



Available Online at EScience Press

Plant Protection

 ISSN: 2617-1287 (Online), 2617-1279 (Print)
<http://esciencepress.net/journals/PP>

STUDIES ON THE EFFICACY OF HETEROGENEOUSLY PRODUCED *PASTEURIA PENETRANS* (PP3) ISOLATE OVER INDIVIDUAL *PASTEURIA* ISOLATES IN THE SPORE ATTACHMENT, AND PATHOGENIC POTENTIAL ON THREE *MELOIDOGYNE* SPECIES

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ARTICLE INFO

Article history

Received: 24th February, 2023Revised: 21st March, 2023Accepted: 27th March, 2023

Keywords

Biocontrol

Pasteuria penetrans

Reproductive factor

Root galling

Root-knot nematode

ABSTRACT

The aim of the present test was to develop a *Pasteuria penetrans* isolate (*Pp3*) on a *Meloidogyne* blend and compare the attachment and pathogenic potential of this heterogeneously produced isolate with other *Pp3* isolates produced on individual *Meloidogyne* species (*Meloidogyne javanica*, *M. incognita* and *M. arenaria*). Number of spores attached varied among *Meloidogyne* spp. *Pp3* isolate originally multiplied on *M. javanica* showed greater attachment level with second stage juveniles (J2s) of *M. javanica* (13.8) than other *Meloidogyne* spp. tested and lesser attachment was observed on J2s of a *M. arenaria*. The *Pp3* isolate showed variable influence on the *Meloidogyne* spp. in suppressing root-knot disease. There was a significant difference in numbers of eggmasses produced by females of *Meloidogyne* spp. Higher numbers of eggmasses were recorded with females of *M. arenaria* (370) while fewer numbers of eggmasses were observed in treatment where *M. javanica* (245) was present. Root galling differed significantly among treatments ($P < 0.01$) and higher gall infestation was recorded in *M. arenaria* treatment (6.2) while lesser galling was observed in *M. javanica* treatment (4.4). Final female population also varied among treatments ($P < 0.05$) showing higher numbers of females in *M. arenaria* treatment (456) and fewer with *M. javanica* (398). Parasitism of females of *Meloidogyne* spp. by *Pp3* differed significantly among treatments. Higher numbers of females infected with *Pp3* spores were recorded among females of *M. javanica* (14.4) while lesser numbers of parasitized females were observed in *M. arenaria* treatment (9.8). There were significant variations in numbers of endospores/female produced by females of *Meloidogyne* spp. however egg set data did not differ in the treatments. The results showed that the *Pp3* isolate was more pathogenic on *M. javanica* compared with other *Meloidogyne* spp. (*M. incognita*, *M. arenaria* and *M* blend). The *Pp3* isolate was found compatible with different *Meloidogyne* spp. while *M. arenaria* proved the least good host to bacterial parasite.

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INTRODUCTION

Root-knot nematodes are microscopic, soil-dwelling roundworms that parasitize plant roots, causing significant losses in agricultural production. They are

considered one of the most damaging plant parasitic nematodes worldwide (Hussain et al., 2012; Kayani et al., 2013; Hussain and Mukhtar, 2019; Tariq-khan et al., 2017, 2020). Root-knot nematodes can reduce the

growth and yield of a wide range of crops, including vegetables, fruits, and grains (Hussain et al., 2011; Kayani et al., 2017; Mukhtar et al., 2017). The nematodes penetrate the roots, causing the formation of galls, which impede the flow of water and nutrients to the rest of the plant. As a result, the plants become stunted, produce fewer fruits or seeds, and are more vulnerable to other stressors, such as drought or disease (Asghar et al., 2020; Aslam and Mukhtar, 2023). Root-knot nematodes can also affect the quality of the crops by altering their chemical composition, taste, and appearance.

Root-knot nematodes can cause significant yield losses in vegetable crops, ranging from 10-100% (Shahid et al., 2007). The economic losses caused by root-knot nematodes can vary depending on several factors, such as the crop species affected, the severity of the infestation, and the control measures used (Hussain et al., 2014, 2016a; Mukhtar et al., 2013a, 2014; Mukhtar and Hussain, 2019; Haq et al., 2022; Tariq-Khan et al., 2020). However, according to some estimates, the annual global losses caused by root-knot nematodes are in the billions of dollars. In addition to the direct losses in crop yield and quality, the costs associated with nematode control measures, such as soil fumigation, crop rotation, and resistant cultivars, can also add up.

Root-knot nematodes are known to have a wide host range, which means that they can infect and parasitize a broad range of plant species. In general, root-knot nematodes are most damaging to vegetables, but they can also affect many other crops, including fruit trees, ornamentals and field crops.

