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Research Article – Biology

Prospects and present status and of Entomopathogenic Nematodes(Steinernematidae and Heterorhabditidae) in Nepal

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Abstract

Naturally occurring entomopathogenic nematodes(EPN) and their symbiotic bacteria are important biotic factor in suppression of insect pest populations in soil and cryptic habitats. These nematodes can control pests due to their mutualistic association with bacteria that kill the hosts by septicemia and make the environment favorable for EPN development and reproduction.The virulent species of EPN can commercially be mass produced as biological control agents all over the world. Nepal has a great potential to exploit these beneficial nematodes for the control of insect pests. Exploration of indigenous EPN is receiving attention around th eworld. Numerous surveys conducted worldwide have detected many indigenous isolates. Altogether 115 species of EPN (97 of *Steinernema* and 18 of *Heterorhabdits* species) have been reported so far worldwide. However, very limited research on EPN in Nepal has been done with 29 species only with some new species. Hence,the isolation and description of the native efficacious species and populationsof EPN is the need of the hour,not only from a biodiversity view point but also from an environmental and biological control perspective.Thei dentification of EPNs, adapted to environmental and climatic conditions of cultivated areas, is important for sustainable pest suppression in integrated management programs in agricultural areas of Nepal. This article provides an overview of recent development on EPN research and evaluates their potential for use and exploitation in Nepal.

Keywords: Entomopathogenic nematodes,insect-pests,biological control, status, prospects, Nepal.

Introduction

Nepal is an agricultural diverse country and most of the people earn their livelihoods directly or indirectly from agriculture and forms a major source of income. Several kinds of crops are grown here in different bio-geographical areas; however, every year there are huge losses to agricultural and horticultural crops due to attack by many insect species. The most important pests of these crops include lepidopteran, coleopteran, dipteran, orthopteran and homopteran species all widely distributed in different agro-climatic conditions in Nepal (Joshi *et al.,*1991). For the control of these pests, large quantities of hazardous chemical insecticides are used every year by Nepalese farmers which

result in serious implications to man and environment, such as insecticide resistance, pest resurgence, residues on crops harmful to beneficial organisms. Thus, alternative measures are therefore mandatory to get rid of these consequences and save this planet earth. In the present scenario, developing countries are intended to increase the awareness and to enhance the better understanding of naturally occurring beneficial microorganisms in the terrestrial environment. Exploration of biological control agents of the insect pests is one of the major thrusts on agricultural research, which can lead to the development of the commercial biological control products at local level, however, currently, there is no biological control agent used commercially in Nepal for pest management. One of the beneficial control agents paying attention of experts worldwide is the use of entomopathogenic nematodes. These are microscopic organisms residing in the soil and cause death of insects which come in contact with them but are not lethal to non-target hosts including humans and other vertebrates. They are one of the important bio-control agents for controlling most of the crop pests all over the world. Since the description of first EPN by Gotthold Steiner (1923), a total of 116 valid species have been described globally and new more species are described every year (Bhat*et al.,*2018; Hunt and Subbortin, 2016). However, only few species of have been commercialized worldwide as biopesticides and are ... the control of different ...oductivity.

EPN comprise two families Steinernematidae represented by the genera; *Steinernema* (97 species) and *Neosteinernema* (1 species) and Heterorhabditidae represented by one genus *Heterorhabditis* (18 species) which are respectively associated with *Xenorhabdus*and *Photorhabdus* (Poinar and Grewal, 2012; Batalla-Carrera *et al.,* 2016) and together are lethal duo. The bacteria are found only in the 3rd stage juveniles and are only infective stage, called infective juveniles (IJs). The IJ is the only stage that can survive outside the host without food for long periods and forages in the soil for alternative hosts (Gaugler, 2002). Once in the vicinity of the host, they enter their body either through natural openings or by abrading the skin. They release their bacteria in the insect haemocoel which multiply and provide food for IJs to complete their life cycle (Poinar, 1990), and kill insect due to septicemia. These nematode families exclusively grow and reproduce inside insect hosts, and IJs leave the cadaver after the food resources get exhausted. EPNs are infective to large number of insect pests such as Lepidoptera, Coleoptera, Hemiptera, Diptera, and Orthoptera, but have been proven non-lethal to humans and other vertebrate and invertebrate hosts. It has been noticed that these IJs locate their insect host via some chemical secretions by the insect body and thus are only parasitoids only to them. In several countries, especially developed countries EPN are used as important biocontrol agents and are sold under different trade names which have provided good results against insect pest fauna. The economic importance of EPNs is increasing due to their potential use in the biocontrol of insect pests, their easy mass production using conventional fermentation technology, safe to non-target organisms and to the environment. Thus, in this high opinion about EPN, the exploration of indigenous species and/or abundance provides a valuable resource not only from biodiversity perspective but also from a more applicable stand point. Indigenous EPN may result more suitable for inundative release against local insect pest because of adaptation to local climate and other population regulators, while exotic EPN may have negative impact on non-target organisms. Numerous surveys conducted worldwide have detected many indigenous isolates of undescribed species. There is also a tremendous opportunity for discovery of new nematode strains and species adapted to local environmental conditions and pests. The objective of this paper is to know the status of entomopathogenic nematode research conducted in Nepal by various scientists, and the possibility of their further research in integrated pest management (IPM) in Nepal.

