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Entomopathogenic Nematodes Associated with Soil Types and Vegetation Cover in Pothwar Region of Pakistan

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Abstract: A total of 120 soil samples were collected from eight different crops. Nematodes were recorded from 18 (15%) samples through *Galleria mellonella* baiting technique. The soil samples were analyzed to see the effect of soil pH and soil texture on the persistence of EPNs. Maximum nematodes (38%) were recovered from the soil with pH 7, sandy loam soil texture followed by loamy soils. EPNs were found maximum in the samples taken from the root base of maize and grasses. Nematodes were also recovered from the soil of cauliflower and orange orchard. The nematodes were identified as *Steinernema* sp. and *Heterorhabditis indicus.*

Key words: Isolation, entomopathogenic nematodes, Pothwar region

Introduction

A number of insect pests cause serious damage to our cash crops like cotton, sugarcane, fruits and vegetables. So far most of the insect pests have been controlled by the extensive use of pesticides. The use of highly toxic chemicals has not only created environmental hazards but also had adverse effects on human health and wild life. Use of different pesticides has also been the major factor in developing insect resistance against different organophosphates and synthetic pyrethroid (Poxenheim and Tgabashnik, 1991). The pesticides are also responsible for creating serious health problems such as muscle stiffness, dry throat, difficulty in breathing, stinging eyes, nausea and chest pain. Their indiscriminate application has been considered a potential threat to the biodiversity. It is due to these factors that biocontrol methods have gained momentum as a major component of Integrated Pest Management System, to minimize the use of pesticides in field conditions. Pests have been suppressed to a greater extent by different biocontrol agents such as predators and parasites, fungi, bacteria, viruses and nematodes (Reddy and Devakar, 1997). Out of different biological control methods entomopathogenic nematodes could be one of the best choice because, these are indigenous to many regions throughout the world, have wide host range, with no adverse effects on animals and plants and are easy to handle during application with conventional pesticide equipments.

Entomopathogenic nematodes of the genera *Heterorhabditis* and *Steinernema* have a worldwide distribution and occur in a variety of soil types and habitats (Travasoss and Sobre, 1927). They are obligate natural parasites of insects (Poinar, 1979) and remain in soil, without feeding until the development of infective juveniles (IJs) completing their life cycle in an insect host. The infective juveniles of nematodes parasites enter insect host through their natural openings (Poinar, 1979). *Heterorhabditis* sp. also enters through inter-segmental membranes (Bedding and Molyneux, 1982). On reaching the host haemocoel, the nematodes release symbiotic bacteria, which results into septicemia and subsequent death of the insect. The nematodes naturally occur in soil and can be isolated by flotation method (Jiang *et al.*, 1995). The most common and efficient method used for isolation of entomopathogenic nematodes is *Galleria mellonella* baiting techniques (Bedding and Akhurst, 1975). Studies on isolation of EPNs through *Galleria mellonella* baiting have been carried out in different parts of the world (Hominick *et al.*, 1995).

Shahina and Maqbool (1996) conducted a study on EPNs distribution in soils of Sindh, Pakistan. However the Pothwar area in the Punjab remained unexplored for EPNs incidence. The present study was planned to undertake survey of EPNs in Pothwar region of Pakistan to inventories species associated with different geographic regions, soil type and vegetation cover and to identify the nematodes isolated up to species level.

Materials and Methods

Collection of materials: Soil samples were collected to isolate entomopathogenic nematodes (EPNs) from ten different localities in Pothwar region of Punjab during August 1998. It included Rawalpindi, Attock, Hasanabdal, Taxilla, Gudwal, Ckakwal, Talagang, Fatehjang, Gojarkhan and Jhelum areas. Diverse habitats like agricultural fields, lawns, parks and grasslands were surveyed. Three fields/grasslands and twelve soil samples from each locality were collected. Soil samples were also taken from the root base of eight different crops including maize, grass, groundnut, mungbean, cauliflower, spinach, loquat and orange orchards. The samples were analyzed to determine the soil texture. In all a total number of 120 soil samples were collected.