There are several methods of controlling root-knot nematodes, but chemicals are mostly relied on (Kayani et al., 2012; Mukhtar et al., 2013b, 2021; Mukhtar, 2018; Khan et al., 2019; Nazir et al., 2019; Azeem et al., 2021; Saeed et al., 2021; Mukhtar et al., 2023; Shahbaz et al., 2023). Chemical control of root-knot nematodes can have several negative effects on the environment and agriculture. They are harmful to non-target organisms and can be expensive. Frequent use of chemical nematicides can lead to the development of resistance in nematode populations, persistence in the environment, and ecological disruption. Among several alternatives to chemical control of root-knot nematodes, biological control can be the most feasible and practicable (Mukhtar et al., 2013c; Mukhtar et al., 2020). Several beneficial organisms like nematode-trapping fungi, predatory mites, and bacteria can be used to control

nematode populations. These organisms attack and kill the nematodes, reducing their numbers and limiting the damage they cause to plants. One of the bacterium, *Pasteuria penetrans* has shown promising effects against root-knot nematodes. However, the effectiveness of *P. penetrans* depends upon several factors (Ahmad and Mukhtar, 2007a, b; Mukhtar and Ahmad, 2000; Mukhtar et al., 2000, 2002, 2005).

Host specificity is generally considered a useful attribute in a biological control agent because it allows a specific pest to be targeted with a specific parasite without direct effects on other species. Host specificity is also an important consideration when the pathogen is released in an inoculate manner and this has been reported for both *Pasteuria* and root-knot nematode populations. Different populations of the bacterium seem to be highly specific not only to the particular nematode genera but also to different populations of the same species. The ability of the *P. penetrans* spores to adhere to the nematode cuticle is dependent on both the population of spores and the population of the nematode being tested. This suggests that great heterogeneity is present in both the cuticle of the nematode and the surface of the bacterium. Some heterogeneity may be retained despite long term culture on particular species (Stirling, 1985, 1991; Oostendorp et al., 1990, 1991). However care is needed in interpreting results based on spore adhesion as *P. penetrans* has also been found to retain its adhering ability though losing its infectivity (Espanol et al., 1997). Field populations of root-knot nematodes are genetically diverse and it is possible that populations of the *P. penetrans* with a restricted host range will be unable to cope with this diversity. Therefore, it may be necessary to select populations with a wide host range, or to mix several populations together to counter the diversity in root-knot nematodes usually found in the field (Channer and Gowen, 1988). The objective of the present study was to investigate the multiplication of *Pasteuria penetrans* on three *Meloidogyne* species and on their mixed population.

MATERIALS AND METHODS

Nematode species

All available *Meloidogyne* spp. were included in the experiment to maximize the heterogeneity of the nematode host. The nematode species having populations from different geographical areas were tested for spore attachment and susceptibility to *P. penetrans* (Pp3). The

species and origins of root-knot nematodes used in these studies were, *Meloidogyne javanica* (Pakistan, Malawi, Zimbabwe), *Meloidogyne incognita* (USA, race 1 and Ecuador) and *Meloidogyne arenaria* (USA, race 2). These species were maintained on tomato in a glasshouse at the University of Reading. Spores of the *P. penetrans* isolate (*Pp3*) which was originally collected in South Africa were multiplied on *M. javanica* on tomato and stored as powder (Stirling and Wachtel, 1980) or in suspensions prepared from this powder.

Spore attachment studies

Root systems bearing eggmasses of the above mentioned *Meloidogyne* spp. were shaken in a jar with sodium hypochlorite to facilitate egg separation. Egg suspensions belonging to all populations of *Meloidogyne* spp. were placed separately in extraction dishes and incubated at 28°C for hatching. Newly hatched juveniles of all populations of *M. javanica* and *M. incognita* were pooled in equal numbers in separate beakers. A spore suspension of the *Pp3* isolate multiplied on *M. javanica* was added to J2s of four nematode populations (*M. javanica*, *M. incognita*, *M. arenaria* and mixture of these *Meloidogyne* spp.) in separate Petri dishes (4.5cm). Six hundred J2s of each *Meloidogyne* species and *Meloidogyne* blend were exposed to (9×10^4) spores suspensions of *Pp3* in four treatments. The final volume in each Petri dish was adjusted to 6 ml by adding tap water. Each treatment was replicated threefold and incubated at 28°C for 24 h. Attachment level was recorded on 10 J2s/replicate.

