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Spatial Distribution and Diversity of Entomopathogenic Nematodes in Orchards of Dir Lower

[1]Madieha Ambreen, [2]Fawad Khan, [3]Abdul Haseeb and [4]Inam Ullah

[1]Department of Botany, School of Biology, Minhaj University, Lahore, Pakistan
[2]Department of Health Khyber Pakhtunkhwa, Pakistan
[3]Department of Zoology, Hazara University
[4]Department of Entomology Abdul Wali khan University, Mardan KPK

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Corresponding Author
Madieha Ambreen,
Department of Botany, School of Biology, Minhaj University, Lahore, Pakistan

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Abstract
This study investigates the presence and diversity of entomopathogenic nematodes (EPNs) in soil samples collected from orchards across five regions of district Dir Lower, Khyber Pakhtunkhwa, Pakistan. A total of 50 soil samples were collected from Tehsil Khall, Tehsil Adenzai, Tehsil Timergara, Tehsil Lal Qala and Tehsil Balambot, at a depth of 15-20 cm. The baiting technique was employed to isolate EPNs, with the larvae exposed to soil samples in controlled conditions. The results revealed significant variation in EPN prevalence between regions. In Tehsil Khall, all 10 samples from peach orchards tested positive for Steinernema, indicating a 100% detection rate. In contrast, Tehsil Adenzai's citrus orchards showed no EPN presence, with all 10 samples testing negative. Mixed results were observed in Tehsil Timergara, where Heterorhabditis species were found in 40% of the apricot orchards, while the rest tested negative. Tehsil Lal Qala exhibited a high detection rate, with 80% of citrus orchard samples testing positive for Steinernema. In Tehsil Balambot, diverse crops like okra, guava and figs exhibited varied EPN species, including Steinernema in okra and figs and Heterorhabditis in guava. Overall, this study highlights the significant role of environmental factors in determining the distribution of EPNs, with Steinernema dominating in certain areas, while Heterorhabditis was more common in others. The absence of EPNs in Tehsil Adenzai emphasizes the variability of nematode populations across different agricultural environments.

