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STUDIES ON THE POSSIBLE ROLE OF PLANT HOST ON THE DEVELOPMENT OF ROOT-KNOT NEMATODE, *MELOIDOGYNE JAVANICA* AND *PASTEURIA PENETRANS* AS AFFECTED BY DIFFERENT HARVESTING DATES

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ABSTRACT

There were strong interactions between plant-hosts and *Pasteuria* (P<0.01) on numbers of eggmasses, root galling and numbers of eggs/eggmass produced by females of *Meloidogyne javanica* after 422, 595 and 767 degree days after first, second and third harvests. The presence and absence of the biocontrol agent (*Pasteuria penetrans*) in the treatments influenced these parameters and it was also influenced by plant hosts. After the first harvest, there were significant differences in the above-mentioned parameters between *P. penetrans* and control treatments (P<0.01). There was lesser root galling (3.13) in the treatments where *P. penetrans* was applied compared with untreated control (5.53). Plant hosts also differed significantly in root galling (P<0.01) and the interaction between plant-host and *Pasteuria* was non-significant (P>0.05). After the second harvest, there were significant differences between *Pasteuria* and control treatments in these parameters. Plant-hosts differed significantly in numbers of eggmasses and root galling (P<0.01) and there was no interaction between plant-host and *Pasteuria* (P>0.05) regarding eggmasses and root galling. After the third harvest, there were significant differences between *Pasteuria* and control treatments in the production of eggmasses (P<0.01), root galling, and the number of eggs/eggmass (P<0.01). There were fewer eggmasses (379) and lesser galling (5.1) in the treatments where *P. penetrans* was applied. Plant-hosts differed significantly in numbers of eggmasses and root galling (P<0.01). However, there was no interaction between plant-host and *Pasteuria* (P>0.05) regarding eggmasses and root galling. Contrarily, a significant interaction between plant-hosts and *Pasteuria* (P<0.01) and greater numbers of eggs were observed among females developed on tomato in the presence (385) and absence (629) of *Pasteuria*. The rate of parasitism of *M. javanica* was very low and there was no *P. penetrans* infection after 422-degree days as neither vegetative stages nor mature endospores were observed in females. After 595-degree days, few females were observed having vegetative stages of the bacterium. The parasitism of females was only observed though very low after 767-degree days and females reared on okra were infected in greater numbers than those reared on tomato and eggplant.

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INTRODUCTION

Root-knot nematodes of the genus *Meloidogyne* are soil-dwelling entities that were found infecting a variety of plants especially vegetables (Hussain and Mukhtar, 2019; Kayani et al., 2017; Kayani et al., 2018; Mukhtar and Hussain, 2019; Mukhtar and Kayani, 2019, 2020; Tariq-Khan et al., 2020; Tariq-Khan et al., 2017). To date, more than 100 species of root-knot nematodes from all over the world have been known, most of which are widely prevalent in tropical and subtropical regions. Root-knot nematodes are extremely pathogenic and have the ability to break the host resistance (Jones et al., 2013; Mukhtar, 2018). It is well documented that the incidence of root-knot nematodes has significant correlation with pathogenic microorganisms. The interactions between different root-knot nematodes species and phytopathogenic microorganisms can be synergistic and antagonistic. The former has resulted in disease complexes and augmented the severity of bacterial wilts (Asghar et al., 2020; Mukhtar et al., 2021). On the other hand, the antagonistic interaction caused reductions in nematode infestations and is used as biocontrol agents (Ahmad and Mukhtar, 2007a; Ahmad and Mukhtar, 2007b; Mukhtar et al., 1999; Mukhtar et al., 2000; Saeed et al., 2021). Some of these bacteria, such as *Pasteuria* can suppress the growth and reproduction of root-knot nematodes (Mukhtar and Ahmad, 2000; Mukhtar et al., 2013).