Diversity and distribution of entomopathogenic nematodes worldwide and in Nepal

The planet earth is enthralled by vast diversity of life and nematodes represent the second most species rich phylum after Arthropoda. Among the nematodes, there is one special class of nematodes which are effective against insect fauna and serve as good bio-control agents. They are referred as entomopathogenic nematodes (EPN) due to their pathogenic nature against entomic fauna. They are used successfully to control a variety of agricultural and horticultural insect pests in different countries. There is an intense interest to isolate these nematodes from different parts of the world where they are adapted climatically and have the potential to control the insect pests in that particular area. Hence, surveys have been conducted in many parts of the world to isolate these beneficial nematodes, with a future aim to exploit them in the management of crop insect pests.

The first entomopathogenic nematode to be described was *S. krausse*(Steiner, 1923) and was isolated from *LydapotrophicaHartig*, a hymenopterous sawfly (Krausse, 1917). The second EPN that was documented was *S. glasseri* (Steiner, 1929) from scarabaeid beetle, *Popillia japonica* and this was the first EPN which was mass produced for the control of white grubs in USA. Later, other species were included like *S. feltiae* (Filipjev, 1934), *S. affinae* (Bovein, 1937) and *S. carpocapsae* (Weiser, 1955). The *S. carpocapsae* is considered one of the most popular steinernematid nematode named after the genus of its host, codling moth larvae, *Carpocapsa pomonella* in Germany and is most heavily researched member of the genus *Steinernema*. For a long time, the different strains of this species were widely used as a potential biocontrol agent.

With the passage of time, more and more species were described throughout the globe and some of which were either synonymised with already existing species while others were described as species *inquirendae* due to inaccurate data and some as *nominanudum*. More and more *Steinernema* species were unearthed and increased in the last decade because of their potential biocontrol properties, particularly in Asia and Africa followed by practicing molecular techniques that help in reliably characterizing species in a group that is increasingly difficult to access using classical methodologies. To date, 97 species (Hunt andSubbortin, 2016, Bhat *et al.,* 2017 and 2018) have been described in all the continents except Antarctica, and this number is growing every year. Till 1920s, only two currently valid species were described, two more in 1930s, one in 1950s, one in 1960s and four in 1980s which constitute a total of ten valid taxa. Later 85 valid species were proposed, 15 of these in the 1990s, 39 from 2000-2009 and 31 from 2010 to the beginning of 2016. From 2017 to 2018, 12 valid species were reported of which two were recently added to the list of new species, one from South Africa, *S. Litchii* (Steyn *et al.,* 2017) and other from Georgia, *S. borjomiense* (Gorgadze *et al.,* 2018).

Among heterorhabditidae, the first valid species to be described was *Heterorhabditis bacteriophora* by Poinar, 1976 and at the same time, he also proposed the family Heterorhabditidae (Poinar, 1976). This was followed by the discovery of one more new species *H. megidis* by Poinar*et al.* (1987) that was recovered from infected Japanese beetle larvae, *Popilla japonica* from Ohio, which was different from *H. bacteriophora*. Thereafter, 6 more species were described: *H. indica* by Poinar*et al.* (1992); *H. argentinensis* by Stock, (1993); *H. brevicaudis* by Liu, (1994); *H. hawaiiensis* by Gardner *et al.* (1994); *H. marelatus* by Liu and Berry, (1996); and *H. hepialius* by Stock *et al.*(1996), the last one was found to be a synonym of *H. marelatus*. Due to the lack of adequate details, *H. hoptha* and *H. hambletoni* were considered as *species inquirendae*. Unlike steinernematids, very few species have been described in this group which indicates that there is not much morphological and genetic biodiversity in heterorhabditids. As far as the number of *Heterorhabditis* species is concerned, 18 valid species have described till date. Since 2007, 11 species of *Heterorhabditis* genus were described (Nguyen and Hunt, 2007) by different authors and later nine more species have been added, all with morpho-taxometrical and molecular data. Among these nine, four species were synonymised with already existing species; a study of the molecular data by Subbotin (pers. comm.). Like steinernematids, a number of species in Heterorhabditidae too fail to justify valid species status. Survey conducted throughout the World showed that steinernematids and heterorhabditids were common to all types of soil and more frequently inhabit agricultural and secondary forest ecosystems which provide the suitable conditions for host insect's populations (Půža*et al.,* 2016). The global distribution of both the genera indicates that they were present when all land masses were combined as the Pangaea supercontinent. This theory was supported by a genetic study where *Heterorhabditis* was found as a sister group of vertebrate parasitic strongylids and both the groups' arose independently from the free living *Rhabditis* group (Kiontke *et al.,* 2007). In 2011, the ancient age of *Heterorhabditis* clade was shown when a 100 million old *Proheterorhabditis burmanicus* fossil was discovered from Early Cretaceous Burmese amber (Poinar, 2011).

