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**Review Article**

# An overview of trends in pest management and the need for a paradigm shift in technologies for the progression of entomopathogenic nematodes in managing crop health

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**ABSTRACT:** Global pesticide usage is 3.5 million tonnes at an average of 1.81kg/ha, while Indian usage is at 55,000 metric tonnes (2023) with an average of 0.517kg/ha. Compared to the pesticide market, the Indian biopesticide market remains small- cumulative annual biopesticide production at 9000 metric tonnes and a growth rate of 3-5% in consumption which is projected to reach a CGR of 8-10% by 2030. The utilization of biopesticides amounts to approximately 9% of overall pesticide use and is projected to increase to 50% of the total pesticide market by 2050. Among several microbial biocontrol agents, Entomopathogenic Nematodes (EPN) has been realised to be dependable IPM component against several insect pests. EPNs are soil-inhabiting beneficial nematodes that parasitize and kill insect pests, with immense potential for ecological services making them valuable tools in IPM. Worldwide, the demand for the development of EPN-containing products is mounting with several companies involved in their production, distribution and sales. India's estimated demand for EPN is 24,000 metric tonnes, while the current production is 1800 metric tonnes from 25-30 firms. In India and other developing countries, the current EPN production and supply chain are in their infancy and operate as a cottage industry. The market is flourishing with products that are spurious, expensive, and unregulated due to the wide gap between demand and availability of EPN products. The authors present an overview of the status and prospects of EPN as an IPM component, contemporary and futuristic issues for the transformation of the upcoming EPN industry to a self-reliant, self-sufficient and profitable enterprise and accomplish better uptake of EPN individually or in IPM.

**KEYWORDS:** Biological control, entomopathogenic nematodes, innovations, paradigm shift, pest management

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**ABBREVIATIONS:** BC - Biological control; BCA - Biological control agents; CAGR - compound annual growth rate; CBD - Convention on Bio-Diversity; CM - carbamates; $DO_2$ - dissolved oxygen; DPPQS - Directorate of Plant Protection, Quarantine and Storage; EPN - entomopathogenic nematodes; EU - European Union; GPS - Geographical position system; IJs - infective juveniles; ICAR-NBAIR - Indian Council of Agricultural Research; ICAR-NBAIR - National Bureau of Agricultural Insect Resources; ICAR-SBI - Sugarcane Breeding Institute; IPM - integrated pest management; Mha - million hectares; NE - natural enemies; OP - organophosphorus; SDG - sustainable development goals; WP - Wettable powder.

## PARADIGM SHIFTS IN CROP PROTECTION PHILOSOPHY

In the current era of futuristic global farming systems and the regulations enshrined through SDGs, CBD, and Biosafety protocols, the authors consider that there have been three major paradigm shifts in theory and practice related to crop protection and crop health management. The three major trends include the paradigm shift in pesticide use for agricultural pest management, the paradigm alteration in IPM strategies and the paradigm shift towards biological control and ecological services.

Firstly, the paradigm shift in pesticide use for agricultural pest management reflects a growing awareness of the limitations and drawbacks associated with conventional pesticide-based approaches. Here are certain points related to pesticide use:

- Reduction of reliance on synthetic pesticides to mitigate the residues, environmental pollution, negative impacts on non-target NEs and organisms and pesticide resistance in pest populations.

- Emphasis has shifted towards Integrated Pest Management (IPM) and biological control such as using NEs (predators, parasitoids, pathogens) and biopesticides

derived from natural sources (microorganisms, botanical extracts), which offer eco-friendly alternatives to chemical pesticides.

- Advancements in technology allow for more precise and targeted application of pesticides, minimizing their overall use and reducing non-target exposure. Techniques like precision agriculture, using drones, and sophisticated equipment help in applying pesticides only where and when necessary.

- Regulatory bodies in many countries are revising regulations governing the approval and use of pesticides. This shift is aimed at ensuring the safety of pesticide use, minimizing environmental contamination, and promoting the scope for the adoption of safer alternatives.

- Growing public concern about pesticide residues in food and their impact on health has influenced consumer preferences towards organically grown produce and sustainable agricultural practices, driving the demand for reduced pesticide use.

- Further, efforts are being made to develop and promote the use of novel reduced-risk pesticides that have lower toxicity, shorter persistence, and specific modes of action.

- Importantly, providing education and training to farmers on the adoption of alternative pest management strategies, sustainable agricultural practices, and the importance of biodiversity conservation is essential to drive this paradigm shift.

- Secondly, in recent years, the strategies for insect pest management have undergone a significant paradigm shift, moving away from heavy reliance on chemical pesticides and embracing more integrated, sustainable, and ecologically friendly approaches. Several key changes have marked this shift:

- IPM emphasizes a holistic approach that integrates multiple strategies to manage pests effectively while minimizing environmental impact, while the main objective is on prevention and monitoring to sustain pest incidence below economically damaging intensities.

- There's been a greater focus on harnessing natural enemies, such as predators, parasitoids, pathogens, and their ecological services for biological control. This involves introducing beneficial organisms, conserving existing natural enemies, or using microbial agents as biopesticides to manage pest populations.

- HPR and cultivating crop varieties with inherent resistance or tolerance to pests have gained traction. This involves utilizing genetic diversity to develop plants with traits that deter pests or reduce susceptibility to damage.

- Emerging technologies such as genetic engineering and genome editing for pest-resistant crops and novel biopesticides derived from microbial sources or botanical extracts continue to be explored.

- Behavioural Manipulation using pheromones, semiochemicals, or other behaviour-modifying substances to disrupt pest mating, for example, through mass trapping, mating disruption, or attract-and-kill strategies, has become more prevalent.

- Precision Agriculture with remote sensing, GPS, drones, and data analytics, have enabled more precise monitoring and targeted application of control measures. This helps optimize resource use and minimize the ecological footprint of pest management practices.

- There's been a push towards the development and use of minimal-risk pesticides, including biopesticides derived from natural sources or using specific modes of action that are less harmful to non-target organisms and the environment.

- Community involvement of the farmers, stakeholders, and communities in understanding the significance of sustainable IPM practices and providing them with knowledge and training has become integral to successful pest management programs.

- With changing climatic conditions affecting pest distributions and behaviour, pest management strategies have evolved to adapt to these shifts, considering the effect of climatic change on pest dynamics.

- Climate change adaptation in NEs and biological control: The impact of climate change is critical not only on pest dynamics, it manifests on the NEs and BCA and their adaptive pest management strategies are increasingly crucial.

This paradigm shift toward sustainable, integrated, and environmentally friendly pest management approaches aims to reduce reliance on chemical pesticides, minimize ecological disruptions, conserve biodiversity, and ensure long-term agricultural sustainability. Adoption of these practices

requires collaboration among researchers, policymakers, farmers, and other stakeholders to promote and implement effective pest management strategies.

Greater emphasis initiated on biological control towards the promotion of on-farm ecological services by re-orienting the approaches with more focus on evolving robust, reliable and practicable technologies for on-farm use of biocontrol agents.

## TRENDS IN THE DEMAND FOR AND ADOPTION OF BIOLOGICAL CONTROL

Growing demand for biological control of crop pests is marked by some key indicators as given below.

### Increased adoption of biological control in IPM practices

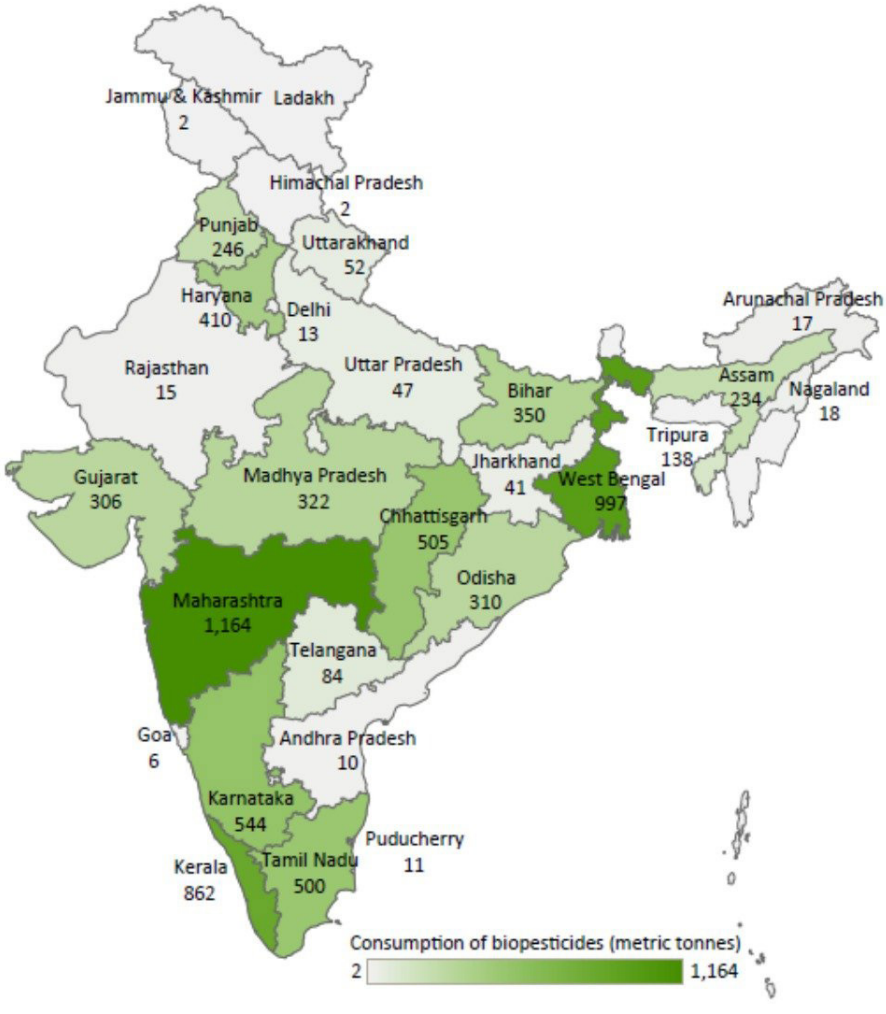
An increase in the adoption/consumption of biocontrol agents in IPM methods by farmers has been recorded by the DPPQS, Faridabad. Figure 1 depicts the state-wise consumption of biopesticides in metric tonnes for the year 2020. It is predicted that the demand would increase annually by 4-6% depending on the crop, pest and the state.

