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Role of bacterial bioagent, *Pasteuria penetrans* in the management of root knot nematode, *Meloidogyne incognita* by altering the lifecycle

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ABSTRACT: *Pasteuria penetrans* is a bacterial hyper parasite of nematodes. The influence of *P. penetrans* infestation on life cycle of root knot nematode was studied in tomato. One day after inoculation, 40.0 per cent of *P. penetrans* encumbered J2 and 63.7 per cent of healthy J2 had penetrated into the roots of tomato (t>0.05) showing that J2 parasitized by the hyper-parasite were less infective than the healthy one. Infection of *M. incognita* by *P. penetrans* resulted in slight delay in the completion of life cycle. The duration of all post-parasitic stages of the parasitizing nematodes took longer time for development and the adult stage was reached in 30 days as against 24 days for non-parasitized nematodes. Sex ratio was affected by *P. penetrans* infection. Number of males produced were high from healthy J2 when compared to the *P. penetrans*-infected. *P. penetrans*-infected females were milky white in colour and males were filled with spores. As a result of lower parasitization by nematodes, fewer galls were formed when plants were inoculated with *P. penetrans*-encumbered J2. The number of galls under inoculation with *P. penetrans*-infected J2 were 18.3/plant as against 35.0/plant when inoculated with healthy juveniles (t>0.05). Influence of *P. penetrans* on delaying nematode lifecycle and various developmental stages are discussed in this research paper.

Keywords: Life cycle, *Meloidogyne incognita*, *Pasteuria penetrans*, tomato

INTRODUCTION

Pasteuria penetrans (Thorne, 1940) Sayre and Star is an obligate nematode parasite which has been the subject of intensive study in recent years as a biological control agent of nematodes. The life cycle, mode of infection, reproduction and survival stages of this bacteria have to be studied to determine its biocontrol potential against plant – parasitic nematode. Life cycle of *P. penetrans* is well documented by several authors in root-knot nematode (Mankau and Imbriani, 1975; Sayre, 1980; Brown and Smart, 1985; Chen *et al.*, 1997) and in cyst nematode (Bhattacharya and Swarup, 1989; Davies *et al.*, 1990; Sayre *et al.*, 1991). Mankau and Imbriani (1975) reported that the life cycle of this bacterium was unlike that of other known protozoa but resembled more of a bacterium and concluded that it developed in the pseudo-coelomic fluid into irregular vegetative thalli or filaments. Sayre (1980) described the life stages of this bacterium as vegetative growth phase, colony fragmentation, sporogenesis, soil phase, spore attachment and penetration. Recently Keith *et al.*, (2011) reevaluated the life-cycle of *P. penetrans*. They have observed similarity between *Bacillus* and described three phases: Phase I: attachment and germination; Phase II: rhizoid production and exponential growth; and Phase III: sporogenesis. A pot cultureexperiment was carried out to study influence of *P. penetrans* on altering the lifecycle of root-knot nematode, *Meloidogyne incognita.*

MATERIALS AND METHODS

Lifecycle of *P. penetrans* was studied using tomato cv. CO 3 as host plant and *M. incognita* as nematode host. Tomato seedlings (15-days-old) were transplanted into two sets of plastic cups (51 cups/set) filled with steam sterilized soil. Native isolate of *P. penetrans* (TNAU-PpM2) was used for this study. *P. penetrans*-infected second stage juvenile (J2) of *M. incognita* (Fig. 1) were inoculated to one set of cups and healthy J2 to another set of cups at 500 J2/cup, three days after transplanting. The juveniles used for inoculation were encumbered by an average number of 10 spores/J2. The percentage of penetration of J2 was recorded at 24 and 48 h intervals. The developmental stages of *P. penetrans* were examined by dissecting the juveniles at 8, 14, 24, 26, 30 and 32 days after inoculation. Number of matured females per g of root was recorded at the end of the observation period. Dimensions of all developmental stages of the bacterium was measured using ocular micrometer and accordingly camera lucida diagrams were also drawn.

RESULTS AND DISCUSSION

One day after inoculation, 63.7 per cent of *P. penetrans*e ncumbered and 40.0 per cent of healthy J2 had penetrated into the roots of tomato (t>0.05) showing that J2 parasitized by the hyper-parasite were less infective than the healthy ones. Penetration of second-stage juveniles was reduced by 37 per cent in the present study which is in agreement with observation of Prasad (1971). *P. penetrans* reduced the multiplication of root-knot nematodes by reducing the numbers that invade roots (Mankau and Prasad, 1977; Stirling, 1984; Brown and Smart, 1985). However no reason has been advanced for reduced penetration of roots by the spore encumbered J2 of root-knot nematodes by the previous workers. The spore germinates only during the parasitic life of juveniles and until such time they remain simply attached without causing any visible pathological changes on the free living J2. It is reasonable suppose that the encumbered juveniles are less active and their locomotion is hindered, affecting their penetration into the roots. This hypothesis is supported by observation made by Davies *et al.,* (1988) who reported that the invading capacity of J2 was reduced as the spore load increased.

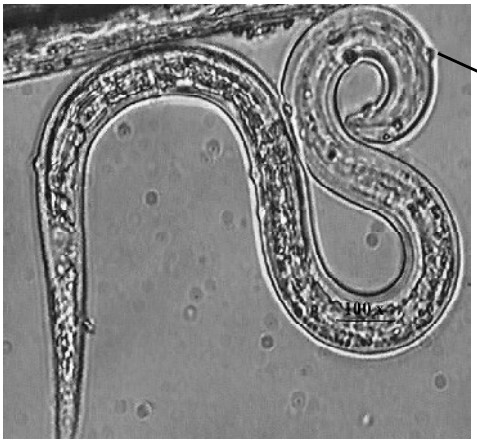

Fig. 1. Endospores of *P. penetrans* attached on the second stage juvenile cuticle of *M. incognita.*

P. penetrans-encumbered J2 resulted in fewer galls due to lower parasitization by nematodes. Number of galls were 18.3 galls /root-system under inoculation with *P. penetrans*-infected J2 against 35.0/root-system when inoculated with healthy juveniles (t>0.05). This is to be expected as already stated, the invading capacity of the J2 was affected by *P. penetrans*. Other workers have also reported similar findings (Brown and Smart, 1985; Davies *et al*., 1988).