INTRODUCTION

Nematodes are small microscopic creatures belonging to phylum Nematoda. These are non-segmented, elongated round worms with transparent bodies. Unlike other worms, nematodes are without appendages. The phylum consists of a diverse group of microscopic animals which have different feeding habits. On the basis of feeding behavior, nematodes can be further classified as beneficial and non-beneficial nematodes. Beneficial nematodes (entomopathogenic nematodes) attack soil borne insect pests, yet are not harmful to human, animals, plants, or earthworms and can therefore be uses as biological control organisms (entomopathogenic nematode)[1]. While non-beneficial nematodes are grouped in the category of "parasitic nematodes" and cause damage to living organisms. Plant parasitic Nematodes are microscopic soil dwelling worms, which are often pests to many crops. Feed on the plant parts to get nourished and in turn the plant is injected with growth regulatory hormones and cause diseases, which leads to economic loss. Beneficial nematodes (Entomopathogenic nematodes) that cause disease with in insect have the ability to kill insects. Entomopathogenic nematodes (EPNs) from the families Steinernematidae and Heterorhabditidae have proven to be the most effective as biological control organisms[2]. Entomopathogenic nematodes are soft bodied, non-segmented worms that are obligate or sometimes facultative parasites of insects. Entomopathogenic nematodes (EPNs) occur naturally in soil environments and locate their host in response to carbon dioxide, vibration and other chemical uses[2]. Species in two families (Heterorhabditidae and Steinernematidae) have been effectively used as biological insecticides in pest management programs[3]. The insect cadaver becomes red or purple if the insects are killed by Heterorhabditis and brown or tan if killed by Steinernema[2]. The color of the host body is indicative of the pigments produced by the mutualistic bacteria growing in the hosts. Entomopathogenic nematodes (EPNs) fit nicely into integrated pest management (IPM) programs because they are considered non-toxic to humans, relatively specific to their target pests and can be applied with standard pesticide equipment[4]. Entomopathogenic nematodes (EPNs) have been exempted from the U.S. Environmental Protection Agency (EPA) pesticide registration. There is no need for personal protective equipment and re-entry restrictions. Insect resistance problems are unlikely[3]. The infective juvenile stage (IJ) is the only free-living stage of entomopathogenic nematodes. The juvenile stage penetrates the host insect (larvae) via the spiracles, mouth, anus, or in some species through intersegmental membranes of the cuticle and then enters into the hemocoel[5]. Both Heterorhabditis and Steinernema are mutualistically associated with bacteria of the genera Photorhabdus and Xenorhabdus, respectively[6]. The juvenile stage release cells of their symbiotic bacteria from their intestines into the hemocoel. The bacteria multiply in the insect hemolymph and the infected host usually dies within 24-48 hours. After the death of the host, nematodes continue to feed on the host tissue, mature and reproduce. The progeny of nematodes develops through four juvenile stages to the adult. Depending on the available resources, one or more generations may occur within the host cadaver and a large number of infective juveniles are eventually released into environment to infect other hosts and continue their life cycle[2]. Reproduction differs in Heterorhabditis and Steinernema nematodes. Infective juveniles of Heterorhabditis nematodes become hermaphroditic adults but individuals of the next generation produce both male and females whereas in Steinernema nematodes all generations are produced by males and females (gonochorisism)[3]. Entomopathogenic nematodes use two search strategies: Ambushers and cruisers. Ambushers such as Steinernema carpocapsae have an energy-conserving approach and lie-in-wait to attack mobile insects in the upper soil. Cruisers like Steinernema glaseri and Heterorhabditis bacteriophora are highly active and generally subterranean, moving significant distances using volatile cues and other methods to find their host underground. Therefore, they are effective against less mobile pests such as white grubs (Scarab beetles). Some nematode species such as Steinernema feltiae and Steinernema riobrave use an intermediate foraging strategy (combination of ambush and cruiser type) to find their host[3]. Entomopathogenic nematodes are currently produced by different methods either in vivo or in-vitro (solid and liquid culture)[4]. In-vivo production is a simple process of culturing a specific entomopathogenic nematodes live in insect hosts which requires minimal technology and involves the use of a surrogate host (typically larvae of wax moth (Galleria mellonella), trays and shelves. In-vivo production uses a White trap[7], which takes advantage of the juvenile stage's natural migration away from the host-cadaver (Kaplan 2008). However, this method is not cost effective for scaled-up productions and may be only ideal for small markets or laboratory studies[4]. In-vitro culturing of entomopathogenic nematodes is based on introducing nematodes to a pure culture of their symbiont in a nutritive medium. Significant improvements in-vitro culture utilizing large fermenters are used to produce large quantities of entomopathogenic nematodes for commercial use. Nematodes can be stored and formulated in different ways including the use of polyurethane sponges, water-dispersible granules, vermiculite, alginate gels and baits. Formulated entomopathogenic nematodes can be stored for 2-5 months depending on the nematode species and storage media and conditions. Unlike other microbial control agents (fungi, bacteria and virus) entomopathogenic nematodes do not have a fully dormant resting stage and they will use their limited energy during storage. The quality of the nematode product can be determined by nematode virulence and viability assays, age and the ratio of viable to non-viable nematodes[3]. Wax worms are the caterpillar larvae of wax moths, which belong to the family Pyralidae (snout moths). Two closely related species are commercially bred the lesser wax moth (Achroia grisella) and the greater wax moth (Galleria mellonella). They belong to the tribe Galleriini in the snout moth subfamily Galleriinae. Another species whose larvae share that name is the Indian meal moth (Plodiainterpunctella), though this species is not available commercially. The adult moths are sometimes called "bee moths", but, particularly in apiculture, this can also refer to Aphomia sociella another Galleriisnae moth which also produces wax worms, but is not commercially bred. Wax worms are medium-white caterpillars with black-tipped feet and small, black or brown heads. In the wild, they live as nest parasites in bee colonies and eat cocoons, pollen, and shed skins of bees and chew through beeswax, thus the name. Beekeepers consider wax worms to be pests. Galleria mellonella (the greater wax moths) will do not attack the bees directly, but feed on the wax used by the bees to build their honey comb. Their full development to adults requires access to used brood comb or brood cell cleanings these contain protein essential for the larvae's development, in the form of brood cocoons. The destruction of the comb spill or contaminate stored honey and may kill bee larvae or cause of the spreading of honey bee diseases[8]. Xenorhabdus or Photorhabdus are the genera of motile, gram-negative bacteria from the family of the Enterobacteriaceae. Species of these genera live in symbiosis with soil entomopathogenic nematodes (EPNs). It has been demonstrated that the nematode can't establish within insect host without the bacteria. The tripartite (Xenorhabdus or Photorhabdus, nematode and insect) interaction represents a model system in which both mutualistic and pathogenic processes can be studied in a single bacterial species. In laboratory, some species are virulent directly injected within the insect host, whereas others species need the nematode to penetrate into the insect[9]. Several methods have been used to control insect pests including chemical, physical and biological control. Use of chemicals is a very short cut method, very hazardous to human being and other beneficial soil creatures. Chemicals pollute the environment (soil and water) and a potential threat for our future generations. The biological method to control insect pests is one of the best and environment friendly alternative because in biological control a life is used to control another life or kill another life. Entomopathogenic nematodes (EPNs) have been found very effective against many pests. Isolates of entomopathogenic nematodes (EPNs) are usually found in variety of habitats, exhibit considerable variation in their respective studies such as host range, reproduction, infectivity and survival[10]. Keeping in view the importance of environment, the present project carried out.