### Consumer demand for sustainable agriculture

Consumers are increasingly concerned about the environmental and health impacts of chemical pesticides. This demand for sustainable and environmentally friendly food production can drive interest in biological control. The market for biopesticides in India is anticipated to grow from USD 69.62 million in 2022 to USD 130.37 million by 2029, with an annual compounded growth rate of 9.38% during the forecast period (Figure 2).

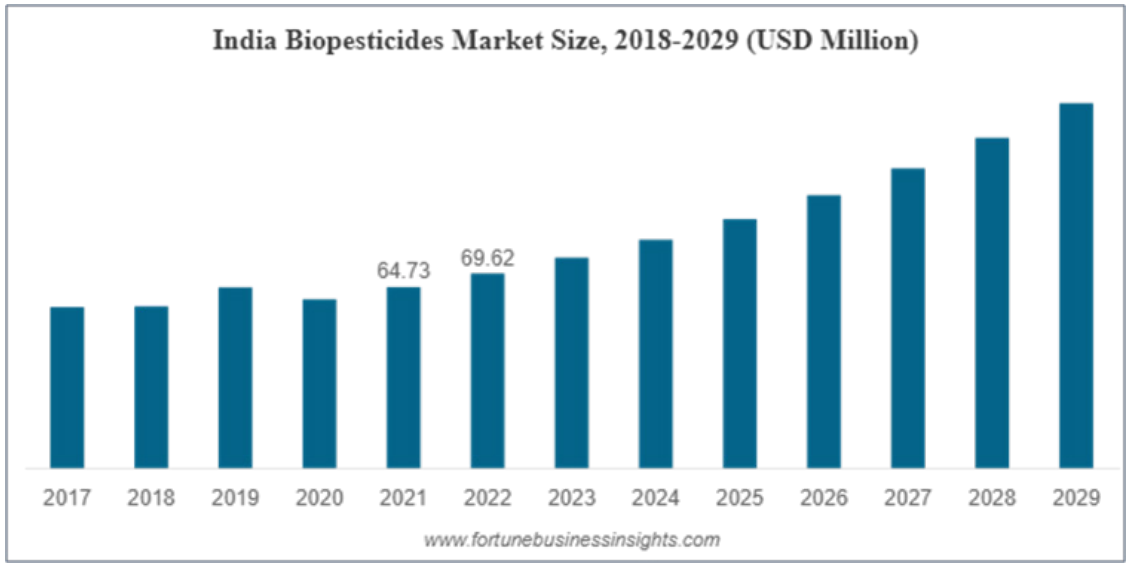
### Availability of commercial biological control products

The presence of a diverse range of commercially available biological control products, including predators, parasites, and biopesticides, is a clear sign of demand (Figure 3).

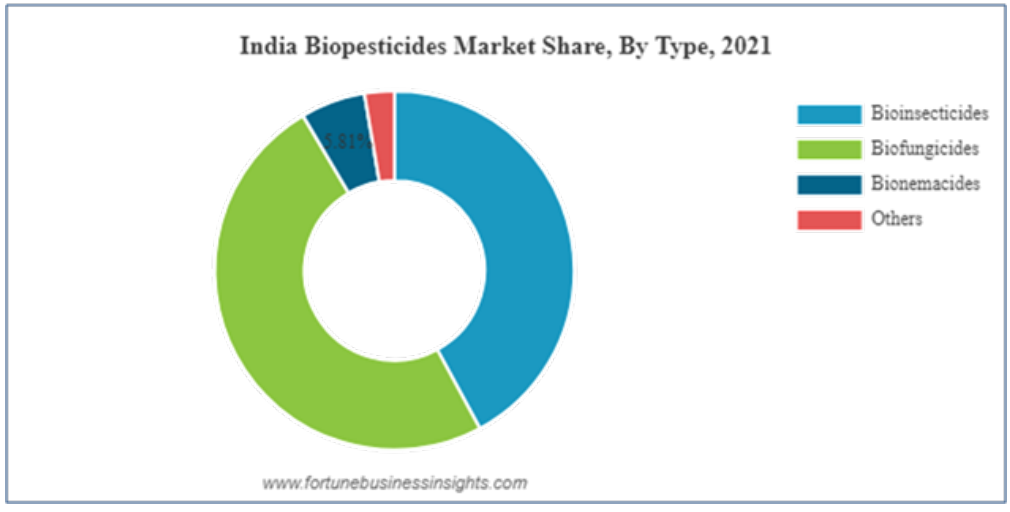



Source: Directorate of Plant Protection, Quarantine and Storage 2020 https://www.ceew.in/publications/sustainable-agriculture-india/integrated-pest-management

**Figure 1.** Utilization of biopesticides in different states in metric tonnes during 2020.




Source: https://www.fortunebusinessinsights.com/india-biopesticides-market-106498
**Figure 2.** Trend in India's biopesticide market size.




Source: https://www.fortunebusinessinsights.com/india-biopesticides-market-106498
**Figure 3.** Trend in India's biopesticide market share.

### Market growth

An expanding market for biological control products and services, including the emergence of new companies and increased sales, is a strong indicator of demand. India has a good number of biocontrol-related establishments and biocontrol labs (Table 1).

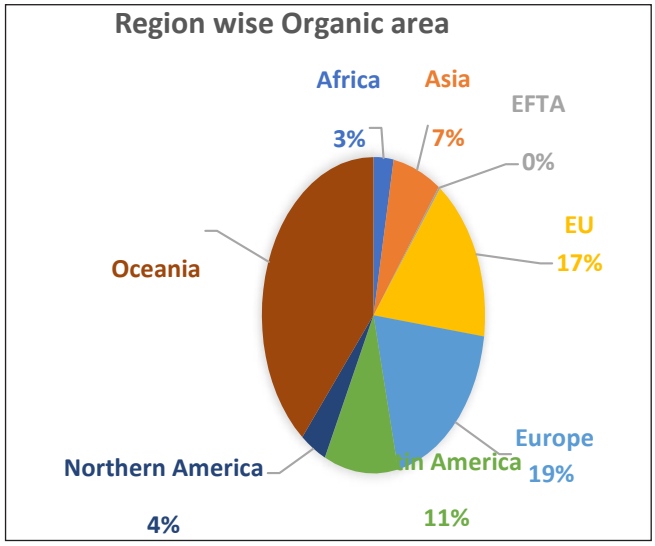
### Expanding trends in organic agriculture

Organic farming relies heavily on biological control methods because there are limitations on the use of chemical pesticides in a package of practices. An increase in the acreage of organic crops suggests a greater demand for biological pest control solutions. The most recent FiBL survey conducted across 191 countries revealed that both organic cropland and retail sales have continued to rise and have now hit yet another record high (The World of Organic Agriculture 2023). Globally about 76.4 million hectares were reported to be organically managed during 2020-2021, with a growth rate of 1.7 per cent or in other terms 1.3 million hectares, compared to 2019-2020.

**Table 1.** Profile of biocontrol labs/companies operating in India

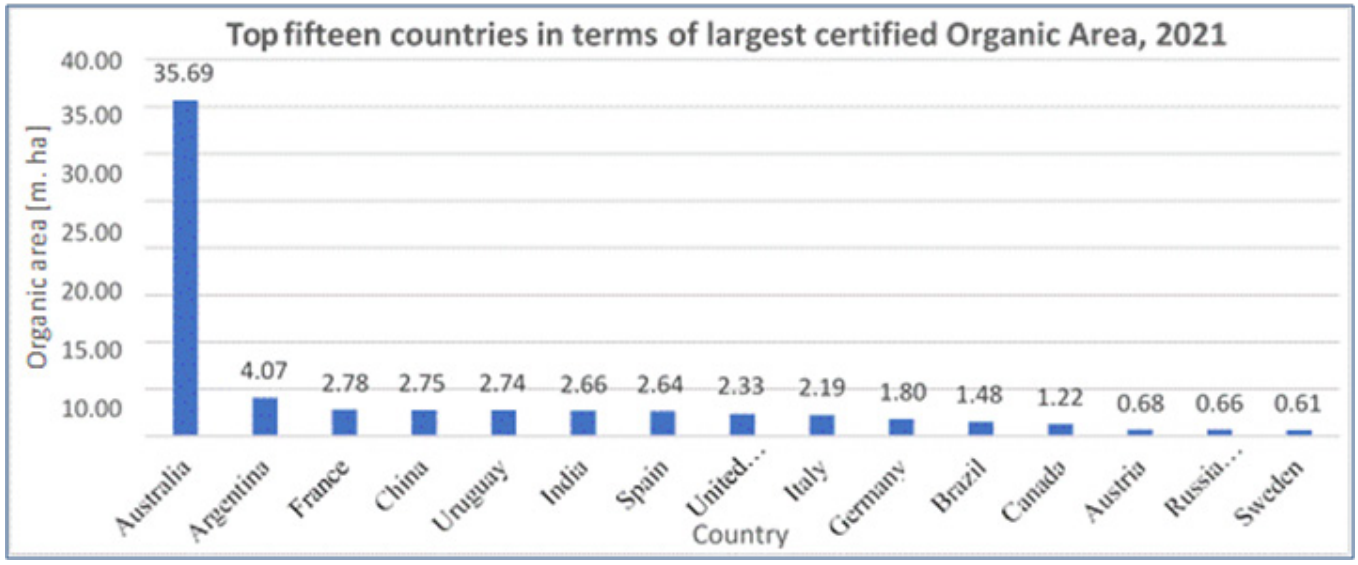
| Biocontrol labs/units | Number |
|---|---|
| ICAR/SAUs/DBT Labs | 49 |
| Central Integrated Pest Management Centres (CIPMCs) | 35 |
| State Biocontrol Labs | 98 |
| Private invested industry labs | 141 |
| Private sector GOI-aided labs | 38 |
| Total biocontrol labs/units in India | 361 |