In Nepal, little EPN diversity has been found during surveys of agricultural soils. A few nematologists/parasitologists are working on entomopathogenic nematodes in Nepal and their contribution to taxonomy of these nematodes is still in their preliminary phase. In Nepal, the initial research on EPN was conducted by Khatri-Chhetri*et al.* (2010). They made a survey of the 276 soil samples during June–December 2007. Twenty nine EPNs strains were recovered from 276 soil samples (10.50%) from different climatic conditions and diverse habitats. The prevalence of different species of EPN seems quite obvious with high diversity on climate, altitude, vegetation and habitats.

Moreover, the recovery of seven species of EPN from agricultural soil (12.75%) and orchards (5.26%) is an important indicator of their association with insect pests of agricultural importance. They isolated EPN from both types of habitats indicating that in countries like Nepal, EPN abundance does not only depend upon type of habitat. Survey was conducted in Nepal for the presence of EPN in 87 locations, and 20 sampling sites (22.98%) yielded EPN. EPNs were observed from all the physiographic regions viz. terai and foothills (12.50%), hills (9%) and in mountains (12%) (Fig. 1), however, their abundance were recorded more in river banks (25%) followed by agricultural land (12.75%), forest (9.09%), orchard (5.26%) and meadow (5.26%).

Currently, a total of nine EPN species (8 steinernematids and 1 heterorhabditids) out of 116 species known globally have been reported from Nepal, of which four new species *viz. S. lamjungense* (Khatri-Chhetri*et al.,* 2011b); *S. Everestense* Khatri-Chhetri *et al.*, 2011a; *S nepalense* and *S.surkhetense*Khatri-Chhetri, Waeyenberge, Spiridonov, Manandhar and Moens, 2011c) has been described from the country. However, one of the previously described new species, *S. everestense* is currently regarded as a junior synonym of *S. akhursti* based on its molecular data (Hunt and Subbotin, 2016). Among heterorhabditids, no new species have been reported to date. The other steinernematid species reported from Nepal are: *S. abbasi*Elawad, Ahmad and Reid 1997 (Khatri-Chhetri*et al.,* 2010), *S. Cholashanense* Nguyen *et al.,* 2008 (Khatri-Chhetri *et al.,* 2010), *S. Feltiae* Filipjev, 1934 **(**Khatri-Chhetri *et al.,* 2010), and *S. Siamkayai* Stock, Somsook & Reid, 1998 (Khatri-Chhetri *et al.,* 2010). Distribution of Steinernematids has followed ecological niches and localized areas.

Among *Heterorhabditis*, only one indigenous nematode species, *H. indica*Poinar, Karunakar& David, 1992 (Khatri-Chhetri *et al.,* 2010) have been reported from Nepal till date. *H. indica* was recovered from diverse habitats. There are very few people working on EPN in Nepal, but there may be a great possibility of finding more species of EPN due to varied biogeographic regions. Thus, it may provide a plenty of room for studying these fascinating, useful and delightful worms and later implement as biopesticides. The summary of the sampling results from Nepal is given in Table 1.