Root-knot nematodes have very wide host range and high rate of multiplication and are extremely difficult to be managed. These sedentary endoparasites are responsible for causing an estimated US $100 billion loss per annum worldwide (Oka et al., 2000a; Oka et al., 2000b). Root-knot nematodes are commonly controlled by using synthetic nematicides and crop varieties resistant to nematodes (Ahmed et al., 2021; Azeem et al., 2021; Haq et al., 2022; Kayani and Mukhtar, 2018; Khan et al., 2019). Although a large number of chemicals are available in the market yet their large scale applications are associated with health and environmental concerns hence not recommended. Furthermore, owing to be costly, small-scale poor farmers cannot afford them (Chitwood, 2002). This situation has increased the urgency and compelled the farming community and agricultural scientists to search for alternative nematode management strategies which should be ecofriendly (Mashela et al., 2008; Pinkerton et al., 2000).

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; Mukhtar et al., 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 (Das et al., 2007; Davies et al., 1988).

Meloidogyne spp. are an important group of plant parasitic nematodes infecting a wide range of host plants. Plant parasitic nematodes derive their nutrition through parasitism and vary in their effects on plant hosts. The same nematode may cause dissimilar damage to different hosts under identical environmental conditions. Similarly, different species of the same genus or races of the same species of nematode may differ in their damage potential and reproductive efficiency on a susceptible host. Some *Meloidogyne* spp. are more pathogenic to certain hosts and even certain crops are non-hosts to the nematode. Existence of biotypes or races within *Meloidogyne* spp. affects the host-parasite relationship. Physiologic variation in the host-parasite relationship within the genus *Meloidogyne* is not only due to the variability within the nematode but also due to the difference in susceptibility between cultivars of the same host. Huang (1986) found that carrot varieties differed in host status to *Meloidogyne arenaria*, the poorest host decreased rates of invasion, female development and fecundity. Some workers have reported differential ability of invasion, plant damage and fecundity by different *Meloidogyne* spp./races among different plant hosts (Arens et al., 1981; Barker et al., 1981; Hadisoeganda and Sasser, 1982; Khan and Haider, 1991a; Khan and Haider, 1991b; Kinloch and Allen, 1972). These are due to greater ability of some *Meloidogyne* spp. to locate and invade the roots of certain plant hosts or cultivars, greater inherent reproductive capacities of some *Meloidogyne* spp. and even differences in numbers of infection sites of different plant hosts (Arens et al., 1981; Khan and Haider, 1991a; Khan and Haider, 1991b).

Keeping in view the differential pathogenic ability of

Meloidogyne spp. and *P. penetrans* isolates, the following studies were carried out to answer these questions a). Does the root-knot nematode, *M. javanica* develop and multiply at the same rates on tomato, eggplant and okra? b). Is the infective potential of *Pasteuria penetrans* influenced by different plant hosts (at a standard temperature)? c). Will the rate of development of nematode and bacterium be similar on different host plants?

MATERIALS AND METHODS

A population of root-knot nematode, *Meloidogyne javanica*, obtained from tobacco field of Kutsaga Research Station, Zimbabwe was used in the study. The nematode was multiplied in pots from single eggmasses from females identified on the basis of perineal patterns. Similarly, an isolate of *Pasteuria penetrans* (Pp3) originated from South Africa was used in the experiments.

Tomato and eggplants were raised in seed trays while okra seeds were sown directly into the pots. The above-mentioned root-knot nematode population of *M. javanica* from Zimbabwe was used in the study and applied @ 2000 J2s/plant encumbered with a mean of 6.8 spores/J2. After inoculations, plants were placed in the growth room. The experiment was run and harvesting was made 27, 38 and 49 days after inoculation when a sum of 422, 595 and 767-degree days were accumulated. Every treatment belonging to each level of host and harvesting date was replicated five times. The pots without *P. penetrans* were treated as control. Therefore, ninety plants were used in a randomised complete block design.

For per cent infection of females by *P. penetrans*, 20 females were picked randomly from each replicate. After squashing between slide and cover slip in a drop of water, each female was examined under a compound microscope (×400) for the presence of mature endospores. *Pasteuria* infected females had intact and dark appearance, except in the head and median bulb regions. This may be due to the fact that the bacterium restricts its growth to the region occupied by the reproductive system of the host (Bird, 1986). When necessary, the stage of *P. penetrans* development was assessed by differentiating vegetative stages from mature endospores.

