006). Host status of six major weeds to *Meloidogyne chitwoodi* and *Pratylenchus penetrans*, including a preliminary field survey concerning other weeds. *Nematology*, *8*(5), 647-657 doi: 10.1163/156854106778877839

Mani, M. P. (1996). Effect of *Pasteuria penetrans* (Thorne) Sayre and Starr and *Pseudomonas fluorescens* (Migula) against *Meloidogyne incognita* (Kofoid and White) Chidwood in grapevine (*Vitis vinifera* Linn.). *M. Sc. (Agri.) thesis, Tamil Nadu Agri. Univ., Coimbatore*.

Oostendorp, M., Dickson, D. W., & Mitchell, D. J. (1990). Host range and ecology of isolates of *Pasteuria* spp. from the southeastern United States. *Journal of Nematology*, *22*(4), 525.

Sayre, R. M., & Starr, M. P. (1985). *Pasteuria penetrans* (ex Thorne, 1940) nom. rev., comb. n., sp. n., a mycelial and endospore-forming bacterium parasitic in plant-parasitic nematodes. *Proceedings of the helminthological Society of Washington*, *52*(2), 149-165.

Stirling, G. R., & Wachtel, M. F. (1980). Mass production of *Bacillus penetrans* for the biological control of root-knot nematodes. *Nematologica*, *26*(3), 308-312.

Stirling, G. R., & White, A. M. (1982). Distribution of a parasite of root-knot nematodes in South Australian vineyards.

Tzortzakakis, E. A., DE. R. Channer, A. G., Gowen, S. R., & Ahmed, R. (1997). Studies on the potential use of *Pasteuria penetrans* as a biocontrol agent of root-knot nematodes (*Meloidogyne* spp.). *Plant Pathology*, *46*(1), 44-55.

Walia, R. K., Bansal, R. K., & Bhatti, D. S. (1990). A new bacterial parasite (*Pasteuria* sp.) isolated from pigeonpea cyst nematode, *Heterodera cajani*.