Rearing of wax moth (*Galleria mollonella*): Beehives infected with *Galleria mellonella* were obtained from bee farm. They were kept in rearing glass jars (19× 12× 12 cm^3), covered with muslin cloth. Last instar larvae of *G. mellonella* were separated for nematode culture, leaving some small sized larvae for moth emergence and egg laying. The fresh laid eggs were transferred to modified artificial diet prepared by mixing oat porridge (20g), yeast granules (50g), in a solution of 80ml warm honey and 100ml glycerol. After reaching last instar, they were taken out from the diet and used for nematode isolation.

Nematode isolation from soil: From each collection site, 500g soil samples were collected with a hand shovel from the top 10cm soil profile within an area of about 1× 1 meter square. These soil samples were sealed in polyethylene bags and transported to the laboratory for processing and extraction of nematodes. For isolation of nematodes "*Galleria* trap" method was used (Bedding and Akhurst, 1975). Soil samples were placed in (9× 6cm^2) plastic pots, 500g in each container. In each pot, five wax moth *G. mellonella* larvae were placed in close contact with soil through the pressure of the lid. Pots were incubated for 5-7 days at 28°C. From day one to onwards, samples were checked daily and dead larvae were removed, rinsed thoroughly with water and transferred to white traps.

White traps: Modified white trap consisted of a plastic container (9x 9x 6cm^3), filled with distilled water to a depth of 1cm (White, 1927). The bottom of an inverted petridish (about 5x 3.5cm^2 depending on plastic container) was placed in container. A sheet of filter paper (about 7cm) was placed on the petridish allowing the edge of the filter paper to come in contact with the water. The dead larvae were placed on filter paper on top of the petridish and the plastic container was closed with the lid. White traps were further incubated at respective infection temperature until nematode progeny emerged. Depending on nematode species, infective juveniles start to leave the cadaver 8-20 days after infection. Infective juveniles that moved down through the filter paper into the water were harvested after two to three days.

Harvesting: Water containing infective juveniles in white trap was poured in 100ml beaker filled with distilled water. Nematodes were left to settle for about 30 minutes at the bottom of the dish. The washing process was repeated three times until water becomes clear.

Identification: Specimens isolated were relaxed in hot water, fixed in F.A 4:10. Permanent mounts were prepared by processing through Lectophenol and finally mounted in glycerin. Nematodes were identified using taxonomic keys available in their respective groups.

Results and Discussion

Five sites were positive for EPNs including Rawalpindi, Attock, Chakwal, Fatehjang and Gojarkhan (Fig. 1). No EPNs were recovered from remaining five sites. Out of 120 soil samples, fifteen percent contained EPNs. These nematodes were identified as *Steinernema* species and *Heterorhabditis indicus*. From Rawalpindi and Chakwal 41.6% soil samples were positive. Nematodes isolated from Rawalpindi belong to the family Steinernematidae. Twenty five percent soil samples from Attock and Fatehjang and 16.6 percent soil samples from Gojarkhan were having EPNs. Soil samples from Chakwal contained *Heterorhabditis indicus*, whereas *Steinernema* species was present in Gojarkhan and Fatehjang. These soil samples were also evaluated for the effect of soil pH and soil texture on the distribution of EPNs. Prevalence of the nematodes varied in four soil textures, sandy loam, loam, clay loam and sandy clay loam. Maximum number of EPNs isolates was recovered from the sandy loam soils i.e.30%, followed by 16.6% in loamy soils. No EPNs were recovered from clay loam and sandy clay loam soils (Fig. 2).

These results were expected as EPNs could have free movement in sandy loam soils and may have easy approach to their targets due to large pore size (Haukeland, 1993). They may also get sufficient amount of oxygen from soil water in loamy soils. Kung *et al.* (1990) also reported that nematode persistence decreased as clay contents in the soil increased. Choo *et al.* (1995) mentioned that EPNs preferred porous soils enriched by humus and hence high in organic matter frequently harbouring the nematodes. Recovery of most EPNs from sandy loam and loamy soils in the present study was therefore expected. The samples taken from the root base of maize crop, contained maximum number of *Steinernema* sp. and *H. indicus*, followed by grass having 15.5% *Steinernema* sp. Nematode isolates recovered from soil samples taken from orange orchard and cauliflower fields were 9.3% each. EPNs were also recovered from the soils taken from the root base of groundnut, mungbean, spinach and loquat orchards (Fig. 3). These results also confirm the findings of Barbercheck *et al.* (1995), who observed that EPNs progeny production was highest in the soils of maize fields due to the presence of plant secondary metabolites.