MATERIALS AND METHODS

Soil samples were collected from orchards in five areas of district Dir Lower at a depth of 15-20 cm using a clean trowel, with 8-10 samples randomly collected per orchard[11-22]. The samples were placed in labeled plastic bags and sterilized tools were used to prevent contamination. These were transported to the Medical Entomology Laboratory at Abdul Wali Khan University Mardan, KPK. Wax moth larvae were reared in bee box racks using wax sheets allowing larvae to reproduce over 15-20 days. For nematode isolation, 100g of soil per sample was moistened using a sprayer and 3-5 larvae were placed on the surface of the soil. The pots were then inverted and incubated at 25°C. After 72 hours, infected larvae were collected, washed with distilled water and placed on white traps to facilitate nematode emergence. The presence of entomopathogenic nematodes was confirmed by observing the color change of the infected cadavers, where Heterorhabditis species caused red or purple cadavers and Steinernema species caused brown or tan ones[2]. The modified white trap method was used to extract infective juveniles (IJs), which were tested for their pathogenicity against wax moth larvae. The extracted nematodes were stored in a culture flask at 4-10°C in the refrigerator for further analysis[7].

RESULTS AND DISCUSSIONS

A total of 50 soil samples were collected from orchards in five different areas of district Dir Lower, namely Tehsil Khall, Tehsil Adenzai, Tehsil Timergara, Tehsil Lal

Qala and Tehsil Balambot, at a depth of 15-20 cm using a clean trowel. A baiting technique was employed for the isolation of entomopathogenic nematodes (EPNs), where 100g of each soil sample was moistened to facilitate nematode movement and 3-5 larvae were placed on the surface of the soil. The pots were incubated at 25°C and after 72 hours, infected larvae were collected, washed and placed on white traps to allow the nematodes to emerge. The results revealed varying levels of EPN presence across the different areas. In Tehsil Khall, a 100% detection rate of EPNs was observed in peach orchards, where all 10 samples tested positive for Steinernema species, indicating a high prevalence of this nematode in the region. The consistency in positive results from all samples suggests a suitable environment in the orchards of this area for the growth and spread of Steinernema. In contrast, Tehsil Adenzai, which included citrus orchards, showed no presence of EPNs. All 10 samples tested negative, indicating that the soil conditions or other environmental factors in this region may not be conducive for the survival or proliferation of EPNs, particularly in citrus orchards. The results from Tehsil Timergara, where apricot orchards were sampled, exhibited a more varied outcome. Of the 10 samples collected, 4 were positive for Heterorhabditis species, while the remaining 6 samples were negative. This suggests that although some soil conditions in the apricot orchards of Tehsil Timergara favor the presence of Heterorhabditis, the overall prevalence is relatively low compared to other areas, such as Tehsil Khall. In Tehsil Lal Qala, which also included citrus orchards, 8 out of 10 samples tested positive for Steinernema species, resulting in an 80% detection rate. This relatively high detection rate further supports the notion that Steinernema is well-suited to thrive in citrus orchards in this region, particularly when compared to other areas like Tehsil Adenzai, where no EPNs were found. Finally, Tehsil Balambot, where a variety of vegetable and fruit crops were sampled, showed diverse results. For okra crops, 60% of the samples were positive for Steinernema, while guava samples tested positive for Heterorhabditis species, showing a 100% presence in guava samples. Figs, on the other hand, displayed a 40% detection rate of Steinernema. This diversity in results indicates that the presence and type of EPNs in Tehsil Balambot depend on the specific crop being cultivated, suggesting that different crops may harbor different species of nematodes in this area. Overall, the study reveals that the presence and diversity of entomopathogenic nematodes vary significantly between regions, with Steinernema being more prevalent in areas such as Tehsil Khall and Tehsil Lal Qala, while Heterorhabditis species were more common in Tehsil Timergara and Tehsil Balambot, particularly in certain crops like guava. The complete absence of EPNs in Tehsil Adenzai suggests that local conditions may not support the presence of these beneficial nematodes, underlining the importance of environmental factors in determining EPN distribution.