Pathogenicity test

Eggs of *M. javanica*, *M. incognita* and *M. arenaria* were

collected from tomato root systems. The egg suspensions were incubated at 28°C and freshly hatched juveniles (2-4 days old) were exposed to the *Pp3* suspension. Three groups of 11×10^3 J2s of *M. javanica*, *M. incognita* and *M. arenaria* were exposed to the *Pp3* suspensions (1.65×10^4 /ml) in plastic trays (10 × 20 cm) and incubated at 28°C. Similarly J2s of *M. javanica*, *M. incognita* and *M. arenaria* were pooled in equal numbers and this *Meloidogyne* mixture was also exposed to the same *Pp3* suspension. Attachment levels were monitored when J2s in all treatments had the desired attachment level (6-12 spores/J2) the suspensions were sieved through 20 µm sieve to separate the nematodes from the spore suspension. Encumbered J2s of *M. javanica*, *M. incognita*, *M. arenaria* and *Meloidogyne* blend were inoculated on 20 (6-week-old) tomato plants @ 1800 J2s/plant. After inoculations, pots were kept in a growth room under uniform conditions. After 750 degree (base temp 10°C) days, plants were harvested from these pots and data regarding eggmasses, root galling, total females and infection on females were recorded. The root systems were dried, chopped, milled and root powders were kept for further studies.

RESULTS

Spore attachment

Number of spores attached varied among *Meloidogyne* spp. ($P < 0.01$) (Table 1). *Pp3* isolate originally multiplied on *M. javanica* showed greater attachment level with J2s of *M. javanica* (13.8) than other *Meloidogyne* spp. tested and lesser attachment was observed on J2s of a *M. arenaria* (7.73).

Table 1: Number of spores of *Pp3* attached to J2s of *Meloidogyne* spp. after 24 h incubation (counts on 10 J2s/replicate).

<i>Meloidogyne</i> spp.	Spore attached
<i>M. javanica</i>	13.8
<i>M. incognita</i>	9.10
<i>M. arenaria</i>	7.73
<i>M</i> blend	9.56
SED	0.78
P value	<0.01

Data are means of 3 replicates.

Pathogenicity test

The *Pp3* isolate showed variable influence on the *Meloidogyne* spp. in suppressing root-knot disease (Table 2). There was a significant difference in numbers

of eggmasses produced by females of *Meloidogyne* spp. ($P < 0.01$). Higher numbers of eggmasses were recorded with females of *M. arenaria* (370) while fewer numbers of eggmasses were observed in treatment where *M.*

javanica (245) was present. Root galling differed significantly among treatments ($P < 0.01$) and higher gall infestation was recorded in *M. arenaria* treatment (6.2) while lesser galling was observed in *M. javanica* treatment (4.4). Final female population also varied among treatments ($P < 0.05$) showing higher numbers of females in *M. arenaria* treatment (456) and fewer with *M. javanica* (398).

Parasitism of females of *Meloidogyne* spp. by *Pp3*

differed significantly among treatments ($P < 0.01$) (Table 3). Higher numbers of females infected with *Pp3* spores were recorded among females of *M. javanica* (14.4) while lesser numbers of parasitized females were observed in *M. arenaria* treatment (9.8). There were significant variations ($P < 0.1$) in numbers of endospores/female produced by females of *Meloidogyne* spp. however egg set data did not differ ($P > 0.05$) in the treatments.

Table 2: Effect of *P. penetrans* isolate (*Pp3*) on numbers of egg masses, root galling intensity and final populations of females of *M. javanica*, *M. incognita*, *M. arenaria* and *M* blend on tomato plants after 750 degree days.

Treatments	Egg masses/plant	Root galling/plant (0-10)	Total females/plant
<i>M. javanica</i> + <i>Pp3</i>	245	4.4	398
<i>M. incognita</i> + <i>Pp3</i>	329	5.0	432
<i>M. arenaria</i> + <i>Pp3</i>	370	6.2	456
<i>M</i> blend + <i>Pp3</i>	326	5.4	455
SED	22.6	0.47	21.7
P Value	<0.01	<0.05	<0.05

Data are means of 5 replicates.

Table 3: Effect of *P. penetrans* isolates on numbers of eggs/egg mass, endospores/female and infection of females of *M. javanica*, *M. incognita*, *M. arenaria* and *M* blend on tomato plants after 750 degree days.

Treatments	Egg/egg mass	Infected females (out of 20)	Endospores/ females ($\times 10^3$)
<i>M. javanica</i> + <i>Pp3</i>	380	14.4	415
<i>M. incognita</i> + <i>Pp3</i>	422	11.2	391
<i>M. arenaria</i> + <i>Pp3</i>	438	9.8	348
<i>M</i> blend + <i>Pp3</i>	396	10.6	402
SED	24.4	0.54	24.5
P Value	>0.05	<0.01	>0.05

Data are means of 5 replicates.