Source: Mishra *et al.*, 2020




Source: FiBL Survey 2023

**Figure 4.** World: Distribution of organic agricultural land by region [%] (2021).



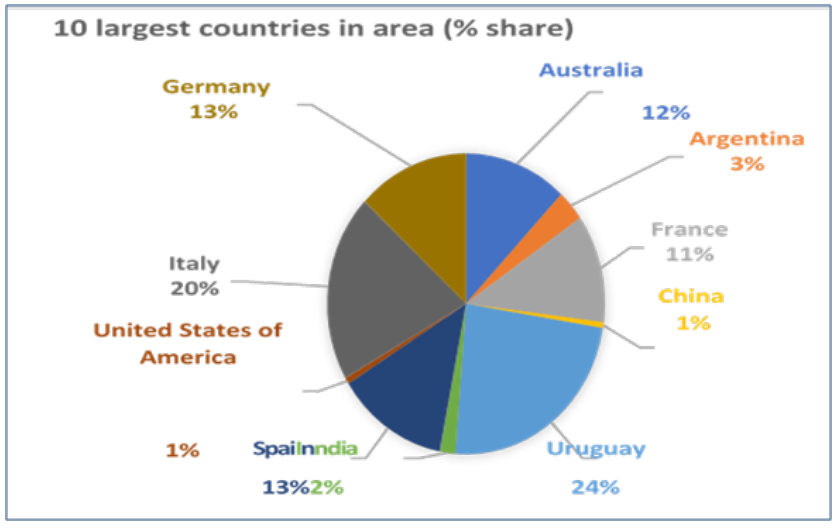

Source: FiBL Survey 2023; Kaur, 2023

**Figure 5.** World: Area under organic agriculture in top ten countries (2021).

Oceania has the greatest organic agricultural land (36.0 Mha, which is nearly half of the world's organic agricultural land, 39 per cent), followed by Europe (17.8 Mha, 19 per cent). Latin America had (9.9 Mha, 11%), followed by Asia (6.5 Mha, 7%), Northern America (3.5 Mha, 4%), and Africa (2.7 Mha, 3%) Figure 4.

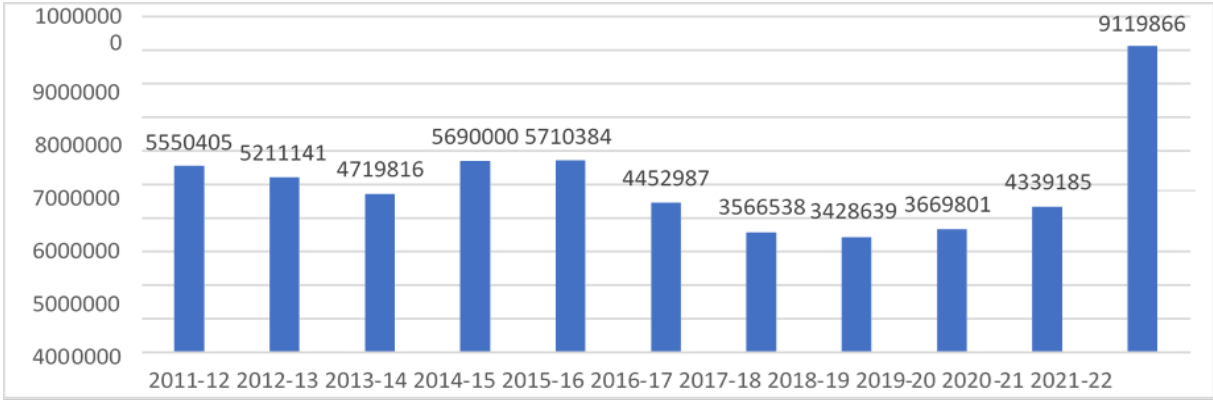
The largest areas under certified organic agriculture in different countries during 2021 are depicted in Figure 5. Significantly, Australia, Argentina, and France occupied the top three positions with 35.7 million, 4.1%, and 2.8 million hectares, respectively. India's total organic agriculture area is 2.66 million hectares, putting it in the sixth position. Likewise, many countries reported a substantial increase, but the biggest increases were in China, France, and Spain. However, the top ten countries in terms of largest area under organic agriculture during 2021 were Uruguay, Italy, Germany, Spain, Australia, France, Argentina, India, the USA and China (Figure 6).

In India, organic agriculture has a very low percentage compared to the total net sown area per se, which is now increasing under certified organic farming. In 2011-2012, the certified organic farming area was 55,50,405 ha, which over the next ten years increased by 1.5 times (Figure 7). The overall period of study has seen fluctuations in the area under organic farming. India is currently ranked among the top 10 countries in terms of global land area, with 91,19,866 ha under certified organic cultivation.




Source: FiBL Survey 2023; Kaur, 2023

**Figure 6.** List of 10 countries with the largest area under organic agriculture during 2021.




Source: FIBL, 2023. Kaur, 2023

**Figure 7.** The area that is being grown organically in India (ha) is between 2012 and 2022.

### Research and development investment

The magnitude of investment in research and development of BCA for farm use directly propels the biopesticide industry growth, which in turn promotes the adoption of biopesticides to foster organic farming.

### Regulatory changes

Stringent regulations on synthetic pesticides and the phase-out of certain chemical pesticides can drive the demand for biological control agents as farmers seek alternative pest management solutions. Such information is available in public domains, viz., https://ppqs.gov.in/divisions/cib-rc/guidelines, and https://cropuser.cgg.gov. in.

### Awareness and training programs

E-media immensely helped in the dissemination of educational and training programs on biological control methods for farmers and agricultural professionals.

### Pest resistance to chemical pesticides

Pesticide resistance among several insect pests urgently called for evolving alternative control methods for pest management, especially in organic farming situations, primarily for biological control and ecological services. Georghiou (1986) summarized that insecticide resistance was most frequently seen in the Diptera (156 species, or 35% of the total), Lepidoptera (67 species at 15%), Coleoptera (66 species at 15%), Acarina (58 species at 13%), Homoptera (46 species at 10%), and Heteroptera (20 species at 4%). Seventeen important species can resist five classes of chemicals, including the Colorado potato beetle; white flies, *Heliothis, Spodoptera* and pink bollworm on cotton; aphids, *Myzus persicae*; Diamondback moth, several mites, thrips, stored grain pests etc. Further, several invasive insect species across the world have developed insecticide resistance (Table 2).

**Table 2.** A record of pesticide-resistant invasive insect species in the world (adopted from Siddiqui *et al.* (2023))

| Scientific name | Common names | Resistance against insecticide | Country | References |
|---|---|---|---|---|
| *Bactrocera dorsalis* | Oriental fruit fly | Organophosphorus (OP) | Pakistan | Khan and Akram (2018); Hsu *et al.* (2004); Vontas *et al.* (2011). |
| | | Carbamates (Cm) | Taiwan | Khan and Akram (2018); Hsu *et al.* (2004); Vontas *et al.* (2011). |
| | | Pyrethroid, Spinosad, Trichlorfon. | unknown | Khan and Akram (2018); Hsu *et al.* (2004); Vontas *et al.* (2011). |
| *Bemisia tabaci* | Cotton white fly | Parathion-methyl, endosulfan | America | Byrne and Devonshire (1993). |
| | | Imidacloprid | Europe | Wang *et al.* (2011). |
| *Cydia pomonella* | Codling moth | Arsenate, DDT, OP, benzoyl urea. | America | Hough (1928); Cutright (1954); Moffit *et al.* (1988); Welter *et al.* (1991). |
| | | Decamethrin, abamectin | France | Bouvier *et al.* (1998); Reyes and Sauphanor (2008). |
| | | Glutathion, Chlopyrifos, Phosalone. | Spanish | Rodríguez *et al.* (2010). |
| *Frankliniella occidentalis* | Western flower thrips | Methiocarb, Bendiocarb (Cm). | Australia | Martin and Workman (1994); Espinosa *et al.* (2002); Herron and James (2005); Götte and Rybak (2011). |
| | | Organochlorine | New Zealand | Martin and Workman (1994); Espinosa *et al.* (2002); Herron and James (2005); Götte and Rybak (2011). |
| | | OP | Spain | Martin and Workman (1994); Espinosa *et al.* (2002); Herron and James (2005); Götte and Rybak (2011). |
| | | Pyrethroid (fenvalerate) | United States of America | Martin and Workman (1994); Espinosa *et al.* (2002); Herron and James (2005); Götte and Rybak (2011). |
| *Leptinotarsa decemlineata* | Colorado Potato beetle | Carbofuran, pyrethroid | Canada | Harris and Svec (1981). |
| *Periplaneta Americana* | Americana cockroach | Imidacloprid | America | Wang *et al.*, 2004, 2006; Ko *et al.* (2016). |
| *Blattella germanica* | German cockroach | Fipronil | America | |
| *Periplaneta australasiae* | Australian cockroach | Abamectin | America | |
| *Spodoptera frugiperda* | fall armyworm | Cyhalothrin, flubendiamide, chlorantraniliprole | America | Gutiérrez-Moreno *et al.* (2019); Yu (1991). |
| *Thrips palmi* | Melon thrips | Organochlorine, OP | America | Zhao *et al.* (1995); Immaraju *et al.* (1992); Broadbent and Pree (1997). |
| | | Pyrethroids | Canada | |

