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Research Article

DIFFERENTIAL RESPONSES OF *MELOIDOGYNE* SPP. TO *PASTEURIA* ISOLATES OVER CROP CYCLES

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ABSTRACT

In the present study examining the interaction between *Meloidogyne* spp. and *Pasteuria* isolates over three crop cycles, significant differences were observed in eggmass production, root galling, plant root weight, endospore production, and parasitism rates. Higher eggmass production was noted in the *Meloidogyne* blend treatment, whereas *M. incognita* showed a marked decline after the third cycle. Root galling varied significantly with nematode populations and *Pasteuria* isolates, showing notable interaction effects ($P<0.01$). The *Meloidogyne* blend treatment exhibited the highest gall rating, while *M. javanica* and *M. incognita* treatments with *Pp*3 and *Pp*Ecu isolates resulted in lower galling. Fresh root weights of tomato plants differed significantly in the presence of *Meloidogyne* spp. and *Pasteuria* isolates, with significant interactions observed ($P<0.05$). Greater root weights were recorded in *M. javanica* and *M. incognita* treatments with *Pp*3 and *Pp*Ecu isolates. The production of *Pasteuria* endospores in *Meloidogyne* females also showed significant variability across crop cycles ($P<0.01$), with *M. incognita* and *Pp*Ecu treatments yielding the highest endospore numbers. Moreover, parasitism of *Meloidogyne* females by *Pasteuria* was significantly influenced by nematode population and isolate presence ($P<0.01$). Higher numbers of infected *M. incognita* females were found in *Pp*Ecu treatments. Final female populations of *Meloidogyne* spp. varied significantly under *Pasteuria* influence, with the *Meloidogyne* blend treatment showing the highest population and *M. incognita* under *Pp*Ecu the lowest. The results highlight the complex interactions between nematodes and the bacteria, emphasizing the potential of *Pasteuria* isolates to influence various aspects of *Meloidogyne* spp. development.

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INTRODUCTION

Root-knot nematodes (*Meloidogyne* spp.) are obligatory plant parasites with a global host range encompassing over 5500 plant species (Trudgill and Blok, 2001; Chen et al., 2017). They were identified among the top 10 plant-parasitic nematodes based on their scientific and economic significance (Jones et al., 2013). Their remarkable adaptability and broad host spectrum render them highly significant in commercial agriculture (Tariq-Khan et al., 2017, 2020; Haq et al., 2022; Mejias et

al., 2022; Yaseen et al., 2023; Saeed and Mukhtar, 2024). Root-knot nematodes induce direct harm to plant roots by inducing distinct galls and aggravate plant diseases by synergizing with other pathogens such as *Thielaviopsis*, *Fusarium*, *Phytophthora*, and *Ralstonia solanacearum*, leading to severe soil-borne plant ailments (Kamalnath et al., 2019; Asghar et al., 2020; Khan and Sharma, 2020; Ahmed et al., 2021; Khan et al., 2022; Aslam and Mukhtar, 2023a, b; 2024; Aziz et al., 2024; Yaseen et al., 2024).

Historically, chemical nematicides have been the primary means of control of root-knot nematodes (Desaeger and Watson, 2019). However, due to the risks that some chemical nematicides pose to human health and the environment, there is a shift towards safer and more environmentally friendly alternatives (Ntalli et al., 2018; Azeem et al., 2021; Mukhtar et al., 2021; Saeed et al., 2021, 2023; Haque and Khan, 2022; Afzal and Mukhtar, 2024). Consequently, the development of efficient and eco-friendly nematode management strategies remains a paramount concern in contemporary agriculture.

Management of root-knot nematodes by using biological control entities has gaining popularity amongst the farmers and nematologists. Among various biological control agents, *Pasteuria penetrans*, a mycelial endospore forming gram positive bacterium, has been widely investigated for having substantial prospective as a biocontrol agent against root-knot nematode, *M. incognita* (Mukhtar et al., 2002, 2005). The endospores of the bacterium in the soil cling to the cuticle of the second stage juveniles of root-knot nematodes and cause reductions in the infection. It is well documented and experimentally proven that an increase in the number of endospores per juvenile significantly decreased the attack of root-knot nematode to plant roots (Davies et al., 1988; Das et al., 2007).