Table1. List of *Steinernema* and *Heterorhabdiits* species reported from Nepalesesoil by Khatri-Chhetri*et al.* from 2007 till date with their isolation source/host geographical location and in associated information

S.No.	EPN species	Isolate/ Genbank No.	Locality	Region	Altitude (m)	Habitat	Vegetation	Soil properties	
								pH	Texture
1	*H. indica*	GQ377407	Baglung, Kalika	Hills	880	Forests	*Shorearobusta*		Loam
2		GQ377408	Kaski, Hemja	Hills	1088	Ag. lands	Maize, cucumber		Silt loam
3		GQ377409	Parbat, Siwalaya	Hills	880	Ag. lands	Maize		Silt loam
4		GQ377410	Chitwan, Kurintar	Foot hills	285	Ag. lands	Ginger		Sandy
5		GQ377411	Chitwan, Kurintar	Foot hills	248	River	*Leuceana* sp.		Sandy loam
6		GQ377412	Chitwan, Kurintar	Foot hills	248	Banks river	*Leuceana* sp.		Sandy loam
7		GQ377413	Chitwan, Kurintar	Foot hills	248	Banks river	*Leuceana* sp.		Sandy loam
8	*S. siamkayai*	GQ377414	Chitwan, Dibyanagar	Inner terai	145	Ag. lands	Black gram		Sandy loam
9		GQ377415	Chitwan, Kurintar	Inner terai	285	Ag. lands	Maize		Sandy loam
10		GQ377416	Chitwan, Meghauli	Inner terai	127	Meadows	Mixed grasses		Sandy loam
11	*S. abbasi*	GQ377417	Chitwan, Shivaghat	Inner terai	160	Banks river	Weeds		Silt loam
12	*S. feltiae*	GQ377418	Jumla, Rajikot	Mountains	2375	Orchards	Walnut		Silt loam
13	*S. cholashanense*	GQ377419	Solukhumbu, Kangel	Hills	1950	Ag. lands	Paddy		Loam
14		GQ377420	Solukhumbu, Garma	Mountains	2125	Ag. lands	Garlic		Sandy
15	*S. lamjungense*	HM000101, HM000102	Lamjung, Tarku	Hills	690	Forests	Mixed deciduous		Loam
16		LMT5	Lamjung, Tarku	Hills	710	Forests	Mixed deciduous		Loam
17		LMT7	Lamjung, Tarku	Hills	700	Forests	Mixed deciduous		Loam
18		LMT8	Lamjung, Tarku	Hills	700	Forests	Mixed deciduous		Silt loam
19		SS4	Syangja, Swarketari	Hills	1133	Forests	Mixed deciduous		Silt loam
20	*S. nepalense*	HQ190044, HQ190045	Sunsari, Itahari	Terai	96	Ag. lands	Lentil		Silt loam

Table 1 conted...

21	*S. surkhetense*	HQ190042, HQ190043	Surkhet, Birendranagar	Hills/valley	674	Ag. lands	Black gram	Loam
22		SKB10	Surkhet, Birendranagar	Hills/valley	690	Forests	Sissoo and weeds	Silt loam
23	*S. everestense*	HM000103, HM000104	Dhankuta, Pakhribas	Hills	1805	Ag. lands	Potato	Sandy
24		SLS3	Solukhumbu, Salleri	Mountains	2350	Orchards	Pear	Loam
25		SLS5	Solukhumbu, Salleri	Mountains	2350	Ag. lands	Radish	Loam
26		SLS6	Solukhumbu, Salleri	Mountains	2350	Ag. lands	Cabbage	Loam
27		SLG1	Solukhumbu, Garma	Mountains	2100	Ag. lands	Buckwheat	Loam
28	*Steinernema*sp. E	PD3	Parbat, Dimuwa	Hills	926	Forests	*Alnusnepalensis*	Sandy
29		KL1	Kaski, Lumle	Hills	1730	Forests	*Mixed deciduous*	Loam

Mass Production of Entomopathogenic Nematodes and work carried in Nepal

For EPN as a successful biocontrol agent, mass production is a key issue in its commercialization. Nematodes of both the genera i.e. *Steinernema* and *Heterorhabditis* can be easily mass produced by *in vivo*, using *G. mellonella* as the host, or by *in vitro* methods in both solid and liquid cultures. EPNs are being mass produced on various substances, such as homogenized animal tissue, peptone-glucose agar and pork kidney, potato mash, dog food, chicken offal homogenate, modified dog biscuit, egg yolk, soy flour and cholesterol medium. In USA and some European countries, *Heterorhabditis* and *Steinernema* spp. are now being produced commercially in monoxenic liquid culture systems by using fermenters (Bedding, 1984). In India, the mass production of EPNs is done on solid substrates on a small scale for laboratory and field experiments (Vyas *et al.,* 1999b).

