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Entomopathogenic nematodes in pest management

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Abstract: Naturally occurring entomopathogenic nematodes and their symbiotic bacteria are important biotic factor in suppression of insect pest populations in soil and cryptic habitats. The virulent species of these nematodes are commercially produced as biological control agents all over the world encompassing North America, Europe, Asia and Australia in glasshouse crops, orchards, ornamentals, turf, lawn, and forestry. India has a great potential to exploit these beneficial nematodes for the suppression of insect pests. Recent emphasis on mass production and formulation technologies of these nematodes in India stresses a need to implement safer and effective pest control methods. This article provides an overview of recent development on formulation and commercialization of entomopathogenic nematodes, and evaluates their potential exploitation in India.

Keywords: Entomopathogenic nematodes, insect pests, biological control, commercial use.

Introduction

Entomopathogenic nematodes (EPNs) are used to control several agriculturally important insect pests of the different orders. The first nematode (*S. carpocapsae*) used successfully in the control of an insect pest was reported 30 years ago from Australia; Commonwealth Scientific and Industrial Research Organization (CSIRO) was the first in the world to use EPNs commercially against black vine weevil in ornamentals and against currant borer moth in black currants (Georgis, 2002). There are several species of EPNs used around the world against a variety of pests in niche markets (e.g. fungus gnats in nurseries, hydroponics, and mushroom; weevils on ornamentals, strawberries, cranberries, citrus and bananas; scarabs of turf, ornamentals and blue berries; cutworms, webworms, billbugs and mole crickets; termites in wooden articles and trees; peach borer moth in apples and carpenter worm in shade trees in China, and fig trees in USA) (Poinar, 1990). Recently out of 13% bio-insecticide sale in industrialised countries, EPN sale was only second to *Bacillus thuringiensis* at 80% (Lisansky & Coombs, 1994). Considerable progress has also been made during the last 20 years on the subject dealing with taxonomy, biology, genetics, ecology, host range, production, application technologies, laboratory and field trials and commercialisation of EPNs and their symbiotic bacteria resulting in over 2000 publications. This aspect was briefly reviewed by Friedman (1990), Akhurst (1996), Kaya and Gaugler (1993) and more comprehensively by Gaugler (2002) and Grewal *et al.* (2005).

Eighty three described EPN species have been identified (64 species of *Steinernema* and 8 species of *Heterorhabditis* and 1 species of *Neosteinernema*) from various insects or from the soil worldwide (Grewal *et al.,* 2001). Some of the important EPN species belonging to Steinernema and Heterorhabditis with their original localities and sources of isolation were listed in the Table 1. The infective juvenile (IJs) of EPN is microscopic organism having 0.5 to 1.5 mm long depending on species. The third stage juvenile of these nematodes have closed mouth and anus and cannot feed until it finds an insect. Usually it is found in soil and is activated by insect movement and then follows a gradient of CO_2 to find the insect larvae (Gaugler *et al.*, 1997) to get into the insect's blood cavity in order to kill it. EPNs enter through the insect's natural body openings, the mouth, anus or respiratory inlets (spiracles) and then penetrate into the blood cavity from the gut (Poinar, 1990); *Heterorhabditis* species can also penetrate through chinks in the insect's armour (the inter skeletal membranes) by scratching away at these with a special tooth (Bedding & Molyneux, 1982). Once in the insect's blood, infective juvenile releases a highly specialised symbiotic bacterium (*Xenorhabdus* spp. in *Steinernema*, *Photorhabdus* spp. in *Heterorhabditis*). These symbiotic bacteria multiply and rapidly kill the insect within a day or two. The bacteria then convert the insect into suitable food for the nematodes and produce a range of antibiotics (Akhurst & Bedding, 1986) and anti-feedants that preserve the dead insect from putrefaction while the nematodes feed and reproduce in it. From a medium-sized insect cadaver 100,000 to 500,000 IJs are produced which leave the decaying cadaver in 7-10 days (unpublished data) and seek out new insect pest hosts.

Nematodes have been applied successfully against soil inhabiting insects (as soil application) as well as above-ground insects (foliar spray) in cryptic habitats (Arthers *et al*., 2004; Shapiro-Ilan *et al*. 2006). They possess many attributes such as wide host spectrum, active host seeking killing the host within 48 h, easy mass production, long-term efficacy, easy application, compatibility with most chemicals, and are environmentally safe. However, the pathogenicity, host searching behaviour, and survivability of different nematode species are varied making them suitable in biological control programs (Kaya, 1990). Indigenous EPN species of Steinernema and Heterorhabditis isolated from Indian natural source with their original localities are listed in the Table 2.