**Government support**

Government incentives, subsidies, or programs that promote biological control practices can stimulate adoption among farmers. In India, most support is provided by the federal government, while the state governments either implement or popularise the programs. The states of Sikkim, Tripura and Uttarakhand have adopted a policy of totally organic agriculture and biological control of pests and diseases.

**Success stories and case studies**

Positive outcomes and success stories of farmers who have effectively used biological control methods can inspire others to adopt similar approaches.

Monitoring these indicators periodically helps stakeholders, including policymakers, researchers, and industry players, gauge the demand for BC of crop pests and respond accordingly to support its spread.

## ENTOMOPATHOGENIC NEMATODES AS A COMPONENT OF BIOLOGICAL CONTROL

Among several microbial biocontrol agents, the EPN has been realised to be a dependable IPM component against several insect pests. EPN are soil-inhabiting beneficial nematodes that parasitize and kill insect pests, with immense potential for ecological services making them valuable tools in IPM. Worldwide, the demand for the development of EPN-containing products is mounting with several companies involved in their production, distribution and sales. India's estimated demand for EPN is 24,000 metric tonnes, while the current production is 1800 metric tonnes from 25-30 firms. In India and other developing countries, the current EPN production and supply chain are in their infancy and operating as a cottage industry.

There are more than 30 nematode families reported with taxa that are associated with insects (Kaya and Stock, 1997). From the point of view of their biocontrol potential, the research studies were largely carried out on seven families belonging to the Phylum Nematoda, including Mermithidae, Allantonematidae, Neotylenchidae, Sphaerularidae, Rhabditidae, Steinernematidae and Heterorhabditidae. *Phasmarhabditis hermaphrodita* (Schneider), a member of the family Rhabditidae, was reported to suppress several slug species besides infecting several insect hosts. Subsequently, it was developed as a biological molluscicide (Wilson *et al.,* 1993). Further, among these 7 families, the nematodes belonging to Heterorhabditidae and Steinernematidae are given the status of EPN by their symbiotic association with lethal obligate bacteria, and for their fitness traits fitting to the IPM and BC programs (Ehlers, 1996; Georgis *et al.,* 2006; Lacey & Shapiro-Ilan, 2008; San-Blas *et al*., 2013; Tofangsazi *et al.,* 2014; van Zyl & Malan, 2014).

### Attributes

The unique features that the EPN inherently possess include quick kill of target pests, broad host range, high virulence, presence of chemoreceptors, host searching ability, tolerance to abiotic stresses, amenability to mass production by *in vitro* method, genetic improvement and longer shelf-life that make them effective BCAs. Further, EPN can be delivered in the field using standard equipment and also are compatible with many chemical insecticides and entomopathogens. EPN, being naturally occurring and beneficial microbial parasites specific to insects and few other invertebrates are not required to register in the USA and many other countries (Kaya & Gaugler, 1993).

### Biology and mode of action of EPN against insects

These EPNs harbour species-specific obligate endosymbionts. The endosymbiotic bacteria from the genera *Xenorhabdus* and *Photorhabdus* are specific to *Steinernema* and *Heterorhabditis* species, respectively (Poinar, 1990). The 3rd instar larvae also known as IJs, are the only free-living stages in the life cycle, with infectivity, host searching ability and survivability in the environment (Grewal *et al.,* 2006). These IJs act as vectors, enter the insect body cavity through natural openings and release the symbiotic bacteria. Bacteria proliferate and release insecticidal metabolites which cause insect mortality in 24-48 hours (Poinar & Grewal, 2012). Subsequently, the larvae develop, reproduce within the host body and complete two or three generations. When there is nutrition is depleted in the host cadaver the IJs are triggered to moult into 3rd instar IJs and exit the host body in large numbers in search of new hosts (Grewal & Georgis, 1999; Shapiro-Ilan *et al.,* 2012).

### Virulence and infectivity of EPN

EPN are ecologically harmonized for the habitats that tender protection from environmental extremes, particularly in the soil, and in cryptic habitats. Virulence of EPN is dependent on several biotic and abiotic factors including type of host, host life stage, nematode species, production method, bacterial strain associated with them, prevailing soil-borne predators of EPN, soil temperature and moisture, etc.

### Host range

EPNs are ubiquitous, infecting and killing more than 200 different insect species. In laboratory tests, *S. carpocapsae* alone infected more than 250 species of insects from over 75 families in 11 orders (Poinar, 1975). Importantly, all insect species pass a part of their life cycle in the soil which gives the insect host a chance to encounter the soil-dwelling EPN. Currently, 6-8 EPN species are utilized commercially for fewer soil-dwelling insect pests. There is vast potential for EPN as BCAs against several other insect pests.

### Soil temperature

Soil temperature is one of the most important factors in determining the efficacy of EPN. Each EPN species has its respective temperature regimes that are suitable for their biological activities including infectivity, development, fecundity, survival, host searching ability etc. The broad range of biologically suitable soil temperature regimes is 18°C and 32°C with a marginal difference of 1°C.

### Soil moisture and desiccation tolerance

Soil moisture is another important abiotic factor that plays a major role in the survival, dispersal and infectivity of EPN. Anhydrobiosis is an inherent physiological feature in most nematode species, like-wise EPN also exhibits anhydrobiosis. Desiccation tolerance among different EPN species is an important survival strategy depending on

their ecological niche. Nematodes use anhydrobiosis and desiccation tolerance strategies under drought/extreme moisture stress conditions and revive once the moisture is restored.

**Foraging behaviour**

Foraging behaviour, in other words, is the host-searching ability which is again a specialized parasitic/feeding adaptation for the survival of the EPN. Characteristically, some EPN species are ambushers that infect the host insect by remaining nearly sedentary at the surface of the soil particles while waiting for the mobile host insects to come in contact. Others are highly mobile and adapted to search for the host deeper in the soil profile, and are referred to as "cruisers". There are some other species which behave intermediate to these two foraging behaviours.

**Effect on non-target organisms**

Glaser and Farrel (1935) were the earliest to use an EPN in the field against an insect pest. *Steinernema glaseri* was used EPN for field control of the white grub, *Popillia japonica* in New Jersey, USA (Glaser & Farrell, 1935). Since then to date, there have been no reports of any kind of hazards caused by the use of EPN on the environment or humans. The use of EPN is safe for the user and Non-Target Organisms (NTO).

**Regulations for entomopathogenic nematodes**

It's important to note that EPNs are naturally occurring organisms, and the strains used for biological pest control are considered safe and environmentally friendly. Nevertheless, the regulatory requirements can vary widely by country, and it's essential to consult the relevant regulatory agencies. Some of the considerations related to biosafety regulations for EPN are as follows.

- **Registration and approval:** Some countries require the registration and approval of EPN-based products before they can be marketed and used for pest control. The approval process typically involves evaluating the safety and efficacy of the product.

- **Import and export regulations:** EPN and products containing EPN may be subject to phytosanitary regulations when imported or exported to prevent the spread of pests or diseases.

- **Labelling and packaging:** Approved EPN products may be subject to labelling and packaging requirements to provide instructions for proper application and safety precautions.

- **Risk assessment:** Some countries may perform risk assessments to evaluate the potential risks associated with the use of EPN for pest control. In India, there are no such regulations defined for risk assessment specifically for the EPN.

## GLOBAL STATUS OF COMMERCIAL EPN PRODUCTION AND SUPPLY SYSTEMS

Comprehensive and accurate data on the global and region-wise consumption patterns of EPN is lacking in the databases to understand the trends. The adoption and consumption of EPN vary from one country to another, depending on factors such as agricultural practices, pest pressures, and the level of awareness and acceptance of EPN in biocontrol practices (Nagesh *et al.,* 2017).

However, some countries are actively utilizing the EPN and have well-developed market systems for these beneficial nematodes. Some of these countries include:

- United States: The United States has a well-established market for EPN, with several companies producing and selling them for agricultural and horticultural pest control.

- Canada: Canada also has a growing market for EPN used in pest management, especially in agriculture.

- European Union: Several European countries, such as the Netherlands, France, and Spain, have adopted EPN in their IPM strategies in agriculture and horticulture.

- Israel: Israel is known for its innovations in agricultural technology and is also the forerunner who inducted the use of EPN in their IPM programs effectively.

- Japan: EPN is used in Japanese agriculture, particularly for controlling soil-dwelling pests in crops like rice and vegetables.