All the data were analysed by SAS and using Analysis of Variance. t, LSD, x^2 tests were used to compare treatment means.

RESULTS AND DISCUSSION

There was strong interaction between plant-hosts and *Pasteuria* (P<0.01) on numbers of eggmasses produced by females of *M. javanica* (Table 1) after 422 degree days. The presence and absence of the biocontrol agent (*P. penetrans)* in the treatments influenced the eggmass production and it was also influenced by plant hosts. There were significant differences between *Pasteuria* applied and control treatments in root galling caused by *M. javanica* (P<0.01) (Table 2). There was lesser root galling (3.13) in the treatments where *P. penetrans* was applied compared with untreated control (5.53). Plant-hosts also differed significantly in root galling (P<0.01) and the interaction between plant host and *Pasteuria* was non-significant (P>0.05). After the first harvesting, there was significant difference in numbers of eggs/eggmass between *P. penetrans* and control treatments (P<0.01) (Table 3). There was no significant difference between plant-hosts regarding egg set data (P>0.05) and there was no interaction between plant-host and *Pasteuria* (P>0.05).

Table 1: Effect of different plant-hosts (tomato, eggplant and okra) on eggmasses/plant of *M. javanica* as affected by *P. penetrans* after 422 degree days.

Treatments	Eggmasses/plant		
	Pasteuria	Control	Mean
Tomato	189	368	280 a
Okra	158	403	279 a
Eggplant	205	307	256 a
Mean	184 b	358 a	

Data are means of 5 replicates. Means with the same letters are not significantly different by least significant difference test.

Treatment effects: Host P>0.05; *Pasteuria* P<0.01; Host **Pasteuria* P<0.01; LSD = 58.67.

Table 2: Effect of different plant-hosts (tomato, eggplant and okra) on galling index (0-10) of *M. javanica* as affected by *P. penetrans* after 422 degree days.

Treatments	Galling index (0-10)		
	Pasteuria	Control	Mean
Tomato	3.4	6.2	4.8 a
Okra	3.0	5.6	4.3 b
Eggplant	3.0	4.8	3.9 b
Mean	3.13 b	5.53 a	

Data are means of 5 replicates. Means with the same letters are not significantly different by least significant difference test.

Treatment effects: Host P<0.01; LSD = 0.43; *Pasteuria* P<0.01; LSD = 0.35; Host **Pasteuria* P>0.05

Table 3: Effect of different plant-hosts (tomato, eggplant and okra) on eggs/eggmass of *M. javanica* as affected by *P. penetrans* after 422 degree days.

Treatments	Eggs/eggmass		
	Pasteuria	Control	Mean
Tomato	412	641	526 a
Okra	369	605	487 a
Eggplant	431	645	538 a
Mean	404 b	630 a	

Data are means of 5 replicates. Means with the same letters are not significantly different by least significant difference test.

Treatment effects: Host P>0.05; *Pasteuria* P<0.01; LSD = 47.74; Host **Pasteuria* P>0.05

After the second harvest, there were significant differences between *Pasteuria* and control treatments in production of eggmasses (P<0.05) and root galling (P<0.01) (Tables 4 and 5). There were fewer eggmasses and lesser galling in the treatments where *P. penetrans* was applied. Plant-hosts differed significantly in numbers of eggmasses and root galling (P<0.01). There was no indication of interaction between plant host and *Pasteuria* (P>0.05) regarding eggmasses and root galling.

After the second harvesting, there was significant difference in numbers of eggs/eggmass between *P. penetrans* and control treatments (P<0.05) (Table 6). There was significant difference between plant-hosts regarding egg set data (P<0.01) and there was no interaction between plant-host and *Pasteuria* (P>0.05).

Table 4: Effect of different plant-hosts (tomato, eggplant and okra) on the number of eggmasses of root-knot nematode, *Meloidogyne javanica* as affected by *Pasteuria penetrans* after 595 degree days.