Genetic diversity among field populations of nematodes is a critical factor influencing their interactions with pathogens, including parasitic bacteria. This diversity manifests in the existence of various biotypes or races within nematode species, some of which exhibit resistance to infection by parasitic bacteria like *Pasteuria penetrans* (*Pp*). Studies by Channer and Gowen (1992) and Tzortzakakis and Gowen (1994a,b) have long suggested that the host-parasite relationship between nematodes and *Pp* is intricate, largely due to the variability in bacterial specificity and nematode susceptibility.

Recent advancements in molecular biology and genomics have further illuminated the complexity of these interactions. For instance, research has shown that genetic variations within nematode populations can lead to significant differences in their immune responses to *Pp* infection. Certain genetic markers in nematodes are associated with heightened resistance to *Pp*. These findings underscore the adaptive potential of nematode populations, enabling them to survive and propagate even in the presence of parasitic bacteria (Davies and Williamson, 2006; Kamran et al., 2019a,b; Mohan et al., 2020).

Moreover, the specificity of the bacterium *Pp* is not uniform across all its strains. Some strains of *Pp* have a narrow host range, infecting only specific nematode biotypes. It was found that *Pp* strains collected from different geographical regions displayed varying levels of infectivity towards local nematode populations. Such variability in bacterial specificity implies that nematode populations can evolve resistance mechanisms, potentially rendering certain *Pp* strains ineffective over time (Liu et al., 2018; Davies et al., 2023).

The host-parasite relationship is further complicated by environmental factors and the presence of other microbial communities in the soil. Soil microbiota composition can influence the effectiveness of *P. penetrans* in controlling nematode populations. Diverse soil environments may either support or hinder the establishment of *P. penetrans*, thereby affecting its ability to infect nematodes (Ciancio et al., 2016).

When considering the practical implications of these findings, it becomes evident that the use of *P. penetrans* as a biological control agent must account for the genetic diversity of both the nematode and the bacterium. If *P. penetrans* populations with restricted host ranges are employed, they may fail to infect resistant nematode biotypes, leading to an unsuccessful control attempt. This scenario is particularly concerning if resistant nematode populations become dominant (Channer and Gowen, 1992; Tzortzakakis and Gowen, 1994a,b).

In light of recent research, it is crucial to adopt an integrated pest management (IPM) approach that combines the use of *Pp* with other control strategies. Such strategies might include crop rotation, use of resistant plant varieties, and soil amendments that enhance beneficial microbial communities. The adaptability of nematodes to various control measures, as highlighted by recent studies, suggests that a multi-

faceted approach is necessary to sustainably manage nematode populations and prevent the development of resistance (Afzal and Mukhtar, 2024). The primary objective of this study was to investigate the interaction effects of crop cycles, nematode populations, and *Pasteuria* isolates on the development of *Meloidogyne* spp. over three crop cycles.

MATERIALS AND METHODS

Three sources of *Pasteuria penetrans* viz. *Pp*3 (South Africa), *Pp*Ecuador, and *Pp* blend (prepared by mixing spores of *Pp*3 and *Pp*Ecuador in equal concentrations) were tested for their pathogenicity on different populations of root-knot nematodes, *M. javanica*, *M. incognita*, and an *M blend* (prepared by mixing J2s of *M. javanica* and *M. incognita* in equal numbers) on a tomato host.

Three groups of 8×10^5 J2s (2-4 days old) of *M. javanica*, *M. incognita*, and the M blend were exposed to three spore suspensions: *Pp*3 (3.65×10^4 spores/ml), *Pp*Ecuador (3.13×10^4 spores/ml), and the *Pp* blend (9×10^4 spores/ml). The bacterium and nematode suspensions were added to plastic trays (10×20 cm) and incubated at 28°C. Control groups of the same numbers of unencumbered J2s of *M. javanica*, *M. incognita*, and the *Meloidogyne* blend were kept in tap water in the same incubator for the same duration. The attachment level of the spores was monitored until 80% of the J2s reached the desired attachment level of 6-12 spores per J2.

Tomato plants cv. 'Tiny Tim' (40 days old) grown in 3 L pots were inoculated with 3000 J2s of *M. javanica*, *M. incognita*, and the *Meloidogyne* blend, each encumbered with spores of *Pp*3, *Pp*Ecuador, and the *Pp* blend. The same numbers of unencumbered J2s of the three *Meloidogyne* populations served as controls. Treatments were replicated five times and randomized on a greenhouse bench with an air temperature of 20-38°C. After 7 weeks, plants were harvested, and fresh root weight, numbers of eggmasses, and galling intensity were recorded.