Several companies around the world are involved in the production and distribution of EPN for pest control under their brand names or trade names. The availability of specific products and brand names may have changed from time to time, and new companies may have entered the market. Here are a few well-known companies that are/were involved in EPN production and their respective trade names are listed in Table 3. Information in Table 4 shows a list of firms that are involved in the commercial production and distribution of EPN.

Several companies in the United States and Canada sell EPN for pest control. These companies offer EPN of various species and strains, depending on the target pests and specific agricultural or horticultural needs.

**Table 3.** List of EPN-producing companies with trade names of their EPN products

| Company Name | Trade Name for EPN products |
|---|---|
| BASF | Nemasys |
| Koppert Biological Systems | Entonem, Capsanem |
| Syngenta | Nemathorin |
| Certis USA | NemaShield |
| ARBICO Organics | Scanmask, NemAttack |
| e-nema GmbH | Beneficial Nematodes |
| BioLogic Company | Grub-Away, Flea-Away |
| AgriLife | AgriLife NemaSeek |
| EcoBuz | Ecomask |
| BioBest | NemaDecide |
| Marrone Bio Innovations | Grandevo |

Here are some companies in the USA and Canada known for selling EPN, along with some of the common EPN species they may offer:

- United States:

| Company | Common EPN Species traded |
|---|---|
| Arbico Organics | *Steinernema feltiae, S. carpocapsae, Heterorhabditis bacteriophora.* |
| Nema Globe USA | *H. bacteriophora, S. feltiae, S. carpocapsae.* |
| Nature's Good Guys | *S. feltiae, S. carpocapsae, H. bacteriophora.* |
| BASF (formerly Becker Underwood) | *S. carpocapsae (Trade Name: Nemasys).* |
| Syngenta | *H. bacteriophora (Trade Name: NemaShield), S. carpocapsae (Trade Name: Nemathorin).* |
| BioLogic Company | *S. carpocapsae, H.bacteriophora.* |

- Canada:

| Company | Common EPN Species traded |
|---|---|
| BioBest Canada | *S. feltiae, S. carpocapsae.* |
| Koppert Biological Systems Canada | *S. feltiae, S. carpocapsae* (Trade Names: Entonem, Capsanem). |
| BioSafe Systems | *S. feltiae, S. carpocapsae.* |
| Nema Globe Canada | *H. bacteriophora, S. feltiae, S. carpocapsae.* |
| Environmental Factor | *S. feltiae, S. carpocapsae.* |

- **India**

The following products traded are of *Heterorhabditis indica*. Some of these companies were primarily granted licenses for *in vivo* and WP formulation technologies by the ICAR-NBAIR, Bengaluru.

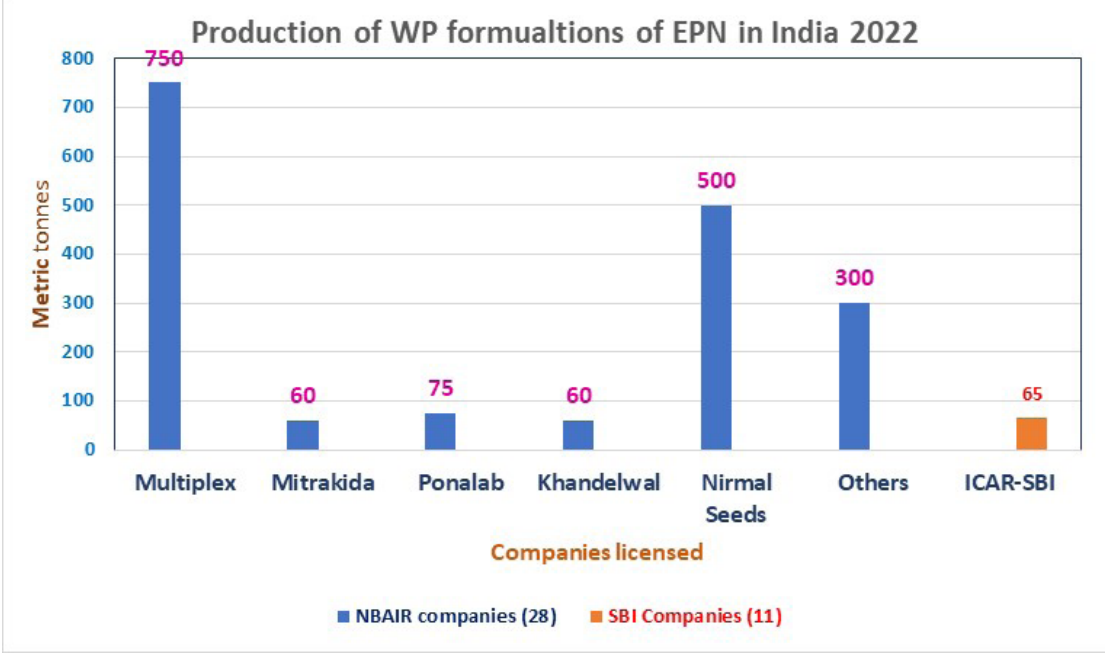
## STATUS OF EPN PRODUCTION AND UTILISATION IN INDIA

Chronologically, during the late 80s Ecomax Pvt. Ltd., Hyderabad made the first bold commercial venture to import, pack and supply aqueous formulations of EPN. Products could not survive in the market and were withdrawn. Significantly, the factors that contributed to this fiasco were that Ecomax imported EPN and formulated them without an actual production system established in India; there was hardly any awareness of biological control of crop pests using EPN in India then and practically no demand among farming communities; limiting ecological suitability of exotic strains of EPN to Indian agro-ecosystems; and very short shelf-life of aqueous and sponge formulations. In the next 3 decades, the research, awareness and innovations on EPN rapidly gained momentum pan India.

Substantively in the last 2 decades, ICAR-NBAIR addressed the challenges involved in evolving the EPN technologies for farm use and then transforming the technologies into commercially viable propositions for the industry. The extensive in-house funding from ICAR and NBAIR, and the grants from the Department of Biotechnology, Ministry of Science and Technology, Government of India bolstered the efforts on systematic cataloguing of native diversity of EPN, studying their bio-ecology, screening their bioefficacy against several crop pests, evolve POPs, technologies for *in vitro* and *in vivo* production, formulation and delivery systems. Tangible innovations in scale-up (*in vivo)* production and devising novel WP formulations with a 12-month shelf-life accomplished the grant of Indian Patent rights to the ICAR-NBAIR team in the year 2018. During the last 10 years (2012-23), ICAR-NBAIR licensed the know-how to 28 commercial entrepreneurs and technically mentored them. ICAR-SBI, Coimbatore licensed another WP formulation of EPN and commenced licensing in 2018. The current production of EPN is depicted in Figure 8 with major support from ICAR-NBAIR followed by ICAR-SBI licensed companies. An area of about 40,000 ha is currently under EPN (Figure 9) in different crops (Figure 10), and in different states (Figure 11), mitigating the use of chemical insecticides.

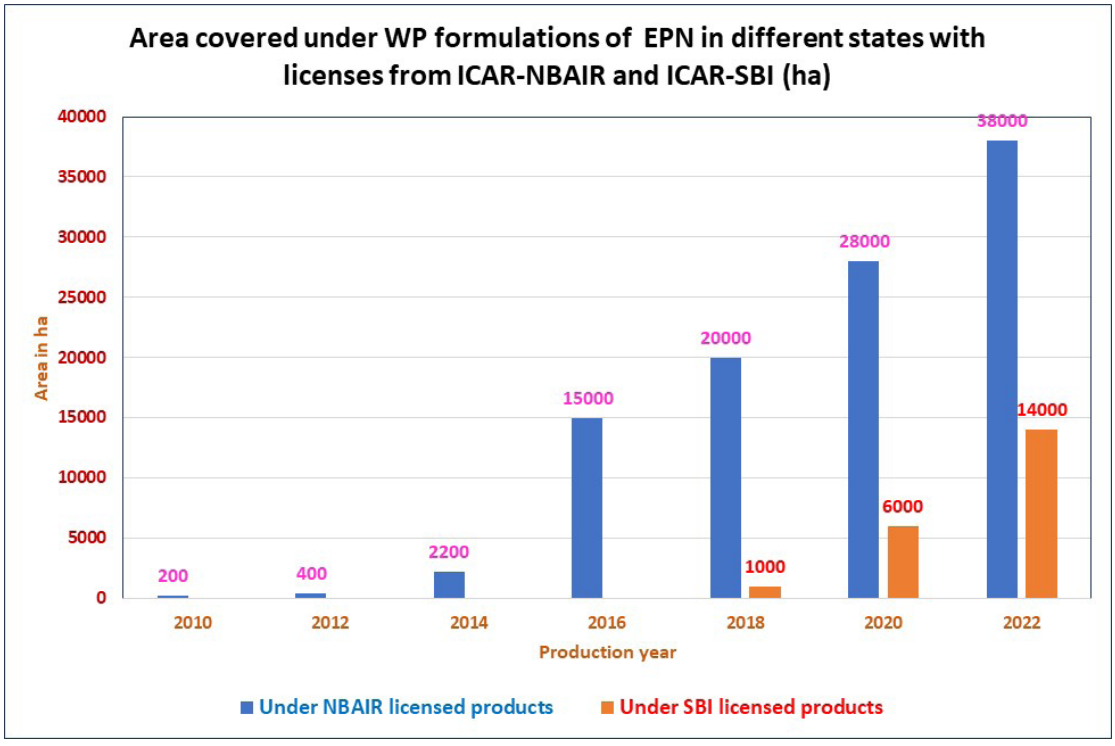
| Product trade name | Formulation | Target pest | Company |
|---|---|---|---|
| Soldier | WP | White grubs, termites | Multiplex, Bengaluru |
| Grub Cure | WP | White grubs, termites | SRI Biotech, Bengaluru |
| Nema power | WP | White grubs, termites | KN Biosciences, Hyderabad |
| Calterm | WP | White grubs, termites | Camson Biotech, Bengaluru |
| BCS-Grub terminator | WP | White grubs, termites | Benzor Crop Science, Sirsi |
| Armour | WP | White grubs, termites | Ponalab, Bengaluru |
| Sniper-WP | WP | White grubs, termites | Nirmal Seeds, Jalgaon |
| Grub Nash | WP | White grubs, termites | Khandelwal Pesticides Pvt Ltd., Ichalkunji |
| Mitrakida-WG | WP | White grubs, termites | Mitrakida Biosolutions Pvt Ltd., Pune |
| Farm Root EPN* | WP | White grubs, termites | Farmroot agritech pvt. ltd., Bengaluru |
| Anshul *Heterorhabditis indica* EPN's Army * | WP | White grubs, termites | Agriplex Pvt. Ltd., Bengaluru |
| EPeN * | WP | White grubs, termites | Nico Orgo, Dacor, Gujarat |
| UPL Kixona Entomopathogenic Nematode* | WP | White grubs, termites | UPL Ltd., Mumbai |
| T-Stanes Crown* | WP | White grubs, termites | T-Stanes Pvt Ltd., Coimbatore |
| SUSCROPS Ranger EPN* | WP | Root grubs, Termites, Root Weevils, cutworms and other soil-borne pests | SusCrops, Jakkur, Bengaluru |