Treatments	Eggmasses/plant		
	Pasteuria	Control	Mean
Tomato	269	529	399 ab
Okra	317	576	446 a
Eggplant	263	467	365 b
Mean	283 b	524 a	

Data are means of 5 replicates Means with the same letters are not significantly different by least significant difference test.

Treatment effects Host P<0.05, LSD = 64.48; *Pasteuria* P<0.01, LSD = 52.65; Host **Pasteuria* P>0.05

Table 5: Effect of different plant-hosts (tomato, eggplant and okra) on root galling of root-knot nematode, *Meloidogyne javanica* as affected by *Pasteuria penetrans* after 595 degree days.

Treatments	Galling index (0-10)		
	Pasteuria	Control	Mean
Tomato	4.6	7.4	6.0 a
Okra	4.8	6.2	5.5 a
Eggplant	3.8	5.4	4.6 b
Mean	4.4 b	6.3 a	

Data are means of 5 replicates Means with the same letters are not significantly different by least significant difference test.
Treatment effects Host P<0.01, LSD = 0.68; *Pasteuria* P<0.01, LSD = 0.56; Host **Pasteuria* P>0.05

Table 6: Effect of different plant-hosts (tomato, eggplant and okra) on eggs/eggmass of root-knot nematode, *Meloidogyne javanica* as affected by *Pasteuria penetrans* after 595 degree days.

Treatments	Eggs/eggmass		
	Pasteuria	Control	Mean
Tomato	591	625	608 b
Okra	694	721	707 a
Eggplant	494	565	530 c
Mean	593 b	637 a	

Data are means of 5 replicates. Means with the same letters are not significantly different by least significant difference test.
Treatment effects Host P<0.01, LSD = 50.18; *Pasteuria* P<0.05, LSD = 40.97; Host **Pasteuria* P>0.05

After the third harvest, there were significant differences between *Pasteuria* and control treatments in the production of eggmasses (P<0.01) and root galling (P<0.01) (Tables 7 and 8). There were fewer eggmasses (379) and lesser galling (5.1) in the treatments where *P. penetrans* was applied. Plant-hosts differed significantly in numbers of eggmasses and root galling (P<0.01). There was no indication of interaction between plant host and *Pasteuria* (P>0.05) regarding eggmasses and root galling.

After the third harvesting, there was significant influence of plant hosts and *Pasteuria* on numbers of eggs produced by females of *M. javanica* (Table 9). Analysis of variance showed significant interaction between plant hosts and *Pasteuria* (P<0.01) and greater numbers of eggs were observed among females developed on tomato in presence (385) and absence (629) of *Pasteuria*.

Table 7: Effect of different plant-hosts (tomato, eggplant and okra) on the number of eggmasses of root-knot nematode, *M. javanica* as affected by *P. penetrans* after 767-degree days.

Treatments	Eggmasses/plant		
	Pasteuria	Control	Mean
Tomato	374	556	465 b
Okra	511	608	560 a
Eggplant	253	464	359 c
Mean	379 b	543 a	

Data are means of 5 replicates Means with the same letters are not significantly different by least significant difference test.
Treatment effects Host P<0.01 LSD = 80.6; *Pasteuria* P<0.01 LSD = 66.08; Host **Pasteuria* P>0.05

Table 8: Effect of different plant-hosts (tomato, eggplant and okra) on root galling of root-knot nematode, *M. javanica* as affected by *P. penetrans* after 767-degree days.

Treatments	Galling index (0-10)		
	Pasteuria	Control	Mean
Tomato	5.0	7.4	6.2 ab
Okra	6.2	7.8	7.0 a
Eggplant	4.2	6.6	5.4 b
Mean	5.1 b	7.2 a	

Data are means of 5 replicates Means with the same letters are not significantly different by least significant difference test. Treatment effects Host P<0.01, LSD = 0.87; *Pasteuria* P<0.01, LSD = 0.71; Host **Pasteuria* P>0.05

Table 9: Effect of different plant-hosts (tomato, eggplant and okra) on eggs/egg mass of root-knot nematode, *M. javanica* as affected by *P. penetrans* after 767-degree days.