Host range

The entomopathogenic activity of steinernematid and heterorhabditid species has been documented against a broad range of insect pests in a variety of habitats (Kaya & Gaugler, 1993). They have been used inundatively in a number of high value cropping systems (Koppenhofer *et al*., 2000). The nematode-bacterium complex kills insects so rapidly that the nematodes do not form the intimate, highly adapted, host-parasite relationship characteristic of other insect nematode associations. This rapid mortality

Table 1. EPN species of Steinernema and Heterorhabditis with their original localities and sources of isolation

	Nematode species	Original locality	Original source
I	Heterorhabditis Poinar 1976		
1	*H. argentinensis* Stock 1993	Rafaela, Argentina	*Graphognathus* sp.
2	*H. bacteriophora* Poinar 1976	Brecon, South Australia	*Heliothis punctigera*
3	*H. brevicaudis* Liu 1994	Fujian Province, China	Soil
4	*H. hawaiiensis* Gardener *et al.* 1994	Hawaii, USA	Soil
5	*H. indica* Poinar *et al.* 1992	Coimbatore, India	Soil*
6	*H. marelata* Liu & Berry 1996	Seaside, Oregon, USA	Soil
7	*H. megidis* Poinar *et al.* 1987	Jeromesville, USA	*Popillia japonica*
8	*H. zealandica* Poinar 1990	Auckland, NewZealand	*Heteronychus arator*
II	Steinernema Travassos 1927		
1	*S. abbasi* Elawad *et al.* 1997	Sultanate of Oman	Soil
2	*S. affine* (Bovien 1937) Wouts et al. 1982	Denmark	*Bibio* sp.
3	*S. arenarium* Wouts *et al.* 1982	Central Russia	Soil
4	S. *bicornutum* Tallosi *et al.* 1995	Strazilovo, Yugoslavia	Soil
5	*S. carpocapsae* (Weiser 1955) Wouts *et al.* 1982	Czechoslovakia	*Cydia pomonella*
6	*S. caudatum* Xu *et al.* 1991	China	Soil
7	*S. ceratophorum* Jian *et al.* 1997	Jining Province, China	Soil
8	*S. cubanum* Mracek *et al.* 1994	Western Cuba	Soil
9	*S. feltiae* (Filipjev 1934) Wouts *et al.* 1982	Denmark	*Agrotis feltiae*
10	*S. glaseri* (Steiner 1929) Wouts *et al.* 1982	New Jersey, USA	*Popillia japonca*
11	*S. intermedium* (Poinar 1985) Mamiya 1988	South Carolina, USA	Soil
12	*S. karii* Waturu *et al.* 1997	Central Province, Kenya	Soil
13	*S. krausei* (Steiner 1923) Travassos 1927	Germany	*Cephaleia abietis*
14	*S. kushidai* Mamiya 1988	Hamikita,	*Anomala cuprea*
15	*S. longicaudatum* Shen & Wang 1991	Guangdong, China	Soil
16	*S. monticolum* Stock *et al.* 1997	Republic of Korea	Soil
17	*S. neocurtillae* Nguyen & Smart 1992	LaCrosse, Florida, USA	*Neocurtilla hexadactylla*
18	*S. orgonense* Liu & Berry 1996	Oregon, USA	Soil
19	*S. puertoricense* Roman & Figueroa 1994	Puerto Rico	Soil
20	*S. rarum* (de Doucet 1986) Mamiya 1988	Cordoba, Argentina	Soil
21	*S. riobrave* Cabanillas *et al.* 1994	Waslaco, Texas, USA	Soil
22	*S. ritteri* de Doucet & Doucet 1990	Cordoba, Argentina	Soil
23	*S. scapterisci* Nguyen & Smart 1990	Rivera, Uruguay	*Scapteriscus vicinus*

**soil baited with Scirpophaga excerptalis Walker, larvae; Source: Grewal et al. (2001)*

permits the nematodes to exploit a range of hosts that spans nearly all insect orders, a spectrum of activity well beyond that of any other microbial control agent. In laboratory tests, *S. carpocapsae* alone infected more than 250 species of insects from over 75 families in 11 orders (Poinar, 1983). The nematodes attack a far wider spectrum of insects in the laboratory where host contact is assured, environmental conditions are optimal, and no ecological or behavioural barriers to infection exist (Gaugler, 1981). Some nematode species may search for hosts at or near the soil surface (e.g., *S. carpocapsae* and *S. scapterisci*), whereas others are adapted to search deeper in the soil profile (e.g., *H. bacteriophora* and *S. glaseri*). The former group has been referred to as "ambusher", which remains nearly sedentary while waiting for the mobile surface dwelling hosts (Campbell & Gaugler, 1993). The latter group has been referred to as "cruiser" which is highly mobile, responds strongly to long-range host chemical cues, and is therefore best adapted to find sedentary hosts (Grewal *et al.*, 1994)

Safety test

Various tests against mammals (mice, rabbits and monkeys have shown that the EPNs tested were harmless when fed, injected or inhaled (Boemare *et al.*, 1996). They are also harmless to earthworms (Capinera *et al.*, 1982; our unpublished data) and other non-insect organisms including plants and they are of course non-polluting. These nematodes have been produced in large scale in various countries for over ten years and large numbers of production workers have been exposed without any adverse effects being recorded. The Environment Protection Agency (EPA) in the India, USA, Australia and many European countries has exempted EPNs from registration (Ehlers & Hokkenen, 1996; Ehlers, 2005). When environmental benefits including safety for humans and other non-target organisms, reduction of pesticide residues in food, increased activity of other natural enemies, and increased biodiversity in managed ecosystems are taken into account, their advantages are numerous.