*Production and formulation technologies not from ICAR-NBAIR, Bengaluru.



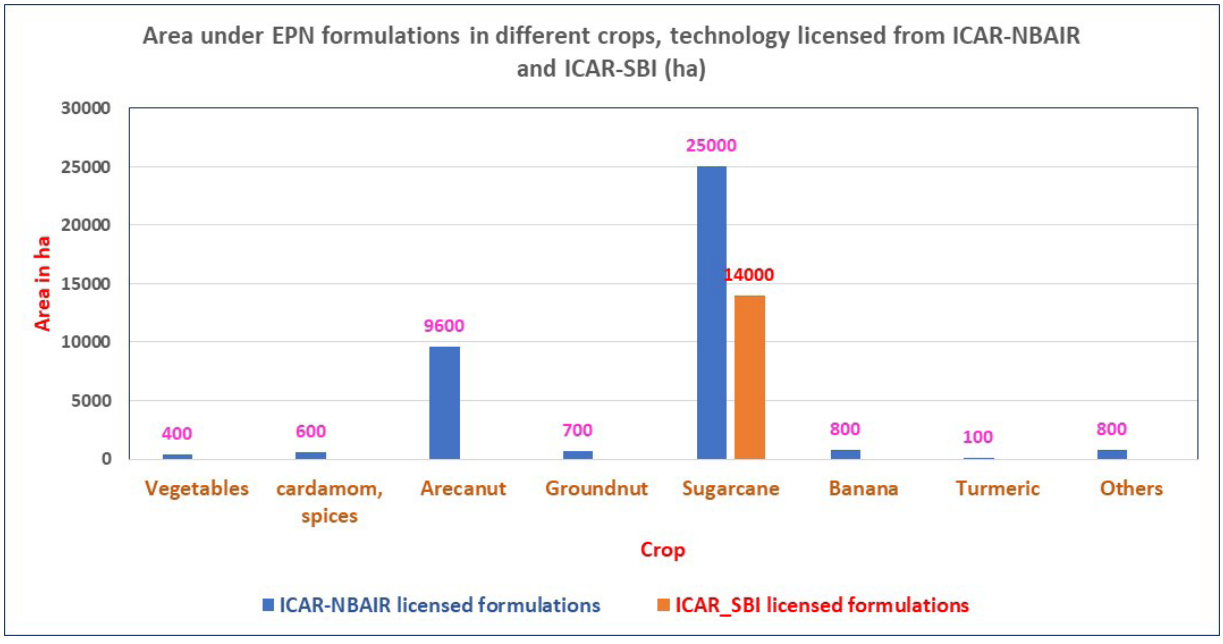

Source: Nagesh *et al.*, 2022

**Figure 8.** Commercial production of WP formulations of EPN for insect pest management in India. Formulation technologies licensed by ICAR-NBAIR and ICAR-SBI.



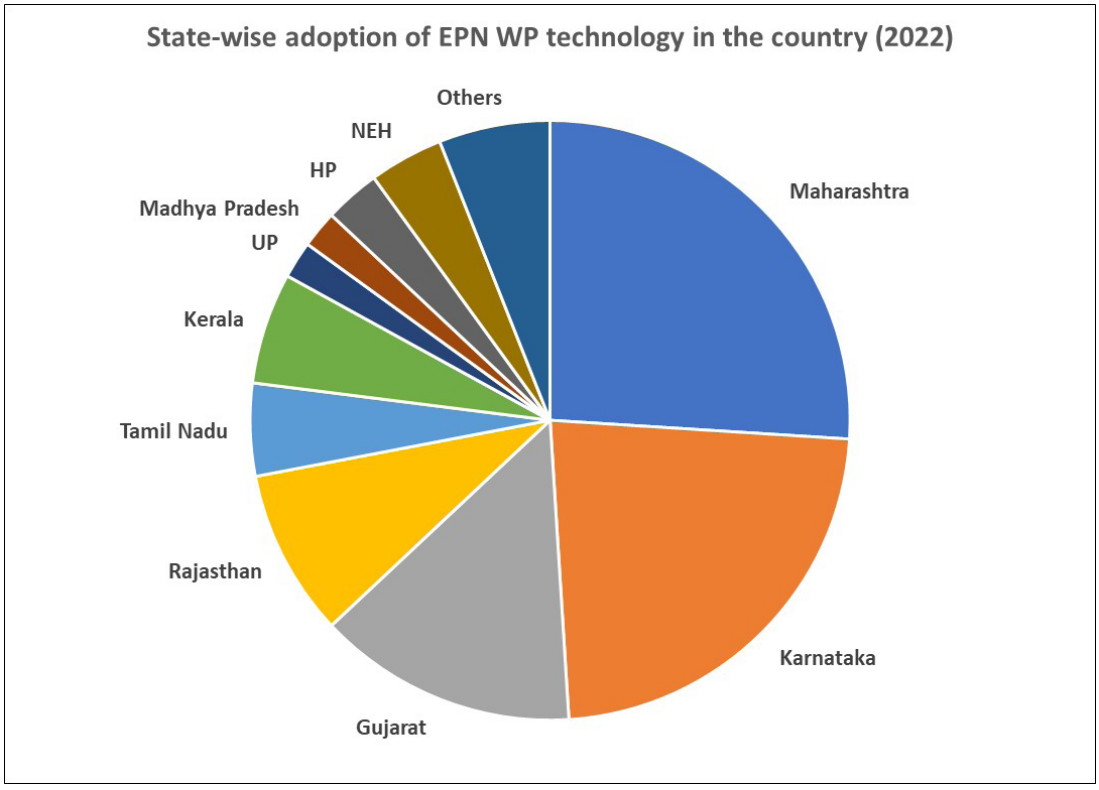

Source: Nagesh *et al.*, 2022.

**Figure 9.** Area covered under WP formulations of EPN for insect pest management in India, Formulation technologies licensed by ICAR-NBAIR and ICAR-SBI.




Source: Nagesh *et al.*, 2022.

**Figure 10.** Crop-wise adoption of WP formulations of EPN across the country (2016-2022).




Source: Nagesh *et al.*, 2022.

**Figure 11.** Adoption of WP formulations of EPN in different states across the country.

## CHALLENGES ENCOUNTERED IN THE PROMOTION OF THE EPN

Indeed, the EPN withstood the test of time as an ecologically safe, pest-specific and tangible BCA in IPM. However, there are some very critical challenges to facilitate maximal utilization by the stakeholders at the farm and the industry. Some of them are as follows.

- Temporal and spatial availability of commercial products of EPN at the grassroots is seriously limiting despite an enormous demand.

- Storage and shelf life: Unlike microbial BCA, EPNs have a limited shelf life depending on the formulation constituted, and require proper storage conditions to maintain their viability. Ensuring that EPNs are formulated, stored and handled correctly can be a challenge for both producers and end-users.

- High costs: Due to high production costs and imbalance in demand and supply the EPN are relatively high-priced, which deter their extensive use, especially in developing countries with limited farm incomes and resources.

- Technical expertise: Effective application of EPN requires technical expertise. Farmers need to understand the optimal conditions for EPN to thrive, such as soil type, moisture levels, and temperature, and know how to apply them correctly. The time, method and dose of application need crop-pest-specific on-farm expertise.

- Environmental factors: EPNs are sensitive to environmental conditions, such as extreme temperatures, moisture regimes and drought. Besides the soil abiotic factors, the biotic stress of parasitic fungi, predatory mites and nematodes affect their performance.

