ABSTRACT

The root-knot nematodes particularly the four major species viz. *Meloidogyne incognita, M. javanica, M. arenaria* and *M. hapla* are world-wide in distribution and infect a large number of plants including vegetable crops, causing substantial yield losses to the growers. Since the realization of this fact various management measures have been in vogue. With increasing understanding of the role of nematodes including root-knot nematodes, a number of nematicides were developed and effectively used for their control. But in the recent past for reasons of economy, availability and particularly health hazards and environmental risks they are no more recommended for large scale use. Currently, biocontrol is the preferred measure for management of root-knot nematodes. Research is in progress in the different parts of the world to search for microorganisms which can be effectively used as biopesticides. In this study, an effort has been made to search for indigenous microorganisms (fungi) and examine their efficiency for management of root-knot nematodes in order to use them as biopesticides.

Some localities of the districts of western part of Uttar Pradesh were surveyed to collect the root samples of vegetable crops. Disease incidence and intensity in the vegetable fields was determined and fungi associated with the egg masses of the nematodes were isolated in order to understand the level of infestation of the vegetable fields by root-knot nematodes and to identify the fungi involved in natural biocontrol.

Root samples of the vegetable crops collected from the districts showed moderate to heavy infection of root-knot nematode. The disease incidence ranged between 36-67% in the samples and on the average disease incidence in the districts was above 50%. Root gall index (GI) and egg mass index (EMI) showed variations ranging from 1-5 and 0-5, respectively. *Meloidogyne incognita, M. javanica* and *M. arenaria* were present in the samples. The former two species were prevalent nematodes on different vegetable crops, occurring singly or concomitantly. *M. arenaria* was less frequent.
Several fungi were found associated with egg masses. The mycoflora of egg masses and frequency of the species varied in different districts. Sixty two species of fungi were isolated from the egg masses of the root-knot nematodes infecting different vegetable crops in the fields. *Fusarium oxysporum, Verticillium chlamydosporium, Paecilomyces lilacinus* and *Rhizoctonia bataticola* were found infecting eggs of the root-knot nematodes.

Culture filtrates of two isolates of *P. lilacinus* (local-isolated from the root samples; foreign-obtained from Peru) and *R. bataticola* effectively suppressed juvenile hatching of *M. incognita* and induced their mortality. The effects of both the fungi, however, differed. *P. lilacinus* isolates were more effective. Time duration of exposure and concentration directly influenced killing of the juveniles and inhibiting their hatch.

Efficiency of *P. lilacinus* (foreign & local) isolates and *R. bataticola* for colonizing egg masses of *M. incognita* was tested in artificial inoculation under aseptic conditions. Both the fungi (*P. lilacinus* isolates and *R. bataticola*) colonized egg masses and destroyed the eggs. *P. lilacinus* isolates were, however, more efficient than *R. bataticola*.

The efficiency of *P. lilacinus* (local isolate) and *R. bataticola* for control of *M. incognita* on tomato was studied under glasshouse conditions by artificial inoculations. In simultaneous and sequential inoculations, *P. lilacinus* and *R. bataticola* suppressed *M. incognita* resulting in improved plant growth. The disease intensity was appreciably reduced as root galling and egg mass production were greatly suppressed. Highest percentage of eggs showed fungal infection when the plants were applied with either of the fungi, two or three weeks prior to nematode inoculations. In combination, *P. lilacinus* and *R. bataticola* were more effective in reducing nematode population and improving the plant growth. In combined inoculation, GI and EMI were lowest and percentage of infected eggs was highest. Nematode population was also greatly reduced.

Root-dipping of tomato seedlings protected their roots from invasion by the juveniles of *M. incognita*. When the roots were dipped in the culture filtrates of *P. lilacinus/ R. bataticola* and inoculated with *M. incognita* juveniles, the lengths, fresh and dry weights of the plants were greater than plants inoculated with the nematode. The time duration of dipping
influenced their effect. The dipping of the roots for 30 min was better than 10 or 20 min dipping. Intensity of the disease was reduced in all the dip time but 30 min dip was the best as root galling, egg mass production and nematode population were greatly reduced. The seedlings developed no adverse effect by dipping in the culture filtrates.

Growth and sporulation of *P. lilacinus* on various organic substrates (agricultural wastes and other organic materials) in order to dispense it in the field showed that gram husk was superior to other materials used in the study as it contained the highest spore count of the fungus. Application of the organic substrates grown with *P. lilacinus* improved growth of tomato plants infected with root-knot nematode, *M. incognita* by suppressing root galling and nematode development. Highest improvement in plant growth of the nematode infected plants occurred when the fungus cultured on gram husk was applied. Growth of tomato plants was highest when 4, 6 and 8 g gram husk cultured with *P. lilacinus* was added to the soil. Root galling and nematode development was also reduced by the application of all the above mentioned doses of *P. lilacinus* cultured on gram husk. The most effective dose was, however, 4g/kg soil.

Effect of varying temperatures on growth of four isolates of *P. lilacinus* designated as PI1, PI2, PI3 and PI4 obtained from Peru (foreign), Bulandshahr (local), Jhansi and Hyderabad respectively and their mixtures designated PI5 was investigated to determine a suitable temperature for their growth. All the isolates showed greatest mean fungal dry weight at 24 to 28 °C.

Comparison of biocontrol efficacy of four isolates of *P. lilacinus* was compared in artificial inoculations. All the four isolates of *P. lilacinus* and their mixture were effective in suppressing the root-knot nematode and improving the plant growth by suppressing the root-knot nematode. The efficacy of the isolates, however, differed both for plant growth and root-knot development. The isolates PI 1, PI 2 and their mixtures were more effective and other isolates showed relatively less efficacy.

*Pasteuria penetrans* a known bacterial parasite of root-knot nematodes was also used in the study, particularly for its combined application with local isolate of *P. lilacinus*. Effect of
temperature and moisture on spore attachment of *Pasteuria penetrans* to the second stage juveniles of *M. incognita* was examined. Attachment of the spores of *P. penetrans* to the nematode body occurred at all the three temperatures (15, 25, 35° C) tested. Lowest attachment was observed at 10° C. The spore attachment increased with the increase in exposure period from 24 to 72 h at all the three temperatures. With regard to moisture, maximum and minimum spore attachment was observed at 50 and 25 per cent moisture levels.

Both *P. lilacinus* and *P. penetrans* and their combination (*P. lilacinus* + *P. penetrans*) were effective in suppressing the root-knot nematode and improved the plant growth. *P. lilacinus* was comparatively more efficient than *P. penetrans* in this respect. The combined efficiency of both the microbes was better than *P. lilacinus* alone. The indigenous isolate of *P. lilacinus* which was found to be efficient, can be used as a biopesticides for root-knot nematodes using gram husk as carrier substrate for field dispensing. *R. bataticola* may be used with care because of biosafety reasons, since this species is parasitic on legumes.