DISCUSSION

As expected, variation among *Meloidogyne* spp. was observed regarding attachment of spores of *Pp3* isolate to J2s of different *Meloidogyne* spp. tested. The *Pp3* isolate was found compatible with different *Meloidogyne* spp. and *M. arenaria* proved the least good host. These results are in agreement with De Silva and Gowen (1995) who reported that *M. arenaria* as poor host to *Pp3* among *Meloidogyne* spp. The *Pp3* isolate was found more pathogenic on *M. javanica* compared with other *Meloidogyne* spp. The reason could be repeated culturing of the isolate on *M. javanica* and has developed host adaptation. Lower levels of parasitism and lesser

numbers of endospores/female in females of *M. arenaria* confirms the observation that rate of build-up of *P. penetrans* is much lower on less susceptible nematode populations (De Silva, 1992).

Spore attachment increased where J2s of *M. javanica*, *M. incognita* and *M. arenaria* were exposed to the selected *Pp3* as compared to *Pp3* isolates multiplied on individual *Meloidogyne* species. The reason behind this increase might be a wider genetic base of selected *Pp3* isolate as it was multiplied on a range of *Meloidogyne* spp. originating from different geographical areas. Lower attachment levels were recorded with *Pp3* isolates which were multiplied on a single *Meloidogyne* species

when exposed to the J2s of particular *Meloidogyne* spp. The results might be expected when *Pp* isolates with a narrow genetic base are exposed to different *Meloidogyne* spp. for attachment. The attachment level with less susceptible nematode, *M. arenaria* was increased and it is in agreement with Channer and Gowen (1992) who reported that when an isolate of *P. penetrans* was cultured on a mixed population of root-knot nematode then spore attachment was subsequently improved in all the nematode populations tested. As spore attachment is the first stage of infection, the increased attachment level recorded with the selected *Pp3* would help to increase infection of nematodes by the bacterium and ultimately resulting in better management of root-knot disease. The numbers of eggmasses and intensity of root galling were reduced in treatments with the selected *Pp3* and this decrease was for all *Meloidogyne* spp.

The presence of endospores in females is generally an indication of infectivity and varied significantly among females of the three *Meloidogyne* spp. The selected *Pp3* increased the infection of females of *Meloidogyne* spp. This supports the suggestion that the *Pp3* isolate has a wide genetic base and is more infective than a *Pp3* multiplied on a single nematode population.

Endospore production was not influenced by the selected *Pp3* isolate while egg productivity was less among females with the selected *Pp3* isolate as compared to other *Pp3* isolates. Invasion of J2s of *Meloidogyne* spp. did not differ under influence of *Pp3* isolates while higher numbers of females were recorded where *M. arenaria* was present. The reason might be because J2s of *M. arenaria* were less encumbered by spores of *Pp3* isolates resulting in greater penetration into roots. Genetic diversity, natural selection of nematodes to resist *Pp* infection, changes in genetic mix of root-knot nematodes by change in cropping pattern and possession of specificity by nematode and bacterium affect the durability of biological control by *P. penetrans*. The complexity of *Pasteuria-Meloidogyne* interaction suggests that *Pasteuria* may give effective control in certain situations in others it may not. Therefore, it is necessary to deploy the bacterium in blend form (mixing several *Pp* populations) or introducing a *Pp* population having a wide host range/genetic base to circumvent the problem of resistance naturally developing in fields. These results support the findings of Channer and Gowen (1988, 1992) that *P. penetrans* when used in

blend form is more effective than a single *Pp3* isolate on the management of root-knot nematodes.

The higher attachment level of the *Pp3* (*M* blend) recorded on juveniles of *M* blend compared with *Pp3* isolates (multiplied on individual nematode populations) strengthens the idea that attachment increases with the nematode host which was used for the *Pp* multiplication (Davies et al., 1988). As the nematode host used in this study was a mixture of *Meloidogyne* spp., therefore variation in the adhering potential of *Pp3* isolates was expected. As higher attachment increases infection (Rao et al., 1997; Giannakou, 1994, 1998; Giannakou et al., 1997) variation in invasion and infection would be expected when deploying these four *Pp3* isolates in pathogenicity test. These studies show that the *Pp3* isolate that was multiplied on the *Meloidogyne* blend is more pathogenic as compared to other *Pp3* isolates multiplied on a single *Meloidogyne* species. These results proved that a *Pp3* isolate having a wider genetic base is more effective as compared to *Pp3* isolates multiplied on single nematode species in suppressing root-knot nematode.

CONCLUSIONS

The results of experiment showed that the *Pp3* isolate was more pathogenic on *M. javanica* as compared with other *Meloidogyne* spp. (*M. incognita*, *M. arenaria* and *M* blend). The *Pp3* isolate was found compatible with different *Meloidogyne* spp. while *M. arenaria* proved the least good host to bacterial parasite.

AUTHORS' CONTRIBUTION

MS and SRG designed the study, MS performed the experiments and collected the data, SRG provided technical assistance, MS, MB, ZN and AH arranged and analyzed the data, SRG supervised the work, MS wrote the manuscript and SRG proofread the paper.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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