## STATUS OF INNOVATIONS IN THE PROMOTION OF ENTOMOPATHOGENIC NEMATODES IN IPM

### Production of EPN

From time to time there were several reviews on EPN production (Bedding 1984; Friedman *et al*., 1990; Gitanjali, 2018, Shapiro-Ilan *et al.,* 2002). EPN production systems have evolved in two major streams: *in vivo* systems that use living insects and *in vitro* systems that use the solid-state or monoxenic liquid fermentation process. *In vivo,* production essentially involves two life cycles, one in insect hosts and the other, EPN-B complex in live insect hosts. The process of *in vitro* culture for entomopathogenic nematodes requires the introduction of nematodes to a culture of their symbiont in the nutritive medium. Entomopathogenic nematodes can now be produced in large quantities for commercial use through

the use of large fermenters due to significant improvements in *in vitro* culture techniques.

### *In vivo* production

the process of cultivating culturing a specific entomopathogenic nematode in live insect hosts is seemingly simple but requires minimal technology and involves using a surrogate lab- or in-house-reared insect hosts including *Corcyra cephalonica*, crickets, *Galleria mellonella* and *Tenebrio molitor,* and finally harvesting the nematodes in bulk from the host cadavers after completion of their multiplication. Several authors (White, 1927; Dutky *et al.*, 1964; Poinar, 1979; Woodring & Kaya, 1988; Lindegren *et al.*, 1993; Flanders *et al.*, 1996; Kaya & Stock, 1997; Shapiro-Ilan *et al.*, 2012) have reported and reviewed *in vivo* production techniques for culturing EPN (Table 5).

The cost of *in vivo* production can be improved significantly by producing the insect hosts "in-house" and mechanising some of the steps (inoculation, harvest, concentration etc.) thus reducing the labour costs (Shapiro-Ilan *et al.,* 2014). Novel methods are available for mechanizing nematode inoculation and harvest. LOTEK is an *in vivo* production system, developed by Gaugler and Brown (2001) that does not depend on the migration of IJs into a stagnant water reservoir for harvesting the IJs (Gaugler *et al*., 2002).

### *In vitro* production of EPN along with their symbiotic bacteria

The method of *in vitro* EPN production evolved into two streams including a solid-state production on solid or semi-solid media, and another on the liquid or submerged liquid production. Solid-state production primarily comprises of solid phase of the nutrient media/nutrient supply media on which the surface-sterilized nematodes are inoculated and allowed to mate and multiply at a specific temperature and incubation conditions. Submerged liquid production involved the introduction of surface sterilized IJs to freshly grown monoxenic symbiotic bacteria in a sterile liquid nutrient medium. The nematodes and the associated bacteria are aerated with filter-sterilized air, agitated mildly with a flow of air bubbles or soft impeller at specific cultivation conditions of temperature, pH, $DO_2$ etc., and allowed to mate and multiply. The nematodes complete 2 generations in 2-5 weeks and the resultant IJs are then harvested in normal water.

### On solid media

Historically, the concept of *in vitro* mass production was attempted for the first time in the USA with *S. glaseri* for the control of *Popillia japonica* (Glaser, 1932, McCoy & Glaser, 1936). Most notably the discovery of the existence of symbiotic bacteria in the dauer-stage juveniles of *S. felitiae* (McCoy & Glaser, 1936), and its isolation and identification as *Xenorhabdus nematophilus* (Poinar & Thomas, 1966)

**Table 5.** Comparative summary of EPN production in *in vivo* and *in vitro* systems

| Features | *In vivo* production | *In vitro* production |
|---|---|---|
| Production on | Insect hosts, primarily *Galleria mellonella, Tenebrio molitor, Corcyra cephalonica, and* crickets. | On solid media.<br>Liquid media. |
| Number of EPN species that can be reared | Almost all nematodes. | Very few species. *H. bacteriophora, H. indica, S. carpocapsae, S. feltiae* |
| Scale-up yields | Limited. 1.0-3.0x$10^5$ IJs per larva | Scalable. Up to 80000 litres per batch with 460×$10^3$ IJs/ml and 252×$10^3$ IJs/ ml, with *H. bacteriophora, and S. carpocapsae*, respectively. |
| Equipment | Simpler, basic and direct equipment. | requires highly sophisticated equipment. |
| Ease of operation | Highly labour-intensive. Low technology. | Mechanized and technology-intensive. |
| Capital investment | Low | Very high. |
| Cost of production | On diet for the rearing of insect hosts and for labour engaged in rearing of insect hosts. | Moderate to low. |
| Production consistency | Not uniform | Uniform. |
| Production cycles | Continuous, long, unless staggered and sometimes inconsistent. | Normally 4-5 weeks or at best 3 weeks. |
| Risk of contamination during production | Low. | Very high. |
| Risk of loss of virulence and infectivity in IJs harvested from | Low. | High. |
| Risk of IJs sensitivity to environmental factors (temperature, moisture, pH) | Very low. | Medium. |

gave a new dimension to the *vitro* production. The second achievement was the commercial-scale *in vitro* production of *Neoaplectana carpocapsae* DD-136 strain on a dog food-based medium (House *et al.*, 1965). Bedding successfully developed the first commercial-scale monoxenic culture which was termed a "solid" culture (Bedding, 1981). The 3rd significant success was the liquid state production of EPN utilizing the nematode-bacterium association.

Subsequently, several studies were carried out on using the solid-state *in vitro* production, each one of them improvising over the other. Hara *et al.* (1981) drew attention towards monoxenicity and produced $125x10^6$ IJs/week from one hundred Petri dishes containing dog food agar for $ 0.28 per million IJs. Bedding (1981) developed methods for the production of *Neoaplectana* spp. Bedding-soaked shredded plastic foam in pig kidney-beef fat homogenate (animal protein and lipid-based medium), and was able to produce about $5.0x10^6$ million IJs of *N. bibionis* in a week. Tabassum and Shahina (2004) mass-produced *S. pakistanense, S. asiaticum, S. feltiae* and *H. indica* using chicken offal media. Other synthetic solid media standardized are Wouts medium, dog biscuit medium or dehydrated and enriched animal tissues.

Solid culture was used to successfully rear several species of neoaplectanid and heterorhabditid nematodes, with an average yield of $6\text{-}10\times10^5$ IJs/g of medium, at a cost of less than $ 0.02 per million. The solid culture methods were found to be economically feasible as long as the production level was approximately $10\times10^{12}$ nematodes/ month (Ramakuwela *et al.,* 2016).

**On liquid media/fermentation**

The current-day production of liquid culture of EPN evolved from using various types of flasks, tissue culture bottles, and mechanical or pneumatically agitated bioreactors with various modified liquid-based culture media. Mass production of EPN in submerged conditions is a highly complex and specialized fermentation process comprising two distinct phases. First, growing of the nematode-specific bacterium, axenically (without any contamination) in sterile media; following the bacterial growth, the inoculation with a synchronous culture of IJs progresses through 2 to 3 generations. The nematode life cycle comprises 6 stages including the egg, four juvenile stages (J1, J2, J3, J4), and the adult. Once the number of IJs attains a maximum the culture is ready to harvest and recover the IJs.

For scale-up commercial production, *in vitro* liquid culture is tangible, due to the prohibitive cost and scale of production involved *in vivo* system, and the ease of downstream processing *in vitro* (Murray *et al.*, 2021). In the beginning, scale-up productions were carried out in Erlenmeyer shake flasks with a combination of aeration and agitation conditions provided, and suitable parameters were arrived at for the *in vitro* submerged culture of EPN. These trials were scaled up to 5-20 L in desktop bioreactors and, thereafter, to 80-120-1000 L in industrial-scale bioreactors (Surrey & Davies, 1996; Hazir *et al.,* 2003; Ehlers & Shapiro-Ilan, 2005). In progression, a new design of the airlift bioreactor (Spier *et al*., 2011), and internal loop bioreactors were developed which yielded the highest IJ concentration (Ehlers & Shapiro-Ilan, 2005).

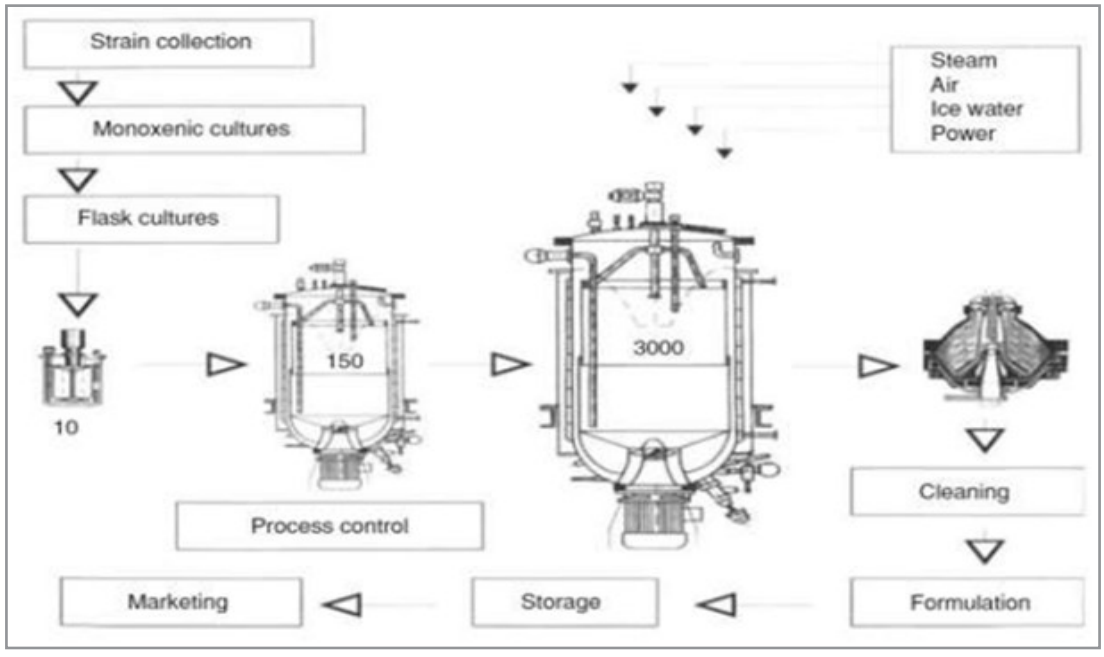



**Figure 12**. Flow chart of the production process.

A schematic outline of EPNs production process is presented in Figure 12, adopted from Spier *et al*. (2011).