In Nepal, negligible work on EPN is carried regarding their biopesticide properties. Pokhrel*et al.* (2016) worked on laboratory tests of two entomopathogenic nematode strains *S. siamkayai* and *S. abbasi* against the 3rd instar larvae of scarabaeid beetle *Chilolobaacuta* (Coleoptera: Cetoninae) in Khulekhani VDC, Makawanpur district, Nepal. In 2011, Khatri-Chhetri*et al.* reported potential of Nepalese EPN as biocontrol agents against *Holotrichialongipennis*Blanch. (Coleoptera: Scarabaeidae). *H. longipennis* is a serious pest of commercial crops in Siduwa, Dhankuta, Nepal. Seven indigenous isolates of entomopathogenic nematodes (*S. lamjungense* LMT5, *S. lamjungense* SS4, *S. everestense* DKP4, *S. abbasi* CS1, S. sp. KL1, *H.indica* CK2 and *H. indica* CK6) were used in a series of bioassays against the insect. All isolates showed an increased dispersal in response to *H. longipennis*. *S. lamjungense* LMT5, *S. everestense* DKP4 and *S. abbasi* CS1 caused greater mortality than other isolates to different developmental stages. Significant differences were observed in LT_{50} values of the isolates against different stages of *H. longipennis*. Three isolates (*S. lamjungense* LMT5, *S. everestense* DKP4 and *S. abbasi* CS1) along with a commonly used insecticide (chlorpyrifos) were tested against this insect in pot and field experiments. In pot experiments using maize and cabbage as a host crop, *S. lamjungense* LMT5 and *S. everestense* DKP4 performed better than *S. abbasi* CS1 and yielded mortality comparable with chlorpyrifos. These experiments suggest that *S. lamjungense* LMT5 to be a promising biocontrol agent against *H. longipennis* followed by *S. everestense* DKP4 and *S. abbasi* CS1.

Potential for exploration of EPN in Nepalese agriculture

Nepal experiences diverse physiography and ecology, thus most of the crops grown in the world are grown in Nepal. Due to this diversity in crops and vegetation, high diversity of insect species in Nepal are reported, therefore it is expected that the diversity of entomopathogenic nematodes will also be high.Further, Nepal offers a variety of ecological niches in which nematode species adapted to different environmental conditions may be found. Discovery of species and strains with greater tolerance to environmental stresses including temperature, UV, and desiccation will expand the biological control potential of entomopathogenic nematodes. Due to the availability of a range of undisturbed habitats including the rain forest, an opportunity exists for the discovery of novel nematode species and strains in Nepal. Therefore, the diversity of EPNs in Nepalese soils requires further study. The identification of EPNs, adapted to environmental and climatic conditions of cultivated areas is important for sustainable pest suppression in integrated management programs in agricultural areas of Nepal. Thus, there is need of concern of young scientists towards this field of nematology and explore new indigenous species from Nepal soils and alternatively use them for the control of insect pests in IPM programme for the welfare of the Nepal and the world as a whole.

Conclusion and future prospects

The pest problems on vegetables and other incredible crops has been observed to be very serious in Nepal and they have been found to be monophagous, oligophagous or polyphagous based on the number of host plants preferred. They play an important role in lowering the yield and are the biggest challenge that confronts agriculture sector in Nepal today. EPN are paying great attention of experts worldwide as one of the beneficial control agents. EPN have proven to be the most effective as biological control organisms with significant results. Entomopathogenic nematodes have been released extensively in crop fields with negligible effects on non target organisms and are regarded as exceptionally safe to the environment. IJs of EPN have been proved to be not harmful to humans and other vertebrates, can be easily mass-produced using conventional fermentation technology, and several formulations of EPN are available in the market for the control of cryptic pests. Therefore, establishment of EPN in Nepal would bring several benefits. On the basis of the work done so far in Nepal, it can be concluded that the future of EPNs in the control of crop insect pests seems to be promising in Nepal. Intensive surveys are needed from different parts and agroclimatic regions of the country. The occurrence of steinernematids and heterorhabditids in most of the surveyed areas in Nepal indicated the potential role of these nematodes in natural regulation of insect populations. This shows the need for further research on host ranges and characterization of these nematodes in view of possible use for biologic control which will contribute to minimize the use of hazardous chemical pesticides in Nepal.

Due to the exceptional successes made with other biological control agents in other countries, Nepal as developing country should begin the research and innovative ideas to implement the use of EPN through holding workshops on entomopathogenic nematodes; promote cooperative international projects; provide training for Nepalese scientists and students. National and international agreements should express the mutual interest of both parts, in terms of exchanging experiences, material and information and respecting the national and international legislation related to conservation of biodiversity and exchange of biological control agents.

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