In vivo production and formulation technologies

It is far too expensive to rear EPNs by *in-vitro* media as they required separate media source for nematodes growth and their associated bacterium with suitable fermentor. The *in-vivo* process, however, lacks any economy of scale; the labour, equipment, and material (insect diet) costs increase as a linear function of production capacity. However, the first successful and commercial scale of mass production of EPN species; *H. indica, S. carpocapsae, S. thermophilum* and *S. glaseri* on larvae of greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae) and formulation of these nematodes have been developed after three years of rigorous research by Multiplex Biotech Pvt. Ltd., Tumkur, Karnataka, India. This is a pioneered organisation commercializing these entomopathogenic nematodes reared *in vivo* method to increase their virulent and survival.

Galleria mellonella are most commonly used host insect to mass produce these beneficial nematodes because of its rich nutrient source available in body and

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Table 2. Steinernema and Heterorhabditis species isolated from original localities and their sources in India

Nematode species	Original locality	Natural source (soil & insect)	References
Heterorhabditis sp.	Dulhera (UP)	Mango and sugarcane	Rishi Pal *et al.*, 2008
Heterorhabditis indica	Pallavpuram (UP)	Guava	Rishi Pal *et al.*, 2008
Steinernema sp.	Horticulture Nursery, Modipuram (UP)	Guava, mango	Rishi Pal *et al.*, 2008
Oscheius sp.	Crop Research Centre, Modipuram (UP)	Chickpea, wheat	Rishi Pal *et al.*, 2008
Heterorhabditis sp.	University Old campus, Modipuram (UP)	Stevia, lemon, okra, mango	Rishi Pal *et al.,* 2008
Steinernema sp.	University Old Campus, Modipuram (UP)	lemon, okra, mango	Rishi Pal *et al.*, 2008
Rhabditis (Oscheius) sp.	Rice soil, DRR, Hyderabad (AP)	Soil	Katti *et al.*, 2003
S. thermophilum	Rice res. Farm, DRR, Hyderabad (AP)	*G. mellonella* larva	Prasad *et al.,* 2005
H. indica	SBRI, Coimbatore (TN)	Sugarcane stem borer	Poinar *et al.* 1992
S. thermophilum	IARI, New Delhi	soil	Gangly & Singh, 2001
Steinernema sp.	PDBC, Bangalore	soil	Hussain *et al.*, 2001
Steinernema masoodi	IIPR, Kanpur	Soil	Ali *et al.*, 2005
Steinernema seemae	IIPR, Kanpur	Soil	Ali *et al.*, 2005
Steinernema siamkayai	IARI, New Delhi	Soil	Ganguly *et al.*, 2005
Steinernema riobrave	IARI, New Delhi	Soil	Ganguly *et al.,* 2002
H. bacteriophora	TNAU,Coimbatore	Soil	Sivakumar *et al.*, 1989

easy to multiply in economical semi-synthetic diet source containing wheat and corn flour based media (Table 4). About 2.5 billion infective juveniles (IJs) of EPN are recommended to treat one hectare where the insect pests are highly infested on any crop (Wilson *et al.*, 2003; Shaprio-Ilan *et al.,* 2006). The latest formulation developed by Multiplex Biotech Pvt. Ltd., is comprised hydro gel based semi solid cream with IJs suspension containing 1 million nematodes in a polythene pouch that can be readily mixed in a water spray tank without blocking the nozzles. Fifty such pouches are recommended to spray in one acre against the highly infested crop. The nematode shelf-life of 2 to 3 months was achieved at room temperature (27-28 ^{o}C) with maximum of 80 % survival by increasing the nematodes' metabolic rate mixing silica powder (0.1 %). The company do have products of entomopathogenic nematodes that are currently marketed satisfactorily in the name of *'Soldier'* recommended as soil application for soil dwelling insect pests and *"Bouncer"* as foliar application for leaf, flower, fruit, pods, stem and borer insect pests against agriculture, horticulture and forest insect pests.

Entomopathogenic nematodes are often applied to sites and ecosystems that routinely receive other inputs that may interact with nematodes including chemical pesticides, surfactants (e.g., wetting agents), fertilizers, and soil amendments. Often it is desirable to tank mix one or more inputs to save time and money. Infective juveniles are tolerant of short exposures (2-8 h) to most agrochemicals including herbicides, fungicides, acaricides, and insecticides (Ishibashi, 1993; Sankar, 2009; Prasad *et al.*, 2009). Some pesticides act synergistically with EPNs and therefore improve nematode efficacy in inundative applications (Koppenhofer & Kaya, 1998; Nishimatsu & Jackson, 1998). Nematodes are also compatible with most inorganic fertilizers when they applied inundatively (Bednarek & Gaugler, 1997).

Application method

Entomopathogenic nematodes should be applied at the first sign that a pest population is initializing to cause damage. Reapplying nematodes depends on the success of the first nematodes released. Their survivorship and success are based on environmental condition, moisture and soil type and percentage of living nematodes actually released during the first application. Nematodes should be reapplied on seven-day intervals if damage continues. In order to ensure maximum effectiveness, it is crucial to apply them at the optimum environmental conditions needed for their better survival. Therefore, it is best to irrigate the target site, both before and after application because they need moist conditions to prevent desiccation and aid with movement to find hosts. Also, the best results are obtained when the relative humidity is high, ambient temperature is neither extremely hot or cold, soil temperature is between 10 to 35 ^{o}C, soil is moist and direct sunlight is minimal. All of these factors help prevent the nematodes from drying out and increase their survival and virulent.