Root systems from all treatments were dried, chopped, and macerated in a pestle and mortar to kill all nematode stages (J2s and eggs) and facilitate the release of bacterial inoculum. The root powders were mixed with the respective soils in plastic bags, which were then returned to their respective pots. After 2 weeks, 40-day-old tomato plants were transplanted into the pots. To prevent powdery mildew (*Erysiphe* spp.) infestation, plants were sprayed with benomyl (Benlate) at 0.5 g/L, which has no effect on nematodes. To support foliage growth, Phostrogen (NPK 14:4.4:22.4) was applied at 2 g/L twice during the second crop cycle.

The second crop cycle was harvested 7 weeks after transplanting, and the same procedure was repeated as described above. For the third cycle, to avoid heavy nematode infestation and nutrient deficiencies, half of the soil from each pot was replaced with fresh John Innes compost and thoroughly mixed. Tomato plants (40 days old) were transplanted and maintained under similar conditions as previously described. Final harvesting occurred 7 weeks after transplanting.

Data on eggmasses per plant, galling intensity, endospores per 100 mg of root powder, total females, and parasitism of *Meloidogyne* spp. females by *Pasteuria* isolates were recorded. All the data were analyzed by SAS and using Analysis of Variance.

RESULTS

Eggmass production

There was a significant difference in numbers of eggmasses produced by *Meloidogyne* spp. developed under the influence of *Pasteuria* isolates over three crop cycles (Table 1). Analysis of variance showed significant interaction between crop cycles and nematode and nematode and bacteria ($P<0.01$). Productivity of eggmasses by *Meloidogyne* spp. was significantly influenced by crop cycles as a strong interaction was observed between crop cycles and nematode populations ($P<0.01$). Higher numbers of eggmasses were recorded with *Meloidogyne* blend (356) while fewer eggmasses were observed with *M. incognita* after third cycle (Figure 1). A strong interaction between nematode and bacteria suggested that eggmass production was significantly influenced by the presence of nematode population and *Pasteuria* isolate in the treatments (Figure 2).

Root galling

Root galling caused by *Meloidogyne* spp. developed under the influence of *Pasteuria* isolates differed significantly over three crop cycles (Table 2). Analysis of variance showed significant interaction ($P<0.01$) between nematode populations and bacterium isolates regarding root galling as higher gall rating was recorded in *Meloidogyne* blend treatment (8.0) while lesser galling (4.4) was caused by juveniles of *M. javanica* and *M. incognita* developed in presence of *Pp*3 and *Pp*Ecu isolates (Figure 3).

Fresh root weights

There were significant differences in fresh root weights of tomato plants grown in presence of *Meloidogyne* spp. and *Pasteuria* isolates over three crop cycles (Table 3). Analysis of variance showed significant interaction (P<0.05) between nematode populations and bacterium isolates regarding fresh root weight of plants. A greater root weight of plants was recorded (10.2) in *M. javanica* and *M. incognita* treatments developed in presence of *Pp*3 and *Pp*Ecu isolates while lesser root weight was observed where *Meloidogyne* blend and *M. incognita* were present in absence of *Pp* isolates (Figure 4).

Table 1. Total numbers of egg masses of *Meloidogyne* spp. (*M. javanica*, *M. incognita* and *M* blend) as influenced by *P. penetrans* isolates (*Pp*3, *Pp*Ecu and *Pp* blend) after the third crop cycle.

Nematode	First Cycle				Second Cycle				Third Cycle			
Meloidogyne spp	*Pp*3	*Pp*Ecu	*Pp* blend	Control	*Pp*3	*Pp*Ecu	*Pp* blend	Control	*Pp*3	*Pp*Ecu	*Pp* blend	Control
M. javanica	252	334	292	503	201	319	290	425	211	246	215	406
M. incognita	344	268	300	477	299	227	264	407	246	182	203	400
*M.*blend	344	323	279	522	298	284	245	454	352	332	253	488

Data are means of 5 replicates.

Table 2. Root galling of females of *Meloidogyne* spp. (*M. javanica*, *M. incognita* and *M* blend) as influenced by *P. penetrans* isolates (*Pp*3, *Pp*Ecu and *Pp* blend) after the third crop cycle.