The endospores of the bacterium started germination and penetrated into the host nematode in about three days after infection. After penetration, bacterial thalli started spreading inside the post-parasitic second-stage juvenile nematode. Spherical colonies of the bacterium were formed with dichotomously branched mycelium in about 14 days after infection, coinciding with the nematode reaching third-juvenile stage. Four-celled stage of the bacterium was observed in about 18 days after infection. This parasite then fragmented into 2 cell stage which developed into unicellular stage on the 24th day by further fragmentation (Fig. 2). The mode of infection, developmental stages, sporogensis are similar as those described by Mankau and Imbriani (1975). Mature spores of the bacterium were formed in about 30 days after infection synchronizing with females reaching the adult stage (Fig. 2). The dimension of the different developmental stages *P. penetrans* are furnished in Table 1.

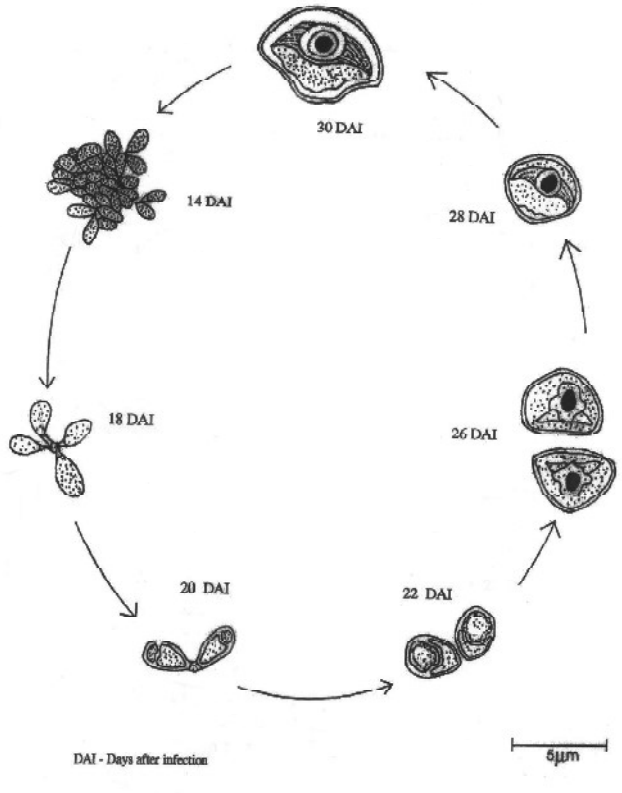

Fig. 2. Developmental stages of *Pasteuria penetrans* inside the root knot nematode, *M. incognita*

Infection of *M. incognita* by *P. penetrans* resulted in slight delay in the completion of life cycle (Table 2).

Table 1. Spore dimension of different development stages of *P. penetrans*

Stage	Dimensions (µm) Mean ± SD
Thallus (or) vegetative	0.75 ± 0.2
4-celled	1.5 ± 0.1
2-celled (early)	2.2 ± 0.14
2-celled (later)	2.6 ± 0.2
Single cell (unicelluar)	2.8 ± 0.2

SD - Standard Deviation of means

Table 2. Duration of different stages and sex ratio of *M. incognita* infected and non infected by *P. penetrans.*

Stages	Duration	
	Healthy (days)	*P. penetrans*-infected (days)
J2	12	14
J3	4	4
J4	6	8
Adult	2	4
Male: female ratio	1:6±0.2	1:15±0.42

The duration of all post-parasitic stages of the parasitizing nematodes took longer time for development and the adult stage was reached in 30 days as against 24 days for non-parasitized nematodes. Sex ratio was affected by *P. penetrans* infection. Number of males produced was high from healthy J2 when compared to the *P. penetrans*-infected (Table 2). *P. penetrans*-infected females were milky white in colourand males were filled with endospores.

The life cycle of *M. incognita* under ideal environmental conditions on a favourable host namely tomato, was prolonged by 6 days. The life cycle of the parasite and its host are so perfectly synchronised to suit the multiplication and survival of the parasite. The results clearly indicate that the increase in the period of post-parasitic development of *M. incognita* infested by *P. penetrans* is only due to the effect of the parasite and not due to any other stress condition..

Stress conditions during the post-parasitic life of *M. incognita* species alter the sex ratio with more number of individual becoming males. Such stress factors include temperature, radiation, chemical, pathogen and over crowding *etc.* (Ellenby, 1954, Wong and Ferris, 1968; Ketudat, 1969). This is the first report that parasitization of *M. incognita* by *P. penetrans* resulting in the production of more number of females. Fewer infected J2 of *M. incognita* became males when compare to the identical environment conditions. This upset in the sex ratio is definitely due to parasitization of *M. incognita* by *P. penetrans* Normally parasitization which is a stress situation which should result in production of more number of males. Development of more number of parasitized J2 into females, is advantageous to *P. penetrans* since it can multiply to a higher level in females because of their larger size. Overcrowding is known to produce more number of males in the case of *Meloidogyne* (Ketudat, 1969). In the presence of *P. penetrans* as already established fewer J2 invade the roots and thus over crowding is avoided and favouring production of more number of females.

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