Successful stories of EPNs used in agriculture pests

Sweet potato weevils: Several weevils attack root and tuber crops, among them those of the genus *Cylas* and *Euscepes* species are the most important on sweet potato and cassava in Brazil and other South American countries. Mannion & Jansson (1992) assessed the virulence of ten entomopathogenic nematodes to *Cylas formicarius*. Most nematodes were more virulent to larvae than to pupae. Adults were less susceptible to nematodes than other stages, and adult males were more susceptible than females. Under field conditions *S. carpocapsae* and *H. bacteriophora* were shown to reduce weevil densities upto 83% and 81% on plants treated with the two species, respectively (Jansson *et al*., 1990).

Table 3. Susceptibility of important crop pests by entomopathogenic nematodes, Heterorhabditis indica and Steinernema carpocapsae

Test insects		*Lethal time (h) of larval mortality by EPN species			
Common name	Scientific name	*H.i*	*S.c*	*S.g*	*S.t*
Gram pod borer	*Helicoverpa armigera*	36	36	42	42
Tobacco caterpillar	*Spodoptera litura*	38	36	40	48
Mustard saw fly	*Athalia lugens proxima*	24	24	36	36
Mulberry leaf folder	*Diapaenia pulvarantalis*	42	36	48	36
Diamond back moth	*Plutella xylostella*	24	24	24	24
Sorghum stem borer	*Chilo partellus*	36.5	36	36	24
Sugarcane borer	*Chilo sacchariphagus indicus*	36	36	48	48
Castor semilooper	*Achaea janata*	47.5	48	48	42
Brinjal fruit borer	*Leucinodes orbonalis*	48	48	36	60
Brinjal spotted beetle	*Epilachna vigintioctopunctata*	50	48	48	56
Bihar harry caterpillar	*Spillosoma obliguae*	48	48	48	48
Fig moth	*Cadra cautella*	48	36	24	36
Termite	*Odontermes obesus*	36	36	24	36
Greater wax moth	*Galleria mellonella*	36	24	20	24
Sorghum grain moth	*Corcyra cephalonica*	24	36	24	42
Cabbage semilooper	*Trichoplusia ni*	24	48	24	48
Bhendi fruit borer	*Earias vittella*	38	24.5	42	24
White grub	*Holotrichia consanguiea*	24	60	48	48
Rhinoceros beetle	*Oryctes rhinoceros*	60	24	52	56
Cabbage leaf webber	*Crocidolomia binotalis*	36	36	24	42
Rice leaf folder	*Cnaphalocrosis medinalis*	36	24	36	42
Rice stem borer	*Scirpophaga incertulas*	48	48	48	36
Coffee white borer	*Xylotrechus quadripes*	48	52	48	48
Banana rhizome weevil	*Cosmopolites sordidus*	48	60	48	56
Cabbage borer	*Hellula undalis*	24	36	24	24

**Mean of 12 replications per treatment; Dosage: 100 infective juveniles/larva; H.i-Heterorhabditis indica; S.c-Steinernema carpocapsae; S. thermophilum; S. glaseri*

Coffee berry borer: Hypothenemus hampei is a major pest of coffee seeds in Brazil and other South American countries (Waterhouse, 1998). Infestations of *H. hampei* occur in coffee seeds while they are enclosed in berries on the trees and in berries that fall to the ground. Spraying of nematodes on fallen berries might remove the need to collect them, leaving them to produce mulch. Dispersal of infected adults may also spread nematodes into the pest population. According to Waterhouse (1998) there appears to be only one record of nematodes attacking *H. hampei* in the field in India (Varaprasad *et al*., 1994). Allard & Moore (1989) showed that a *Heterorhabditis* sp. could cause high mortality of both adult and larvae and that infective juvenile were produced from adults and large larvae. Castillo & Marban-Mendoza (1996) reported differences in the infectivity of eight nematode strains to *H. hampei* larvae and found that three strains of *Heterorhabditis* sp. and one of *S. carpocapsae* caused high mortality of the larvae.

Armyworms, Cutworms and Earworms: Several species of cutworms, *Agrotis* spp., *Spodoptera frugiperda*, *S. exigua* and *S. litorallis* cause serious problems to agricultural, vegetable and forage crops, worldwide. Cutworms are highly susceptible to a number of entomopathogenic nematode species and strains (Morris & Converse, 1991). Control of *Agrotis segetum*, with *S. feltiae* (=*N. bibionis*) in lettuce was equivalent to endosulfan (Lossbroek & Theunissen, 1985) under field conditions. *A. ipsilon* has been effectively managed with *S. carpocapsae* on golf course greens. Larvae and pupae of armyworms are very susceptible to entomopathogenic nematodes (Kaya & Grieve, 1982), and can be effectively managed by nematodes. Richter & Fuxa (1990) reported 33-43% infection of *S. frugiperda* by *S. carpocapsae* in field corn. They also found that spraying of nematodes onto corn ears caused up to 71% infection of *S. frugiperda* and they concluded that *S. carpocapsae*, *S. riobrave*, and *H. megidis* have potential for controlling *S. frugiperda*.

