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Global Standing of EPN for Insect Pest Management *vis-a-vis* Indian Contribution: The Missing Links

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ABSTRACT: Entomopathogenic nematodes (EPNs) provide a quick, reliable and environmentally safe mode for controlling insect pests. Focused research in the US and Europe has led to a fast-growing global market of EPN-based products in the past couple of decades. The global revenues of EPN products match the returns from *Beauveria*, *Metarhizium* and *Trichoderma*. Although EPNs are considered to be broad spectrum, their tremendous international success is due to products that target specific stages of a specific pest in a specific crop in a specific agro-climatic region. Targeted products are launched in cruiser and ambusher EPNs to manage sedentary and mobile pests, respectively. The EPN products are largely available in the form of wettable powder and water-dispersible granules with six to ten months of shelf-life under refrigerated conditions. Unfortunately, indigenous products do not match up to international standards and therefore, do not contribute to the fast-growing global market. Several products are marketed with unrealistic claims on their shelf-life. The powder formulations are developed without adequate technical knowledge of the survival mechanism of EPNs, and invariably anhydrobiosis is not induced in these. The development of formulations by untrained EPN specialists is a major factor why our products do not meet the international quality parameters, and storage and shipment stability. Only trained nematologists having sound knowledge of the survival strategy of EPNs and an understanding of the biochemical processes involved in the inducement of anhydrobiosis should handle product development. Majority of the local products are non-selective to the pest, crop or geographical location. A one-size-fits-all approach to the promotion of EPN products defies the concept of niche specificity and insect-specificity. Field application should be synchronous with the biology and behaviour of the target pest. Scientists must understand and integrate insect behaviour and soil dynamics.

Keywords: Entomopathogenic nematodes, foraging, formulations, *Heterorhabditis*, *Steinernema*, survival strategies

INTRODUCTION

The Entomopathogenic nematodes (EPNs) are obligate insect parasites in the families Heterorhabditidae and Steinernematidae. The two important genera *Heterorhabditis* and *Steinernema* have been successfully utilized as biopesticides as they are capable of controlling a variety of economically important insect pests (Koppenhöffer, 2007; Půža, 2015). Extensive global soil surveys have resulted in isolating 100 *Steinernema* and 21 *Heterorhabditis* species (Bhat *et al.*, 2020). Besides these two genera, *Neosteinernema longicurvicauda* (family Steinernematidae) (Nguyen and Smart Jr, 1994) and a few species belonging to the genus *Oscheius* (family: Rhabditidae) have also been identified with entomopathogenic ability (Torres-Barragan *et al.*, 2011). Among all the known species, currently, five species of *Heterorhabditis* and eight species of *Steinernema* are commercially produced for managing insect pests. The only free-living, non-feeding stages of *Heterorhabditis* and *Steinernema* are the third-stage infective juveniles (IJ) or the dauer larvae. The IJ range from 418 μm and 1283 μm in length for different species (Nguyen and Hunt, 2007). The IJ of *Steinernema* and *Heterorhabditis* carry lethal gram-negative symbiotic bacteria *Xenorhabdus* and *Photorhabdus* (family Morganellaceae) (Adeolu *et al.*, 2016), respectively, which are responsible for the death of the insect. In their natural habitat, the IJ with the help of their advanced chemoreception and mechanoreception are capable of finding and penetrating an insect host (Riga, 2004). The IJ of *Steinernema* gain entry via the natural openings of the insect *i.e.*, mouth, anus, and spiracles, whereas those of *Heterorhabditis*, in addition to the natural openings, can also penetrate directly into the insect haemocoel by puncturing the intersegmental membranes of the cuticle using a mural tooth (Popiel and Hominick, 1992). The ultimate destination of the IJ is the insect hemocoel where they release their symbiotic bacteria. The bacteria multiply exponentially to cause fatal septicaemia, thus, killing the insect within 24–48 hrs (Poinar and Grewal, 2012). The IJ continue to multiply for two to three generations, following which they leave the cadaver in search of another insect host. The IJ are of commercial significance as they are used for developing commercial formulations. For the development of stable and effective products for insect pest management, certain attributes of the IJ are taken into consideration which are described below.