Nematode	First Cycle				Second Cycle				Third Cycle			
Meloidogyne spp	*Pp*3	*Pp*Ecu	*Pp*	Control	*Pp*3	*Pp*Ecu	*Pp*	Control	*Pp*3	*Pp*Ecu	*Pp*	Control
M. javanica	4.6	5.6	5.2	7.8	4.2	5.6	5.0	7.2	4.6	5.6	4.6	7.8
M. incognita	6.0	4.6	5.4	7.8	5.0	4.2	4.4	7.2	5.8	4.4	4.8	6.4
*M.*blend	5.8	5.2	4.6	8.2	5.4	5.4	4.4	7.6	6.4	5.8	5.6	8.2

Data are means of 5 replicates.

Table 3. Total numbers of females of *Meloidogyne* spp. (*M. javanica*, *M. incognita* and *M* blend) as influenced by *P. penetrans* isolates (*Pp*3, *Pp*Ecu and *Pp* blend) after the third crop cycle.

Nematode	First Cycle				Second Cycle				Third Cycle			
Meloidogyne spp	*Pp*3	*Pp*Ecu	*Pp* blend	Control	*Pp*3	*Pp*Ecu	*Pp* blend	Control	*Pp*3	*Pp*Ecu	*Pp* blend	Control
M. javanica	10.6	9.93	10.4	9.44	9.78	9.55	9.57	8.56	10.2	9.95	10.2	9.56
M. incognita	9.84	10.53	10.0	9.58	9.61	9.87	9.51	8.78	10.0	10.4	10.3	9.18
*M.*blend	9.70	9.85	10.3	9.37	9.50	9.65	9.94	8.69	9.74	9.45	9.94	9.44

Data are means of 5 replicates.

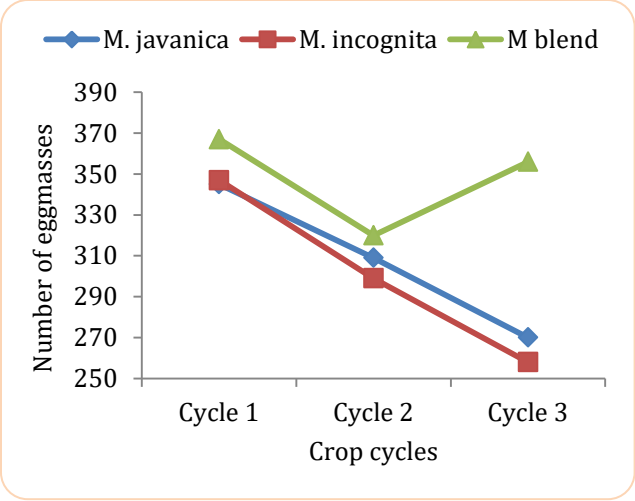

Figure 1: Effect of the interaction of nematode populations x crop cycles on egg mass production. SED for comparing the points is 11.42.

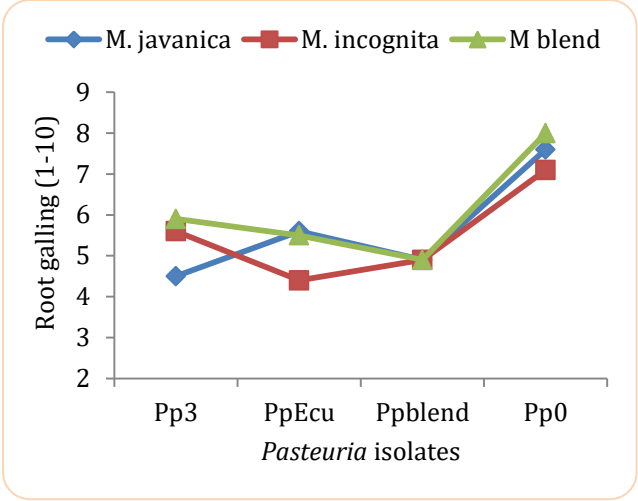

Figure 3: Effect of the interaction of nematode populations x *Pasteuria* isolates on root galling. SED for comparing the points is 0.26.