Corn rootworms: The corn rootworms, *Diabrotica* spp., are important pests of corn. In North America *D. virgifera virgifera* and *D. barberi* are the two dominant species that cause significant economic losses to maize. Nematode applications for rootworm suppression were ineffective in the early experiments (Munson & Helms, 1970), but more recently in field studies, *S. carpocapsae* significantly reduced maize root damage (Ellsbury *et al*., 1996), reduced rootworm larval population (Jackson, 1996), and rootworm adult emergence (Ellsbury *et al*., 1996). In some cases, nematode performance was equal to, or better than, insecticides (Wright *et al*., 1993). The limiting factors of efficacy are the need for timing of application to coincide with the phenology of susceptible stages of

Diabrotica spp. (Jackson & Brooks, 1995) and the adverse effects of desiccation on survival of the nematodes. Recent work (Nishimatsu & Jackson 1998) showed that the combined use of insecticides (tefluthrin) with entomopathogenic nematode may offer an integrated approach to increase nematode of rootworm.

Currant borer moth: One of the first insects to be controlled commercially using EPNs was the black currant borer moth, the caterpillars of which bore through the stems of blackcurrants often halving the yield and also halving the vitamin-C content of the remaining berries (Bedding & Miller, 1981a). Over a million cuttings were stacked in walls and sprayed all over with a concentrated suspension of *S. feltiae* infective juveniles and nearly 100% of the larvae had been killed by nematodes. Several plantations that had serious infestations of currant borer moth were treated by blast spraying the currant bushes with a suspension of *S. feltiae* infective juveniles and this reduced borer moth populations by over 70% (Miller & Bedding, 1982).

Black vine weevil in ornamentals: The black vine weevil, *Otiorrhynchus sulcatus,* is the major pest of the potted plant industry, worth over $10 billion annually worldwide, and in the larval stage is also one of the most susceptible insects to EPNs (Bedding & Miller, 1981b). Suspensions of various *Heterorhabditis* species simply applied to the surface of soil within pots usually results in complete control. Currently control of this pest around the world provides one of the major markets for EPNs and CSIRO is selling it in Australia and New Zealand.

Fungus gnats on seedlings, hydroponically grown flowers and mushrooms: The suitability of using *S. feltiae* against fungus gnats was first discovered by Biotech Australia working on the mushroom pest *Lycoriella mali*. Control of fungus gnats on mushrooms and in other situations using this species of EPN is another major market for EPN around the world and they are currently selling it in Australia.

Banana weevils: As an example of how EPNs can be manipulated to control even insects that are the least susceptible to them, consider the banana weevil, which is one of the most important pests of banana. Even though adults of this insect can each be covered with thousands of EPNs without becoming infected, they were able to develop a system that resulted in nearly 100% mortality of insects coming to bait: briefly, the weevils are attracted to damaged banana tissue and will remain in crevices (Treverrow & Bedding, 1993). EPNs cannot penetrate the weevils through the long proboscis or through the anus which is closed like a vice and they cannot get into the breathing holes (spiracles) because these are tightly covered with the wing cases.

For that reason the solution was found at the time of harvesting when the tree is cut down, to have the grower. 1) Using a de suckering tool, cut out a cone of banana tissue from the residual corm. 2) To place in the resulting hole, poly acrylamide gel crystals together with EPNs and 1% mineral oil. 3). To replace the cone of banana tissue. The poly acrylamide gel absorbs sap from the hole which would have stopped the EPNs working and provides a large surface area from which the EPNs can contact the weevils. Replacing the cone encourages attracted weevils to remain. The 1% oil smears at the edge of the wing covers reducing air intake and the weevil has to lift its wing covers to let air in whereupon EPNs are easily able to enter the now exposed spiracles (Treverrow & Bedding, 1993).

Table 4. Diet ingredients and CBR to rear G. mellonella larvae

Ingredients	Quantity /set to rear 2000 larva	Approx. cost of production (in Indian Rs.)
Wheat flour	250 g	06.00
Wheat bran	350 g	05.00
Maize flour	450 g	07.00
Milk powder	100 g	10.00
Honey	100 ml	25.00
Glycerine	100 ml	07.00
Hot water	200 ml	-
Total=	1550	60.00

Apple borer moth: The worst pest of China's one million hectares of apples is a moth (*Carposina niponensis*) rather like our codling moth except that it over-winters as a grub in the soil rather than in crevices in the bark and it was found that EPNs could be used to control this pest better and more cheaply than conventional insecticides. *Steinernema carpocapsae* IJs sprayed beneath apple tree canopies just after the first summer rains (a signal for the caterpillars to migrate from their over winter sites deep in the soil to near the surface to pupate) gave well over 95% kill and the few moths emerging were insufficient to produce economically damaging levels of infestation during that season. That the Chinese replaced most of their older trees (that favour infestation with the moth) with new ones has so far stopped the EPNs being a major success story. However, the new trees will grow and the moth will once again require controlling.

Carpenter worm in shade trees: A carpenter worm, *Holcocerus insularis* is the major pest of trees such as ash *(Fraxinus pennsylvannica),* the Chinese scholar tree *(Sophora japonica)* and willows *(Salix* spp.) that are planted to provide shade for pedestrians and cyclists in the streets of cities in northern China. Hundreds and sometimes thousands of caterpillars eat out the inside of these trees. In 12 cities affected by *H. insularis*, 30-80% of shade trees were infested and 5% killed annually. Now instead of fumigating the trees which is quite complicated and dangerous, council workers inject upper most holes with a suspension of *S. carpocapsae*. The EPNs kill many of the caterpillars after a few days; then nematodes breed up within the cadavers and the next generation kills the remainder. Several million trees have now been treated and the pest has been totally eliminated from several large cities.