Identification: Entomopathogenic nematodes isolated were identified as *Steinernema* sp. and *Heterorhabditis indicus*. The characteristic features of both the groups are described as under.

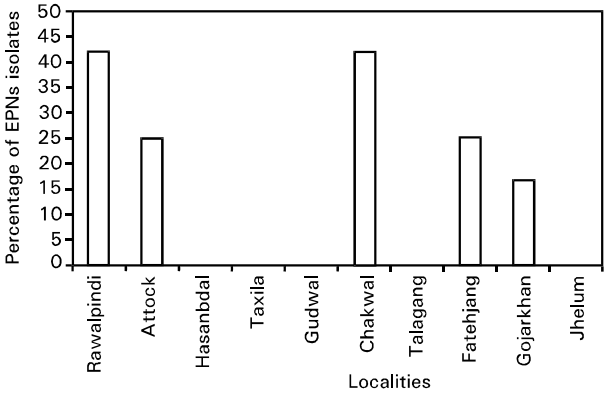

Fig. 1: Percentage of EPNs isolates recorded at selected localities of Pothwar region, Punjab

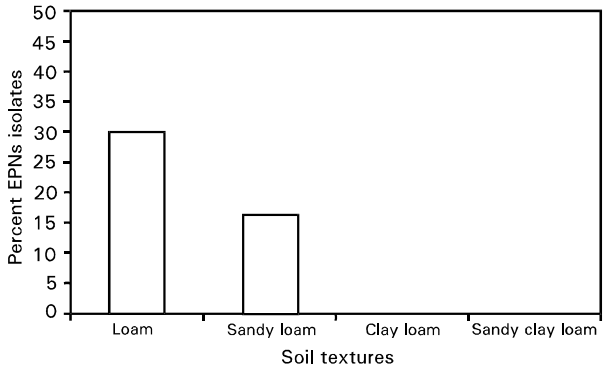

Fig. 2: Percentage of EPNs isolates recorded in different soil textures

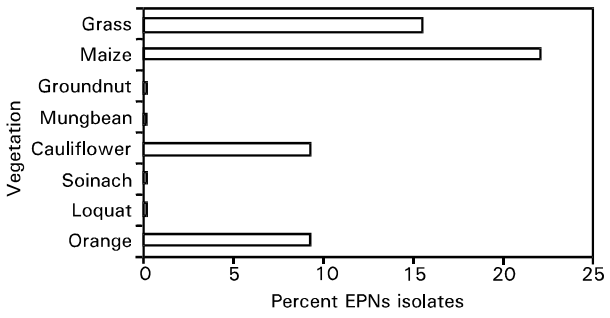

Fig. 3: Percent EPNs isolated from different vegetation cover

Genus *Steinernema*:

- Excretory pore located posterior to swollen part of metacarpus.
- Females without double valve epiptygma.
- Spicule about 58 μm long.
- Infective juveniles EP 78-86 μm from the anterior end.
- Tail less than 58 μm long.
- Infective juveniles have two cephalic horns.

Genus *Heterorhabditis*:

- Average body length less than 700 μm.
- Infective juveniles have short tail averaging 76 μm.
- Lamina of spicule with ventral expansion.
- Mail body with averaging 41μm.
- Spicule length averaging 47μm with ventral expansion of lamina.

Prevalence of EPNs in Pothwar Region of Pakistan and in other parts of the world indicate that entomopathogenic nematodes could be one of the best choice as biological control agents against different insect pests. In Pakistan still there is a need to explore the use of these nematodes in different crops because scientists in developed countries are using these EPNs as biological pesticides against a variety of insect pests.

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