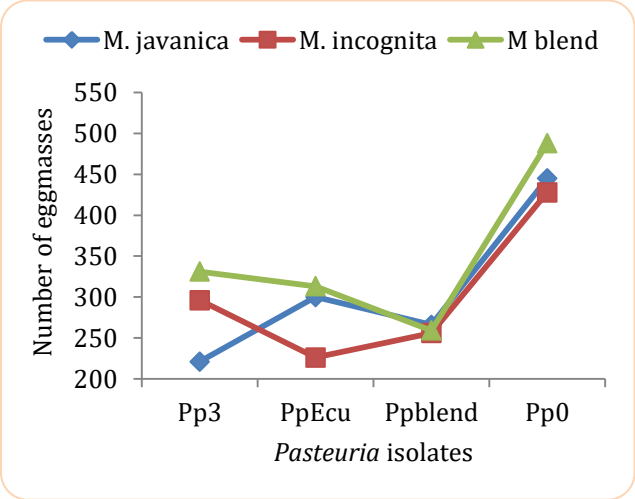

Figure 2: Effect of the interaction of nematode populations x *Pasteuria* isolates on egg mass production. SED for comparing the points is 13.1.

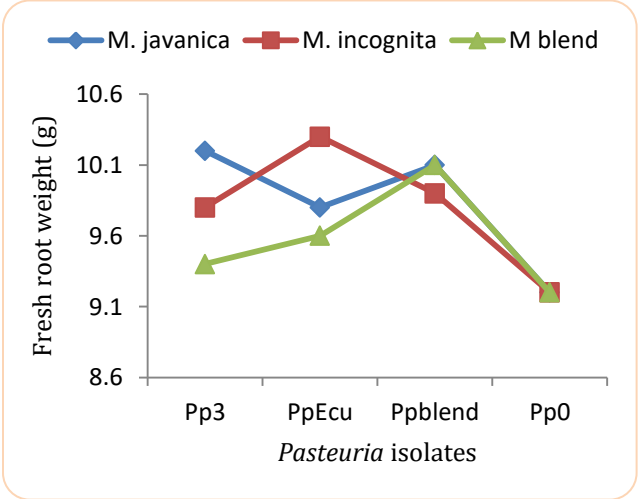

Figure 4: Effect of the interaction of nematode populations x *Pasteuria* isolates on fresh root weight. SED for comparing the points is 0.21.

Endospore production

The production of endospores in females of *Meloidogyne* spp. developed in presence of *Pasteuria* isolates varied significantly over crop cycles (P<0.01) (Table 4). Analysis of variance showed significant interaction between nematode and bacteria (P<0.01) as higher numbers of endospores/100 mg root system (39.5 × 10^6) were recorded in *M. incognita* and *Pp*Ecu isolate treatment while fewer numbers of endospores (30 × 10^6) were observed where *M. incognita* and *Pp*3 isolate were present (Figure 5). There was a significant interaction between crop cycles and nematode populations (P<0.01). Greater numbers of endospores (38.1 × 10^6) were produced by *M. incognita* after third crop cycle while lesser numbers of endospores were also recorded with *M. incognita* (33.8 × 10^6) after second crop cycle (Figure 6).

Parasitism of the females of *Meloidogyne* spp.

Parasitism of the females of *Meloidogyne* spp. by

Pasteuria was significantly influenced by the presence of nematode population and *Pasteuria* isolate in the treatments as a strong interaction (P<0.01) between nematodes and *Pasteuria* isolates was observed (Table 5). Higher numbers of infected females of *M. incognita* (13.8) were recorded in *Pp*Ecu treatment while greater numbers of *M. javanica* females (13.0) were parasitized than other nematode populations.

Table 4. endospores of *Meloidogyne* spp. (*M. javanica*, *M. incognita* and *M* blend) as influenced by *P. penetrans* isolates (*Pp*3, *Pp*Ecu and *Pp* blend) after the third crop cycle.

Meloidogyne spp	Second Cycle			Third Cycle		
	*Pp*3	*Pp*Ecu	*Pp* blend	*Pp*3	*Pp*Ecu	*Pp* blend
M. javanica	37	30	34	41.2	32.7	39.6
M. incognita	28	38	34	32.5	41.8	40.0
M.blend	32	31	38	29.8	30.0	39.4

Data are means of 5 replicates.