BEHAVIOUR AND FORAGING STRATEGIES

To make EPNs a successful bioagent, it is important to understand the foraging behaviour and ecology of the individual isolates *vis-a-vis* their original locality. The host-specificity of EPN is directly correlated with their foraging strategy. The EPNs broadly adopt two distinct foraging strategies - cruiser (move towards a host stimulus) and ambusher (sit-and-wait for the host to arrive). Cruiser can more efficiently infect sedentary, slow-moving pests or those with cryptic behaviour; whereas, ambushers have a higher possibility of infecting mobile pests. Cruiser EPNs depend on the volatile cues in the soil which are released by the host insect as excretory/secretory products, by the plant roots in the form of exudates, and by certain specific compounds released due to insect damage. These cues get dissolved in the capillary water to form a gradient in the soil to allow cruisers to orient their movement (Bird and Bird, 1986; van Tol *et al*., 2001). The EPNs *H. bacteriophora*, *H. megadis* and *S. glaseri* are cruising foragers, while *H. zealandica*, *S. carpocapsae, S. scapterisci,* and *S. siamkayai* are ambush foragers (Campbell and Kaya, 2002). An intermediate foraging behaviour is also evident in some EPNs like *S. riobrave*, which are initially attracted to volatile cues of the host, followed by localized search in response to contact with host body surface (Campbell *et al*., 2003). Different insects feed at different soil depths, different plant parts or cryptic habitats. Therefore, a specific application technique is required for specific EPN products to make easy access for the IJ to their preferred host. *Steinernema* IJ spread horizontally in the upper soil layers, thus they are more effective against insects that are active in the sub-soil. On the other hand, *Heterorhabditis* have the tendency to move downwards, and therefore, are more suited for insects that are active at a depth (Campbell and Gaugler, 1993; Lewis, 2002). The global EPN market is largely successful as the products are specific to insect behaviour and foraging strategies of the IJ.

SURVIVAL STRATEGIES

Being specialized metazoans having symbiotic associations with bacteria, the EPNs require specific conditions to survive and express their virulence. Soil moisture and temperature are the two most important factors which influence the survival of EPNs. All EPNs are most efficient within a favourable narrow range of temperature. This is one of the reasons why commercial products of indigenous EPN isolate belonging to a particular agro-climatic zone are recommended and introduced in the locality of their origin for efficient management of native insect pests. For example, *Heterorhabditis* spp. are efficient between 7–35°C, *S. feltiae* between 2–30°C, while at 10°C *S. carpocapsae* becomes inactive (Georgis *et al*., 2006; Lacey et al., 2006). Till the IJ finds a suitable host, they survive on the lipid reserves which are present in the form of triacylglycerides and glycogen reserves (Grewal and

Georgis, 1999). In *Steinernema* the unsaturated fatty acid is comparatively higher (up to 70%) as compared to *Heterorhabditis* which has more saturated fatty acids (up to 62%) (Selvan *et al*., 1993a,b; Patel and Wright, 1997a). The non-feeding IJ of *Steinernema* utilize oleic acid, palmitic acid and stearic acid which decline rapidly within 100 days of storage, while the glycogen reserves present between 8–18 per cent of nematode dry weight decline more slowly than the lipids (Patel and Wright, 1997b). However, once the lipid is consumed the glycogen starts declining at a faster rate which results in reduced infection ability of the IJ. The formulations with active IJ have a limited shelf-life of less than three months which is extended to some extent by refrigerated storage of the products that allows the slowing down of the metabolic processes. Due to an active searching behaviour, the metabolic process in *H. bacteriophora* is higher as compared to *S. feltiae*, although both are cruise foragers. When formulated, *H. bacteriophora* having a smaller IJ (588 μm) has a poorer shelf-life than *S. glaseri* (879 μm). Due to their smaller size, the lipids reserves are less which deplete faster (Lewis *et al*.,1995).