There is also a tremendous opportunity for discovery of new nematode strains and species adapted to local environmental conditions and pests. Due to the high diversity of insect species in India, a variety of ecological niches in which nematode species adapted to different environmental conditions. A series of laboratory tests were conducted by "*Multiplex Biotech Pvt. Ltd., Tumkur, Karnataka* with *H. indica, S. carpocapsae, S. glaseri and S. thermophilum* against 25 agriculturally important insect pests collected from various cropping systems in Karnataka. The pathogenicity test conducted by these nematodes caused absolutely 100% mortality of this insect pests within 24 to 60 h of post application (Table 3).

Conclusions and recommendations

The great advantages of using entomopathogenic nematodes as biopesticides for insect pest control are in operator and end-user safety, absence of withholding periods, the advantage of minimising the treated area by monitoring insect populations, minimal harm to natural enemies and lack of environmental pollution. The establishment of entomopathogenic nematodes in India would bring several benefits such as firmly establishing insect nematodes will promote the sustained use of agriculture and develop a better understanding of biodiversity. Due to the exceptional successes made with other biological control agents, India is poised for developing innovative ideas to implement the use of EPNs. This may be accomplished through holding workshops on entomopathogenic nematodes; promote cooperative international projects involving scientists could provide training for Indian scientists and students in university programs. 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.

References

1. Akhurst RJ (1996) From then to now- A brief review of entomopathogenic nematodes and their symbiotic bacteria. 2nd *Intl. Sym.on Entomopathogenic Nematodes & Their Symbiotic Bacteria*, pp: 3-8.
2. Akhurst RJ and Bedding RA (1986) Natural occurrence of insect pathogenic nematodes (Steinernematidae and Heterorhabditidae) in soil in Australia. *J. Aust. Entom. Soc.* 25(3), 241-244.
3. Ali SS, Shaheen A, Pervez R and Hussain MA (2005) *Steinernema masoodi* sp. n. and *Steinernema seemae* sp. n. (Nematoda: Rhabditida: Steinernematidae) from India. *Intl. J. Nematol.* 15, 89-99.
4. Allard GB and Moore D (1989) *Heterorhabditis* sp. nematodes as control agents for the coffee berry borer, *Hypothenemus hampei* (Scolytidae). *J. Invertebr. Pathol.* 54, 45-48.
5. Arthurs S, Heinz KM, Prasifka JR. 2004. An analysis of using entomopathogenic nematodes against above-ground pests. *Bull. Entomol. Res.* 94, 297-306.
6. Bedding RA and Miller LA (1981a) Disinfesting blackcurrant cuttings of *Synanthedon tipuliformis*, using the insect parasitic nematode, *Neoaplectana Bibionis*. *Environ. Entomol*. 10, 449-53.
7. Bedding RA and Miller LA (1981b) Use of a nematode, *Heterorhabditis heliothidis,* to control black vine weevil, *Otiorhynchus sulcatus*, in potted plants. *Ann. Appl. Biol.* 99, 211-16.
8. Bedding RA and Molyneux AS (1982) Penetration of insect cuticle by infective juveniles of *Heterorhabditis* spp. (Heterorhabditidae: Nematoda). *Nematologica.* 28, 354–359.
9. Bednarek A and Gaugler R (1997) Compatibility of soil amendments with entomopathogenic nematodes. *J. Nematol.* 29, 220-227.
10. Boemare N, Laumond C and Mauleon H (1996) The entomopathogenic nematode-bacterium complex: biology, life cycle and vertebrate safety. *Biocontrol Sci. Technol.* 6(3), 333-345.
11. Campbell JF and Gaugler R (1993) Nictation behaviour and its ecological implications in the host search strategies of entomopathogenic nematodes (Heterorhabditidae and Steinernematidae). *Behaviour.* 126, 3-14.
12. Capinera JL, Blue SL and Wheeler GS (1982) Survival of earthworms exposed to Neoaplectana carpocapsae nematodes. *J. Invert. Pathol.* 39(3), 419-421.
13. Castillo A and Marban NM (1996) Laboratory evaluation of Steinernematid and Heterorhabditid nematodes for biological control of the coffee berry borer, *Hypothenemus hampei* Ferr. *Nematropica.* 26, 101-109.
14. Ehlers RU (2005) Forum on safety and regulation, In: *Nematodes as biocontrol agents* (Grewal PS, Ehlers R-U, Shapiro-Ilan DI Eds.) CABI Publ, Wallingford, UK. pp: 107-114.
15. Ehlers RU, Hokkanen HMT (1996) Insect biocontrol with non-endemic entomopathogenic nematodes (*Steinernema* and *Heterorhabditis* spp.): Conclusions and recommendations of a combined OECD and COST workshop on scientific and regulatory policy issues. *Biocontrol Sci. Technol.* 6, 295–302.
16. Ellsbury MM, Jackson JJ, Woodson WD, Beck DL, and Stange KA (1996) Efficacy, application distribution, and concentration by stem flow of *Steinernema carpocapsae* (Rhabditida: Steinernematidae) suspensions applied with a lateral-move irrigation system for corn rootworm (Coleoptera: Chrysomelidae) control in maize. *J. Econ. Entomol.* 89, 71-81.
17. Friedman MJ. 1990. Commercial production and development, In: *Entomopathogenic nematodes in biological control*. Gaugler R and Kaya HK (eds.), Boca Raton, FL, CRC Press. pp: 153-172.