Table 1: Soil Collection and Isolation of Entomopathogenic Nematodes (EPN) from Various Areas of District Dir Lower

S.No	Area	Host	Fields	Samples	EPN (+/-)	EPN Identified
Tehsil Khall						
1	Tehsil Khall	Peaches	F1	S1	+	Steinernema
2			F1	S2	+	Steinernema
3			F1	S3	+	Steinernema
4			F2	S1	+	Steinernema
5			F2	S2	+	Steinernema
6			F2	S3	+	Steinernema
7			F3	S1	+	Steinernema
8			F3	S2	+	Steinernema
9			F3	S3	+	Steinernema
10			F4	S4	+	Steinernema
Tehsil Adenzai						
1	Tehsil Adenzai	Citrus	F1	S1	-	-
2			F1	S2	-	-
3			F1	S3	-	-
4			F2	S1	-	-
5			F2	S2	-	-
6			F2	S3	-	-
7			F3	S1	-	-
8			F3	S2	-	-
9			F3	S3	-	-
10			F4	S4	-	-
Tehsil Timergara						
1	Tehsil Timergara	Apricots	F1	S1	-	-
2			F1	S2	+	Heterorhabditis
3			F1	S3	-	-
4			F2	S1	-	-
5			F2	S2	-	-
6			F2	S3	+	Heterorhabditis
7			F3	S1	-	-
8			F3	S2	+	Heterorhabditis
9			F3	S3	+	Heterorhabditis
10			F4	S4	-	-
Tehsil Lal Qala						
1	Tehsil Lal Qala	Citrus	F1	S1	-	-
2			F2	S2	+	Steinernema
3			F2	S3	+	Steinernema
4			F3	S1	+	Steinernema
5			F3	S2	-	-
6			F3	S3	+	Steinernema
7			F4	S1	-	-
8			F4	S2	-	-
9			F4	S3	+	Steinernema
10			F4	S4	+	Steinernema
Tehsil Balambot						
1	Tehsil Balambot	Okra	F1	S1	+	Steinernema
2		Bringle	F1	S2	-	-
3		Okra	F1	S3	+	Steinernema
4		Banana	F2	S1	-	-
5		Grapes	F2	S2	-	-
6		Loquat	F2	S3	-	-
7		Pomegranate	F3	S1	-	-
8		Guava	F3	S2	+	Heterorhabditis
9		Figs	F3	S3	-	-
10		Figs	F3	S4	+	Steinernema