EPNs are highly prone to desiccation due to soil moisture stress. A unique ability of *Steinernema* and *Heterorhabditis* IJ is to survive dry conditions by entering into partial anhydrobiosis which has no or limited effect on their pathogenic potential (Grewal, 2000b; Matadamas-Ortiz *et al*., 2014). This attribute has been successfully exploited for the development of commercial formulations of EPNs with an extended shelf-life of over six months. During induction of anhydrobiosis, physically, the IJ aggregate and coil to reduce the body surface exposure to the environment, and thus, reduce the rate of water loss (Womersley 1990a,b; Glazer 2002; Balakumaran *et al*., 2022). Biochemically, their metabolism is slowed down and their oxygen consumption is reduced by up to 80 per cent (Grewal, 2000a). The glycogen decreases rapidly while trehalose and glycerol synthesis increases (Qiu *et al*., 2000). Trehalose replaces the bound water in nematode membranes and maintains the integrity (Behm, 1997; Crowe *et al*., 1998b; Gal *et al*., 2001). An in-depth molecular and biochemical investigation in *H. indica* IJ has revealed a complex process during which 1584 transcripts get upregulated and 340 downregulated. A large number of genes involved in detoxification pathways, antioxidant defense, signal transduction, unfolded protein response and molecular chaperones and ubiquitin-proteasome system get activated. Genes involved in gluconeogenesis- β-oxidation of fatty acids, glyoxylate pathway; glyceroneogenesis; fatty acid biosynthesis; amino-acid metabolism - shikimate pathway, sachharopine pathway, kyneurine pathway, lysine biosynthesis; one-carbon metabolism, polyamine pathway, transsulfuration pathway, folate cycle, methionine cycle, nucleotide biosynthesis; mevalonate pathway; and glyceraldehyde-3-phosphate dehydrogenase express differential expression. The shikimate pathway, sachharopine pathway and glyceroneogenesis play an important role during the induction of anhydrobiosis (Balakumaran *et al*., 2022). This information is of immense significance in enhancing the shelf-life of water dispersible formulations.

GLOBAL EPN FORMULATIONS AND COMMERCIALIZATION

The commercialization of EPNs started in the 1980s. Presently, nearly 40 countries are involved in the development of EPN formulations to control insect pests in high-value agricultural crops, horticultural crops, turfs, lawns and home gardens using an inundate approach. The success rate of any EPN product cannot match that of chemical insecticides. Farmers will never replace insecticide with EPN products unless they are convinced with the on-field efficacy of the nematodes. Focussed research has reduced the gap between the two to some extent by targeting the appropriate EPN species against specific-pest, specific-crop and specific agroecological habitat. In addition, emphasis has been given to advancements in formulation technology, quality control, delivery systems and application schedules yielding promising results. Research efforts are mainly directed towards enhancing the efficacy of the EPNs in the fields. Targeted products are released in compatible environments of the EPNs in terms of soil type, soil moisture, salinity, organic matter, temperature and exposure to ultraviolet light. Formulations released for commercialization are rigid and uncompromised in terms of the carrier materials used for entrapping the IJ in various gels or clays. While making formulations with active IJ, the significance of viscosity, moisture, dissolved oxygen availability, pH, UV protectants *etc*. are given importance. Likewise, products with partially anhydrobiotic IJ are based on the appropriate combination of clays, their particle size, distribution, compactness, hygroscopic moisture content, pH *etc*. for sustained survival of the IJ. Gradual water loss is critical for inducing partial anhydrobiosis in the formulation which allows the IJ to adapt to the moisture stress and the metabolism goes to undetected levels and upon rehydration the IJ revive. The wettable powder (WP) and water dispersible granule (WDG) formulations with six to ten months shelf-life, when stored under refrigerated conditions, dominate the international market. The past couple of decades have seen focused and stringent research in the US and Europe which has led to a fast-growing global market of EPN-based products. The International Biocontrol Agents Market Value for EPN products was USD 17 million in 2016 which was estimated to grow to USD 28 million in 2022 (BBC Research Report, 2018; CPL Business Consultant Report, 2018). The global revenues of EPN products match the returns from Bt, *Beauveria*, *Metarhizium* and *Trichoderma*.

EPN HOST RANGE

In an inundative biocontrol strategy, the EPNs are highly effective against a wide range of soil-borne pests as soil is their natural habitat, and to a lesser extent against above-ground pests (Arthurs *et al*., 2004; Shapiro-Ilan *et al*., 2006). The entomopathogenic activity of *Heterorhabditis* and *Steinernema* species has been documented against a broad range of insect pests infecting a wide range of crops cultivated in several different habitats. Table 1 elaborates on the major crops and pests against which the EPN products have been successfully commercialized or have shown promise in terms of valid reports with ≥ 70 per cent reduction of the pest in the field trials.

MISSING LINKS: WHY DO WE FALL SHORT OF MEETING THE INTERNATIONAL STANDARDS?