18. Ganguly S and Singh LK (2000) *Steinernema thermophilum* sp. n. (Rhabditida: Steinernematidae) from India. *Intl. J. Nematol.* 10, 183-191.
19. Ganguly S, Rathour KS and Pandey J (2005) New records of *Steinernema siamkayai* Stock from Champawat District of Uttaranchal and its biochemical characterization. *Indian J. Nematol.* 35, 203-204.
20. Ganguly S, Singh M, Lal M, Singh LK, Vyas RV and Patel DJ (2002) New record of an entomopathogenic nematode, *Steinernema riobrave* Cabanillas, Poinar & Raulston, 994 from Gujarat, India. *Indian J. Nematol.* 32, 223.
21. Gaugler R (1981) Biological control potential of neoaplectanid nematodes. *Journal of Nematology.* 13, 241-249.
22. Gaugler R, Lewis E and Stuart RJ (1997). Ecology in the service of biological control: the case of entomopathogenic nematodes. *Oecologia.* 109, 483-489.
23. Gaugler R, Wilson M and Shearer P (1997) Field release and environmental fate of a transgenic entomopathogenic nematode. *Biol. Control.* 9, 75–80.
24. Georgis R (2002) The Biosys experience: an insider's perspective. In: *Entomopathogenic Nematology*. Gaugler R. (ed.) CABI Publishing, Wallingford, UK. pp. 357–372.
25. Grewal PS, Ehlers R-U, Shapiro-Ilan DI (2005) *Nematodes as biocontrol agents*. CABI Publishing, Wallingford, UK, pp. 505.
26. Grewal PS, Gaugler R and Wang Y (1994) Enhanced cold tolerance of the entomopathogenic nematode *Steinernema feltiae* through genetic selection. *Ann. Appl.Biol.* 129 (2), 335 -341.
27. Grewal PS, Nardo ED and Aguillera MM (2001) Entomopathogenic Nematodes: Potential for exploration and use in South America. *Neotropical Entomology.* 30(2), 191-205.
28. Hussaini SS, Ansari MA, Ahmad W and Subbotin SA (2001) Identification of some Indian populations of *Steinernema* species (Nematoda) by RFLP analysis of ITS region of rDNA. *Intl. J. Nematol.* 11, 73-76.
29. Ishibashi N (1993) Integrated control of insect pests by *Steinernema carpocapsae.* In: Nematodes and Biological Control of Insects. Bedding, RA., Akhurst R and Kaya K (ed.) CSIRO, East Melbourne. pp. 105-113.
30. Jackson JJ (1996) Field performance of entomopathogenic nematodes for suppression of Western corn rootworm (Coleoptera: Chrysomelidae). *J. Econ. Entomol*. 89, 366- 372.
31. Jackson JJ and Brooks MA (1995) Parasitism of western corn rootworm larvae and pupae by *Steinernema carpocapsae*. *J. Nematol.* 27, 15-20.
32. Jansson RK, Lecrone SH, Gaugler R and Smart GC (1990) Potential of entomopathogenic nematodes as biological control agents of sweet potato weevil (Coleoptera: Curculionidae). *J. Econ. Entomol*. 83, 1818-1826.
33. Katti G, Padmakumari AP and Prasad JS (2003) *Oscheius* sp., an entomopathogenic nematode of rice yellow stem borer, Scirpophaga incertulas. *Indian J. Nematol*. 32, 44-46.
34. Kaya HK (1990) Soil ecology. In: Entomopathogenic nematodes in biological control. Gaugler R and Kaya HK (ed.). CRC Press, Boca Raton, Florida. pp. 93-115.
35. Kaya HK and Gaugler R (1993) Entomopathogenic nematodes. *Ann. Rev. Entomol.* 38, 181-206.
36. Kaya HK and Grieve BJ (1982) The nematode *Neoplectana carpocapsae* and the beet armyworm *Spodoptera exigua*: Infectivity of pre pupae and pupae in soil and of adults during emergence from soil. *J. Invertebr. Pathol*. 39, 192-197.
37. Klein MG (1990) Efficacy against soil-inhabiting insect pests. In: Entomopathogenic nematodes in biological control. Gaugler R and Kaya HK (ed.). CRC, Boca Raton, Florida. pp. 195-214.
38. Koppenhofer AM, Grewal PS and Kaya HK (2000) Synergism of imidacloprid and entomopathogenic nematodes against white grubs: The mechanism. *Entomol. Exp. Appl.* 94, 283-293.
39. Koppenhofer AM and Kaya HK (1998) Synergism of imidacloprid and an entomopathogenic nematode: A novel approach to white grub (Coleoptera: Scarabaeidae) control in turfgrass. *J. Econ. Entomol.* 91, 618-623.
40. Koppenhofer AM, Grewal PS and Kaya HK (2000) Synergism of imidacloprid and entomopathogenic nematodes against white grubs: The mechanism. *Entomol. Exp. Appl.* 94, 283–293.
41. Lisansky SG and Coombs J (1994) Developments in the market for biopesticides. *Brighton Crop Protection Conference - Pests and Diseases.* 1049-1054.
42. Lossbroek TG and Theunissen J (1985) The entomogenous nematode *Neoplectana bibionis* as a biological control agent of *Agrotis segetum* in lettuce. *Exp. Appl. Nematol.* 39, 261-264.
43. Mannion CM and Jansson RK (1992) Comparison of ten entomopathogenic nematodes for control of sweetpotato weevil (Coleoptera: Apionidae). *J. Econ. Entomol.* 85, 1642-1650.
44. Miller LA and Bedding RA (1982) Field testing of the insect parasitic nematode, *Neoaplectana bibionis* (Nematoda: Steinernematidae) against currant borer moth, *Synanthedon tipuliformis* (Lep.: Sesiidae) in blackcurrants. *Entomophaga.* 27(1), 109-114.
45. Morris ON and Converse V (1991) Effectiveness of steinernematid and heterorhabditid nematodes against noctuid, pyralid, geometrid species in soil. *Can. Entomol*. 123, 55-61.
46. Munson JD and Helms TJ (1970) Field evaluation of a nematode (DD-136) for control of corn rootworm