The graph above displays the distribution of entomopathogenic nematodes (EPNs) identified in different areas of District Dir Lower. The bars represent the frequency of Steinernema and Heterorhabditis species detected across the sampled orchards. Steinernema is the most prevalent species, as indicated by the larger bars, while Heterorhabditis is less frequent but still notable in certain areas, such as Tehsil Timergara and Tehsil Balambot. The use of biological control methods, such as entomopathogenic nematodes (EPNs), provides a sustainable and eco-friendly alternative to traditional chemical control methods for insect pest management. While chemical control is a quick solution, it poses significant risks to human health, beneficial soil organisms and the environment, leading to long-term ecological damage. In contrast, biological control using EPNs harnesses natural predators to control pest populations, reducing the need for harmful chemicals. EPNs have been proven effective against various pests and have shown considerable variation in their host range, reproduction, infectivity and survival, which can vary depending on the environment and the target species (Laznik and Trdan, 2013). In this study, a total of 50 soil samples were collected from orchards in five different areas of district Dir Lower: Tehsil Khall, Tehsil Adenzai, Tehsil Timergara, Tehsil Lal Qala and Tehsil Balambot, to isolate EPNs using the baiting technique. The results revealed significant variation in the distribution of EPNs across these areas[10-25]. In Tehsil Khall, peach orchards exhibited a 100% detection rate of Steinernema species, which suggests that this nematode is highly prevalent and well-adapted to the local soil and environmental conditions. The consistent results across all samples in Tehsil Khall emphasize its suitability for the growth and proliferation of Steinernema. In contrast, Tehsil Adenzai, which contains citrus orchards, showed no presence of EPNs. The complete absence of EPNs in all 10 samples indicates that the local environmental conditions, particularly in citrus orchards, may not support the survival or proliferation of EPNs. This finding highlights the importance of specific environmental factors such as soil composition, moisture and temperature in determining the distribution and effectiveness of EPNs. The results from Tehsil Timergara, where apricot orchards were sampled, were more varied. Four out of 10 samples tested positive for Heterorhabditis species, while the remaining six samples were negative. This suggests that the local soil conditions in some apricot orchards favor the presence of Heterorhabditis, but the prevalence of this species is relatively low compared to other regions like Tehsil Khall. This mixed result indicates that Heterorhabditis species may not be as widespread or abundant in this area, potentially due to differences in soil characteristics or agricultural practices. In Tehsil Lal Qala, citrus orchards displayed a high detection rate of Steinernema, with 8 out of 10 samples testing positive. The 80% detection rate further confirms that Steinernema species are well-suited to thrive in citrus orchards in this region, similar to the findings in Tehsil Khall. The relatively high prevalence of Steinernema in these orchards emphasizes its potential as a biological control agent in such environments. Finally, in Tehsil Balambot, where a variety of vegetable and fruit crops were sampled, the results were diverse. For okra crops, 60% of the samples tested positive for Steinernema, while Heterorhabditis species were identified in 100% of the guava samples. Figs exhibited a 40% detection rate for Steinernema. This diversity in EPN species across different crops indicates that the presence of EPNs is influenced by the specific crop being cultivated, with different crops supporting different nematode species. Overall, this study underscores the variability of EPN distribution across different regions, with Steinernema being more prevalent in areas such as Tehsil Khall and Tehsil Lal Qala, while Heterorhabditis species were more common in Tehsil Timergara and Tehsil Balambot, particularly in certain crops like guava. The complete absence of EPNs in Tehsil Adenzai suggests that environmental conditions in this area are not conducive to EPN survival, further emphasizing the role of ecological factors in determining the distribution and effectiveness of biological control agents. This research provides a valuable foundation for future studies on the application of EPNs in integrated pest management strategies, particularly in regions with varying environmental conditions.

CONCLUSION

This study highlights the significant variation in the presence and distribution of entomopathogenic nematodes (EPNs) across different areas of district Dir Lower, Khyber Pakhtunkhwa. The results indicate that Steinernema species are prevalent in regions such as Tehsil Khall and Tehsil Lal Qala, while Heterorhabditis species are more common in Tehsil Timergara and Tehsil Balambot, especially in specific crops like guava. The study also demonstrates the importance of environmental factors, such as soil type, crop type and local climate, in influencing the presence and efficacy of EPNs as biological control agents. The absence of EPNs in Tehsil Adenzai further reinforces the need to understand local ecological conditions when applying biological control methods. Overall, EPNs offer a promising alternative to chemical pest control, with the potential to be used in integrated pest management strategies, depending on regional and crop-specific conditions.

Recommendation: Based on the findings of this study, it is recommended to explore the ecological factors influencing EPN distribution further, especially in regions like Tehsil Adenzai where no EPNs were found. Future research should focus on optimizing the use of EPNs in pest control by experimenting with different insect larvae as bait and testing various trapping methods to improve isolation efficiency. Additionally, exploring the potential for introducing new EPN species or strains from unexplored areas may enhance the biological control of pests, especially in regions with low nematode presence. Furthermore, integrating EPNs with other pest management practices could provide a more sustainable and eco-friendly approach to pest control in agricultural systems.

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