In the 1980s, two EPN products, namely Green Commandos (*S. carpocapsae)* and Soil Commandos (*H. bacteriophora*) were imported to India by Ecomax Company. These products were withdrawn due to their poor sustenance under field conditions as the nematodes were not native to India. In the past two decades, several EPN products have been developed and commercialized in India using indigenous isolates. Wettable powder formulations developed for *H. indica* strain NBAII HI1 and *H. bacteriophora* NBAII HB5 at ICAR-NBAIR were licensed to 16 Indian companies between 2012–2017. The *H. indica*-based products (Soldier, Nema Power, BCS-Grub Terminator, Grubcure, Calterm, Aarmour) have been released to control grubs, cutworms, root weevils, different pests belonging to order Lepidoptera and termites in crops like sugarcane, corn, arecanut, cardamom, groundnut, potato, banana, guava, turmeric, pulses, vegetables *etc*. The shelf-life of these products is 10–12 months at normal temperature (https://www.nbair.res.in/sites/default/files/2018-12/EPN%20brochure_0.pdf). Some of the products claim at least viable 90 per cent IJ even after eight to twelve months of storage at temperatures between 25°C and 37°C (https://nbair.res.in/sites/default/files/left%20menu/icbc2018/ICARNBAIRtechnologiesforAgribusiness.pdf).

Table 1. Major crops managed by various entomopathogenic nematodes (Adapted from Koppenhöffer *et al*., 2020)

Crop	Pest order	Common name	EPN used
Vegetables	Diptera	Leaf miners	Sc, Sf
Tomato	Lepidoptera	Tomato leaf miner	Hb, Sc, Sf
Vegetables		Armyworms Black cutworm Corn earworm Turnip cutworm Artichoke plume moth	Sc, Sf, Sr Sc Sc, Sf, Sr Sc, Sf Sc
Sugarbeet	Coleoptera	Sugarbeet weevil	Hb, Sc
Sweet potato		Sweet potato weevil	Hb, Sc, Sf
Maize	Coleoptera	Corn rootworms	Hb, Sc
Mushrooms	Diptera	Fungus gnats	Sf, Hb, Hi
Greenhouse	Thysanoptera	Western flower thrips	Sc, Sf
Berries	Coleoptera	Black vine weevil Strawberry root weevil	Hb, Hd, Hmeg, Hm, Sc, Sg, Sf Hm, Sc
Cranberry		Cranberry rootworm	Hb
Blueberries		Scarab grubs	Ssc
Cranberry	Lepidoptera	Cranberry girdler	Sc
Banana	Coleoptera	Banana weevil	Hb, Sc, Sf
Citrus		Citrus root weevils Diaprepes root weevil	Hb, Sr Hb, Hi, Sr
Pecan		Pecan weevil	Sc
Fruit trees		Plum curculio	Sr
Palms		Red palm weevil	Sc
Stone fruit		Flat-headed root borer	Sf
Citrus	Lepidoptera	False codling moth	Hb
Nut/fruit trees		Navel orange worm	Sc
Fruit trees		Clearwing borer moths	Hb, Sc, Sf
Pome fruit		Codling moth	Hz, Sc, Sf
Fruits various	Diptera	Fruit flies	Hi, Sc

Hb, Heterorhabditis bacteriophora; Hd, H. downesi; Hm, H. marelata; Hmeg, H. megidis; Hz, H. zealandica; Sc, Steinernema carpocapsae; Sf, S. feltiae; Sg, S. glaseri; Sk, S. kushidai; Sr, S. riobrave; Ss, S. scapterisci; Ssc, S. scarabaei

Recently, powder formulations of *H. indica* strain SBITND78 and *S. glaseri* strain SBILN1were released against white grubs and other pests by *ICAR-SBI*. The formulations can be stored at room temperature in sealed aluminium-lined high-density polyethylene sachets. The shelf-life for *H. indica* formulation is of nine months with 92 per cent survival of the IJ and *S. glaseri* is of 12 months having 90 per cent survival of IJ at 30±5°C (https://sugarcane.icar.gov.in/index.php/epn_biopesticide_formulation/). A couple of products *Grubicide* (*H. bacteriophora*) released by NBAII and Pusa nemagel (*S. thermophilum*) by IARI were also commercialized but later withdrawn. Besides, several other government departments, agricultural universities, private companies, small start-up companies, non-government organizations (NGOs) *etc*. are also actively involved in formulating and releasing their products in the form of gels and wettable powder. Unfortunately, only a few products have shown sustained efficacy at the field level and a large number of these have failed to deliver and satisfy the farmers. The claims made by a majority of such products do not match with the international standards. Several gel and powder-based products are sold across the country with unrealistic claims on their shelf-life. Some major concerns as to why our products fall short of meeting international standards are discussed below.

1. Unlike the international markets which follow a strict regime of refrigerated storage of the products, the local markets do not follow temperature regulation of the product at all. It is evident from random analysis that the IJ die and appear straight within a short span of time after being formulated because the products are subjected to variable temperatures during storage and shipment across the states. Therefore, the products reaching the farmers are sub-standard. Invariably, the claims on the number of IJ present in the formulation are also far less. Thus, we lag behind on the desired international quality parameters, storage and shipment stability.