larvae. (*Diabrotica*). *Entomol. Soc. Amer. N. Cent. Br. Proc*. 25, 97-99.
47. Nishimatsu T and Jackson JJ (1998) Interaction of insecticides, entomopathogenic nematodes, and larvae of the western corn rootworm (Coleoptera: Chrysomelidae). *J. Econ. Entomol*. 91, 410-418.
48. Parwinder S, Grewal L, Elizabeth AB, Nardo D and Aguillera MM (2001) Entomopathogenic Nematodes: Potential for Exploration and use in South America. *Neotropical Entomol.* 30(2), 191-205.
49. Poinar GO (1983) *The Natural History of Nematodes*. Prentice Hall, New Jersey. pp: 323.
50. Poinar GO (1990) Taxonomy and biology of Steinernematidae and Heterorhabditidae. In: Entomopathogenic Nematodes in Biological Control. Gaugler R and Kaya HK (ed.). CRC Press: Boca Raton, Florida. pp: 23-61.
51. Poinar GO Jr, Karunakar GK and David H (1992) *Heterorhabditis indicus* n. sp. (Rhabditida: Nematoda) from India: separation of *Heterorhabditis* spp. By infective juveniles. *Fundamental & Appl. Nematol.* 15, 467-472.
52. Prasad JS, Sankar M, Padmakumari AP and Gururaj Katti (2009) Compatibility of Entomopathogenic Nematode, *Heterorhabditis Indica w*ith insecticides used in rice. Intl. Sym. on Biopesticides, TERI, New Delhi, Abs. 64. pp: 55.
53. Richter AR and Fuxa JR (1990) Effect of *Steinernema feltiae* on *Spodoptera frugiperda* and *Heliothis zea* (Lepidoptera: Noctuidae) in corn. *J. Econ. Entomol*. 83: 1286-1291.
54. Rishi Pal, M, Abid Hussain and Prasad CS (2008) Natural occurrence of entomopathogenic nematodes in meerut district, North India. *Intl. J.Nematol.* 18(2), 198-202.
55. Sankar M (2009) Investigation of an indigenous entomopathogenic nematode, *Heterorhabditis indica* Poinar, Karunakar and David (1992) as a potential biocontrol agent of insect pest in rice. PhD thesis, Osmania Univ., Hyderabad. pp: 168-79.
56. Shapiro-Ilan DI, Gough DH, Piggott SJ, Fife JP (2006) Application technology and environmental considerations for use of entomopathogenic nematodes in biological control. *Biol. Control.* 38, 124–133.
57. Sivakumar CV, Jayaraj S and Subramanian S (1989) Observations on an Indian population of the entomopathogenic nematode, *Heterorhabditis bacteriophora* Poinar, 1976. *J. Biol. Control.* 2, 112-113.
58. Treverrow NL and Bedding RA (1993) Development of a system for the control of the banana weevil borer, *Cosmopolites sordidus* Germar (Coleoptera: Curculionidae) with entomopathogenic nematodes. In: Nematodes and biological control of insect pests. Bedding R, Akhrust and Kaya HK (eds.). Melbourne, CSIRO. pp: 41- 47.
59. Varaprasad KS, Balasubramanian S, Diwakar BJ and Rao CVR (1994) First report of an entomogenous nematode, *Paragrolaimus* sp. from coffee-berry borer*, Hypothenemus hampei* (Ferrari) from Karnataka, India. *Plant Prot. Bull.* 46, 2-3.
60. Waterhouse DF (1998) Biological control of insect pests: Southeast Asian prospects. Canberra, Australian Center for International Agricultural Research, ACIAR Monograph Series, no. 51, pp:548
61. Wilson MJ, Lewis EE, Yoder F and Gaugler R (2003) Application pattern and persistence of the entomopathogenic nematode *Heterorhabditis bacteriophora*. *Biol. Control.* 26, 180-188.
62. Wright RJ, Witkowski JF, Echtenkamp G and Georgis R (1993) Efficacy and persistence of *Steinernema carpocapsae* (Rhabditida: Steinernematidae) applied through a center-pivot irrigation system against larval corn rootworms (Coleoptera: Chrysomelidae). *J. Econ. Entomol*. 86, 1348-1354.