Treatments	Eggs/eggmass		
	Pasteuria	Control	Mean
Tomato	385	629	512 b
Okra	414	531	472 c
Eggplant	584	608	596 a
Mean	469 b	585 a	

Data are means of 5 replicates Means with the same letters are not significantly different by least significant difference test. Treatment effects Host P<0.01; *Pasteuria* P<0.01, Host **Pasteuria* P<0.01; LSD = 47.18

The rate of parasitism of *M. javanica* was very low and there was not an indication of *P. penetrans* infection after 422-degree days as neither vegetative stages nor mature endospores were observed in females (Table 10). After 595-degree days, few females were observed having vegetative stages of the bacterium (Table 11). The parasitism of females was only observed though very low after 767-degree days and females reared on okra were infected in great numbers than those reared on tomato and eggplant (Table 12).

Table 10: Numbers of females of *M. javanica* infected by *P. penetrans* as influenced by plant-hosts after 422-degree days (20 females/replication).

Infection of females	Plant Hosts		
	Tomato	Okra	Eggplant
Vegetative stages	-	-	-
Mature endospores	-	-	-
Total	-	-	-

Data are means of 5 replicates

Table11: Numbers of females of *M. javanica* infected by *P. penetrans* as influenced by plant-hosts after 595-degree days (20 females/replication).

Infection of females	Plant Hosts		
	Tomato	Okra	Eggplant
Vegetative stages	7	12	9
Mature endospores	-	-	-
Total	7	12	9

Data are means of 5 replicates

Table 12: Numbers of females of *M. javanica* infected by *P. penetrans* as influenced by plant-hosts after 767-degree days (20 females/replication).

Infection of females	Plant Hosts		
	Tomato	Okra	Eggplant
Vegetative stages	12	16	8
Mature endospores	3	9	2
Total	15	25	10

Data are means of 5 replicates

When working with *P. penetrans* in investigation of biological control of root-knot nematodes, the main difficulty is to predict its life cycle under the conditions of the experiment and the development of the nematodes. The biocontrol agent *P. penetrans* was found effective in controlling the root knot disease on okra, eggplant and tomato at three harvesting dates. The biocontrol agent has significantly reduced root galling, eggmasses, and eggs per eggmass production on tested three hosts when compared with control after harvesting at 422, 595 and 750 degree days. Okra was found to be the best host for root-knot nematode development. In all three harvestings, higher numbers of eggmasses and root galling was recorded on okra. The life cycle of *P. penetrans* can be completed on *M. javanica* infecting all the plant-hosts examined in this study. Among the plant-hosts, the percentages of *P. penetrans* infected females were found low. There was no parasitism observed after first and second harvestings among females of *M. javanica* but very few females were recorded having mature endospores after the third harvest. Data of this investigation suggests that the time of 422 degree days and 595 degree days in experiments investigating the influence of *P. penetrans* to *M. javanica* is too short period and not enough for an accurate conclusion. It is clear that under these specific experimental conditions, at least 750 degree days are needed for most of the parasitized females to have mature endospores. This is crucial when an *in vivo* technique is used for the multiplication of spore inoculum, although early harvesting is preferred for infectivity test to avoid confusion with subsequent generations of root-knot nematodes. Although many juveniles seem to escape infection but there is a higher reduction to the numbers of eggs and eggmasses in the treatments where nematodes encumbered with *P. penetrans* spores were inoculated. This is probably due to latent infection of juveniles by *P. penetrans* spores which although it is not easily distinguishable; it influences the productivity of eggs/eggmass. The numbers of females infected by *P. penetrans* should be higher than these found in this investigation after the third harvesting. Also checking twenty females per root system for infection may not be enough for an accurate observation. Larger numbers of females would have given a more precise infection percentage. There is suspicion that while nematodes were infected by *P. penetrans* they did not show distinguishable endospores. This is supported by the evidence that some females contained microcolonies but not endospores.

AUTHORS' CONTRIBUTION

MS and SRG designed the study, MS performed the experiments and collected the data, SRG provided technical assistance, MS and MB analysed the data, MS wrote the manuscript and SRG proofread the paper.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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