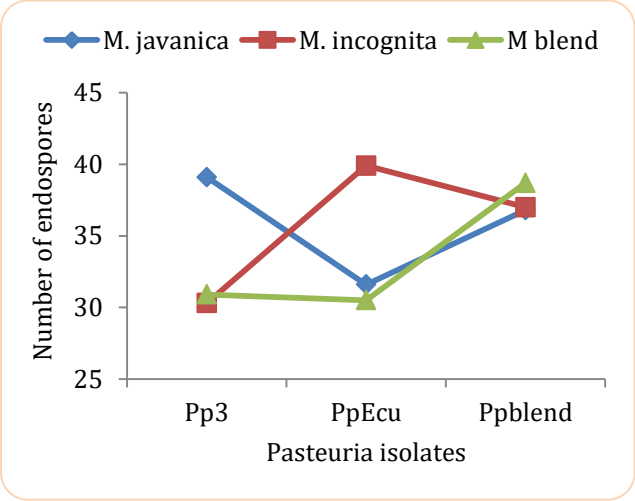

Figure 5: Effect of the interaction of nematode populations x *Pasteuria* isolates on endospore production. SED for comparing the points is 1.26.

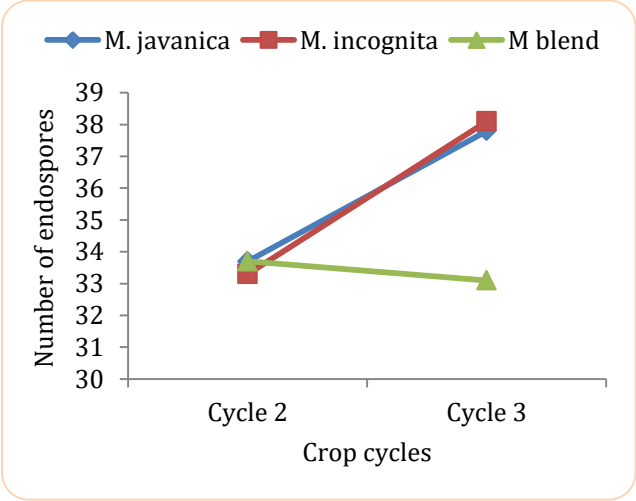

Figure 6: Effect of the interaction of nematode populations x crop cycle on number of endospores. SED for comparing the points is 1.62.

Table 5. Infection of females of *Meloidogyne* spp. (*M. javanica*, *M. incognita* and *M.*blend) by spores of *P. penetrans* isolates (*Pp*3, *Pp*Ecu and *Pp* blend) after third crop cycle (out of 20 females/replicate).

Meloidogyne spp.	*Pp* isolates			
	*Pp*3	*Pp*Ecu	*Pp* blend	Mean
M. javanica	13.6	11.8	13.2	13.0 a
M. incognita	11.8	13.8	13.4	12.8 a
*M.*blend	11.8	12.0	13.8	12.5 a
Mean	12.4 b	12.5 b	13.4 a	

Data are means of 5 replicates. Means with the same letters in columns are not statistically different by Least significant difference test. Treatment effect Nematode = P>0.05; *Pasteuria* = P<0.01; Nematode * *Pasteuria* = P<0.01 LSD = 1.10.

Final female populations

Analysis of variance showed significant interaction (P<0.01) between nematode and bacteria regarding final female populations of *Meloidogyne* spp. developed under the influence of *Pp* isolates over three crop cycles (Table 6). After the final harvest, significant variations in final populations of females were recorded. The higher numbers of females (574) were recorded in the *Meloidogyne* blend treatment while lesser numbers of *M. incognita* females (315) were recorded which developed under *Pp*Ecu over three crop cycles.

Table 6. Total numbers of females of *Meloidogyne* spp. (*M. javanica*, *M. incognita* and *M* blend) as influenced by *P. penetrans* isolates (*Pp*3, *Pp*Ecu and *Pp* blend) after the third crop cycle.

Meloidogyne spp.	*Pp* isolates				
	*Pp*3	*Pp*Ecu	*Pp* blend	Control	Mean
M. javanica	335	406	352	551	411b
M. incognita	401	372	315	482	392b
M blend	469	444	362	574	462a
Mean	401b	388bc	362c	536a	

Data are means of 5 replicates. Means with the same letters in columns are not statistically different by Least significant difference test.