2. Unlike the fungal or bacterial bioagents, the EPNs (*Heterorhabditis*, *Steinernema*) are unique metazoans having specialized symbiotic associations with the bacteria. Only trained entomopathogenic nematologists possessing a sound understanding of the biology, behaviour, ecology and pathogenesis of the EPNs should handle them. Mushrooming of personnel who have trivial or below par comprehension of EPNs are involved in the formulation development which has been a major concern over the past decade.

3. Adequate knowledge of the survival mechanism of EPNs and understanding of the biochemical processes involved in the inducement of partial anhydrobiosis during formulating the EPNs is of utmost importance. There is a subtle difference between true anhydrobiosis exhibited by several plant parasitic nematodes (*Anguina, Ditylenchus, Aphelenchoides etc.*) and partial anhydrobiosis exhibited by the EPNs. The water-dispersible granules and wettable powder products in the international market claim a shelf-life of 6–10 months under refrigerated storage conditions, wherein partial anhydrobiosis is induced. The powder formulations in the local markets are developed without adequate technical knowledge of the survival mechanism of EPNs and, invariably, anhydrobiosis is not induced. International products follow a gradual pan-drying approach to induce partial anhydrobiosis by simulating the natural water-stress that the IJ encounters in the field. Thus, the IJ appears coiled in the dry state which revives and straightens upon rehydration. The indigenous products adopt the fan-drying approach in which the majority of the IJ lose their water content at a faster rate, rarely coil, remain straight and die. A major concern is that the majority of the personnel involved in the development of EPN formulations lack in the knowledge, adopt unscientific approaches, and make unrealistic claims about the shelf-life. Such claims should be strictly scrutinized before licensing a product.

4. The immense global success of EPNs is due to the products which cater to ‘specific pests’ attacking ‘specific crop’ at a ‘specific agro-climatic region’. Unfortunately, the local products do not figure and

contribute to the international market as we are ignoring the standard baseline research approach to develop and commercialize crop-specific, pest-specific and location-specific products. Selection of an EPN is based on the foraging strategy, host range, and biotic factors affecting their survival and efficacy. A stringent proof of concept and multilocation validation of native isolates to manage native pests is imperative before the commercialization of any product. Majority of the researchers ignore the basic conceptualization and adopt easy shortcut plans that entirely defy the biological concept of niche-specificity, survival and pathological efficacy of EPNs in alien environments. This is one of the main reasons which has drastically hampered the commercial success of EPNs. With vast variations in the biotic and abiotic factors that govern Indian agriculture, it is therefore imperative to develop, validate and recommend insect-specific/crop-specific/location-specific products. Unfortunately, the local companies, are indiscriminately promoting EPN products of any nematode species, *Heterorhabditis* or *Steinernema*, for multi-purpose applications against a wide range of insect pests countrywide, irrespective of the nematode's origin and its host preference. The Institutions that sign MOUs with companies, with specific claims on their product, should keep a watchdog approach to prevent unscientific and unethical doings.

5. Targeted products with cruiser/ambusher are not considered. The targeted strategy yields the benefits of accuracy and economy as higher insect mortality is achieved at a lower dose. Appropriate dose of application depending upon the magnitude of pest incidence based on different pests and crops is not considered.

6. Application directives coinciding with the biology and behaviour of the target pest is disregarded. Different EPN species possess differential pathogenicity against different pests and different stages of the same pest. The scientists must understand and integrate insect behaviour (site of egg-laying, feeding site of neonates, feeding site of advanced stages) with appropriate application schedules. The time of application should merge with the availability of the vulnerable stages of the pest.

In summary, to exploit the full potential of EPNs, our research should be designed to conform to our unique ecological parameters and our demographic realities. We have to chart our own path and create our own milestones. We must refrain from and discourage 'borrow-cut and paste' technology. We should exploit insect-specific and location-specific indigenous nematodes and design innovative application procedures. Unscientific research by untrained personnel involved in the commercialization of EPNs should be strictly discouraged. We need to strengthen our basic research to cross the borderline from failure to success.

CONFLICT OF INTEREST

The opinions and statements in this article are solely those of the author based on his research experience and interactions with researchers in government organizations, NGOs, R&D personnel of private companies and farmers across India. There is no intention to disregard the research of entomopathogenic nematode scientists with genuine research out-puts, but to raise concern and caution against deceptive claims.

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