Treatment effect Nematode = P<0.01; *Pasteuria* =P<0.01, Nematode * *Pasteuria* =P<0.01; LSD = 46.78

DISCUSSION

Root-knot nematodes of the genus *Meloidogyne* are soil-dwelling entities that were found infecting a variety of plants especially vegetables and several control strategies were devised for their management (Mukhtar et al., 2013; Kayani et al., 2017, 2018; Kayani and Mukhtar, 2018; Hussain and Mukhtar, 2019; Khan et al., 2019; Mukhtar, 2018; Mukhtar and Hussain, 2019; Mukhtar and Kayani, 2019, 2020; Nazir et al., 2019). Among various biocontrol agents, *P. penetrans* has been investigated widely by researchers and found effective in suppressing root-knot nematode populations (Mukhtar and Ahmad, 2000; Mukhtar et al., 1999, 2000, 2002, 2005; Shahid et al., 2007).

In the present investigation, the suppression of root-knot disease was strongly influenced by the presence of nematode populations and *Pasteuria* isolates over three crop cycles. *P. penetrans* was effective in reducing root-knot disease, as fewer eggmasses and less root galling were recorded where *Pasteuria* was present (Ahmad and Mukhtar, 2007a,b). Incorporating roots containing *P. penetrans* spores proved effective in suppressing nematode buildup in the pots, significantly influenced by the presence of nematode populations, *Pasteuria* isolates, and crop cycles. It seems likely that the spores of the parasite accumulate in the soil when these roots are incorporated (Daudi et al., 1990; Gowen and Ahmed, 1990; Daudi, 1991). This work indicates that with this practice, a high spore concentration per gram of root will develop when plants are uprooted at the end of the third crop cycle.

Eddaoudi and Bourijate (1998) found that *Pasteuria* suppressed eggmasses and gall indices over three crop cycles, suggesting that it takes time to establish a bacterial population in the soil (Atibalentja et al., 1998). Infected females inside the roots may also degrade and release spores into the soil during plant growth. Juvenile nematodes invading a root system from applied inoculum or from a residual soil population are more likely to come into contact with spores than juveniles subsequently hatching from a root system (Stirling, 1981). The rate of parasite buildup is much lower on less susceptible nematode populations (De Silva et al., 1996).

Higher numbers of spores were recorded in *M. incognita* and *P. penetrans* treatments, which proved more effective compared to other *P. penetrans* isolates on single root-knot populations. *P. penetrans* has maintained its reproduction potential despite an increase in nematode population in the same treatment, and it has been reported by several workers that the *Pasteuria* isolate from Ecuador was found more effective in controlling *M. incognita* than *Pp*3 (Trivino and Gowen, 1996). These results contradict previous findings where *Pp*Ecu was recorded to be less effective in controlling the root-knot nematode *M. javanica* than other *Pasteuria* isolates.

The *Pasteuria* blend has shown consistency in controlling all *Meloidogyne* populations tested in the experiment, which aligns with findings of other workers (Channer and Gowen, 1992), who showed that using blends of *P. penetrans* could develop aggressive populations. In field situations, it may need to be investigated whether the aggressiveness of a natural or single population increases. Similarly, blends of *Pasteuria* populations might be deployed where root-knot nematodes become resistant to or fail to be encumbered by single populations (Tzortzakakis and Gowen, 1994b). Blends attaining lower attachment levels than single populations were more infective, suggesting there is no relationship between the number of spores attaching and the subsequent infection of the developing host nematode.

An increase in the number of eggmasses and root galling was recorded in *Meloidogyne* blend treatments over three crop cycles; however, this increase was significantly influenced by the presence of different *Pasteuria* isolates. This might be expected if single *Pasteuria* isolates become unable to overcome resistance development among nematode populations. As diversity occurs in *Meloidogyne* spp., the presence of more than one species resulted in resistance to *Pasteuria* spores and ultimately increased disease severity. The population constitution determined after the final harvest suggested that *M. incognita* might have been dominant as it was less susceptible to *Pp*3 and *Pp* blend. Working with *Pasteuria* as a biological control agent presents a significant challenge. Deploying *Pasteuria* blends rather than a single isolate can minimize this challenge, or using isolates with a wider host range can overcome this problem and sustain the control potential.

AUTHORS' CONTRIBUTION

MS and SRG designed the study; MS performed the experiments and collected the data; SRG provided technical assistance; KM and MB analyzed the data; MS wrote the manuscript and MZN and MAH proofread the paper.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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