It is crucial to select the ingredients of the liquid culture media used for mass-culturing, which has the dual requirement of sustaining the development of EPN and their respective symbiotic bacteria. Therefore, the media need to be typically comprised of vital nutrients that are close to the composition of the natural insect host, including protein, carbohydrates, lipids, minerals, vitamins etc. Media-related factors such as pH, viscosity, osmolarity, dynamics of metabolites, contamination, etc., are other complex but precise requirements for each nematode species. Additionally, regulation of shear damage to the eggs and adults is a challenge during aeration and agitation with rotating impeller blades. Optimization is necessary to attain economically viable yield levels with the quality of IJs, as parameters vary based on the EPN species involved.

Shapiro-Ilan *et al.* (2012) and Cortés-Martínez and Chavarría-Hernández (2020) published exhaustive reviews on the submerged monoxenic culture of EPN. Liquid culture for EPN was attempted for the first time by Stoll in 1952. He cultured them in the shaker by using liver extracts yielding 400 DJ/ml at 21°C-25°C and pH of 6.0-6.5, and he had an important observation that, reproduction was more in the dark. Buecher and Hansen (1971) examined the effects of airflow rate, quantity and shear stress on the growth of EPN after air was supplied to the liquid culture media. The monoxenic liquid culture system described here established a baseline for media and process optimization studies for *S. feltiae* and other steinernematid nematodes. It has paved the way for large-volume liquid culture in fermenters and the provision of low-cost high yields of nematodes required for the commercialization of this insect pest-control agent.

Pace *et al.* (1986) attached a flat blade impeller to the 10 L bioreactor and then inoculated *X. nematophilus*. After 24-hour incubation, *S. carpocapsae* was inoculated at 2,000 DJ/ml and kept their oxygen saturation at 20% at 23-28˚C and 180 rpm for a total of 10 days. *S. feltiae* strain 42 was reared in liquid culture along with its bacterial symbiont, *X. nematophilus*. According to Friedman *et al.* (1989), the liquid fermentation technique can produce about $50\times10^{12}$ IJs/month with a rapid reduction in production costs. Using this method, scalpe-up production conditions were arrived at for *S. carpocapsae, S. riobrave, S. scapterisci, S. feltiae, S. kushidai* and *S. glaseri* at 80,000 L scale, and *H. bacteriophora, H. indica* and *H. megidis* at 300-2000L scale with yield capacity as high as 250,000 IJs /ml (depending on the nematode species).

Lunau *et al.* (1993) developed an improved method that involves culturing the axenic nematode eggs on a pure culture of the symbiotic bacterium. Upadhyay *et al.* (2013) reported the efficiency of the fed-batch culture process with *X. nematophila*. Results from the fed-batch process were on par with the yields from the standard batch process as is the case with *S. carpocapsae* production. The nematode density obtained was $2.02x10^5$ IJs/ml, while the batch culture mode resulted in a nematode density of $2.30x10^4$ IJs per ml. Compared to the batch process, the fed-batches process resulted in an 88.5 % increase in IJs yield in a shorter period. Fed-batch, therefore, seems to make the process more reliable and economically viable.

Gil *et al.* (2002) improved the *in vitro* production of *H. bacteriophora* by growing it on a Feb- batch with glucose supplementation. The time for a production cycle was found to be 3 weeks and determined by the media and species. However, many species can achieve maximum IJ production in two weeks or less (Ehlers *et al.*, 2000). Large-scale production was further advanced through several measures including using bags with gas-permeable Tyvac® strips for ventilation, automated mixing and autoclaving, simultaneous inoculation of nematodes and bacteria, sterile room technology, and automated harvest through centrifugal sifters (Gaugler & Han, 2002; Neves *et al.,* 2001; Wang *et al.,* 2007).

**Media selection and improvement**

Nematodes, like any other invertebrate, require suitable sources of lipid and protein for their growth, development, robustness and fecundity. In general, a medium contains yeast extract (nitrogen source), carbon source (e.g. soy flour, glucose or glycerol), various proteins and lipids (of animal and plant origin), and salts (Han *et al.,* 1995; Surrey & Davies, 1996; Ehlers *et al.,* 1998; Hazir *et al.,* 2003; Ehlers and Shapiro-Ilan, 2005). Its osmotic strength is not above 600 milliosmoles per kilogram (Ehlers & Shapiro-Ilan, 2005). Several lipid sources including vegetable oils such as corn, palm, groundnut, sunflower, and canola, besides animal oil sources such as fish, pork lard, etc., have been worked out. Similarly, important protein or nitrogen sources that have been tried are egg yolk, egg white, soy extract, yeast extract, chicken offal, beef extract, fish collagen etc., while the dog biscuits were used as the combined source of lipid and protein (Table 6). These foods were tried at different proportions for different nematode species with varying yield figures. Media that come from plants are generally reported to have lower productivity than those that come from animals (Abe, 1987; Wouts, 1981; Ehlers *et al.,*1998; Vyas *et al.,*1999; Shapiro-Ilan & McCoy, 2000; Vyas *et al.,* 2001; Hussaini *et al.,* 2000, 2002, 2007; Prabhu *et al.,* 2006; Somwong & Petcharat, 2012; Sunanda & Siddiqui, 2013; Banu & Meena, 2015; Yadav *et al.,* 2015).

**Table 6**. Sources of media ingredients used for scale-up of solid-state production systems for EPN

| Element | Source | Ingredient | Nutrition |
|---|---|---|---|
| Nitrogen | Vegetable | Soy flour, trypticase | Protein, amino acids |
| | | Corn powder | Protein, essential amino acid |
| | Animal | Dehydrated egg yolk | Protein, cholesterol, emulsifier |
| | | Nutrient broth | |
| | | Milk powder of different grades | |
| | | Lactalbumin hydrolysate | Protein, carbohydrate, Iron |
| | | Casein | Protein |
| | | Lecithin | Emulsifier |
| | | Liver extract | Protein, vitamins |
| | | Beef peptone | Protein |
| | | Meat offal, blood. | Protein, Iron |
| Fat | Vegetable | Corn, canola, coconut, olive, palm, peanut, safflower, soybean, sunflower | |
| Minerals | Salts | NaCl, KCl, CaCl2, FeSO4, MgSO4 | Osmolarity and ionic balance |
| Microbial | | Yeast extract, seaweed products | Nutritional factors for the xenobiotics and bacteria |
| | | Cholesterol | Fat, growth factor |
| Complete foods | Synthetic | Whey protein | Fat, proteins, cholesterol, minerals |

**Table 7.** Nematode species studied for suitability to liquid/submerged fermentation and scaleup.

| Nematode, bacterium | Culture, Composition | References |
|---|---|---|
| *S. feltiae* | Egg yolk + egg white supplemented with Glucose | Ehlers *et al*. (1998). |
| *H. megidis/ P. luminescens* | Liquid culture | |
| *H. bacteriophora / P. luminescens* | Submerged monoxenic culture and fermentor | Hatab and Gaugler (2001); Cho *et al.* (2011). |
| *H. megidis/ P. luminescens* | *In vitro* liquid culture. | Ehlers *et al.* (1998). |
| *H. bacteriophora* | *In vitro* liquid fed-batch culture + glucose | Gil *et al.* (2002). |
| *S. carpocapsae* | *In vitro* production | Han *et al*. (1993). |
| *S. feltiae* | *In vitro* production, nutrient concentration, addition of thickeners, and agitation speed. | Leite *et al*. (2016). |
| *H. bacteriophora* | *In vitro* production, lipid source. | Yoo *et al.* (2000). |
| *H. zealandica, S. yirgalemense* | *In vitro* liquid culture. | Ferreira (2013). |

Murray *et al.* (2021) and Cortés-Martínez and Chavarría-Hernández (2020) summarized the information related to *in vitro* submerged production of EPN and also the considerations required for optimal production in liquid culture for different EPN species (Table 7).

Despite the encouraging results of the *in vitro* production system, it further requires technologies to address optimisation, rationalize the production costs, production time and scale-up for high-quality nematodes (Shapiro-Ilan & Dolinski., 2015).

A summarised data on the productivity of different nematode species under different liquid culture conditions is presented in Table 8 (Cortés-Martínez & Chavarría-Hernández, 2020). Importantly, the information established

**Table 8.** Productivity of *Steinernema* and *Heterorhabditis* IJs in submerged *in vitro* production during 1986 and 2020.

| EPN species | IJ recovered (×$10^3$ IJ/ml) | IJ productivity (IJ/ml/day) | Technology used | Agitation method | References |
|---|---|---|---|---|---|
| *H. bacteriophora* | 362 | 29,667 | Flask | Orbital shaker | Gil *et al.* (2002). |
| *H. indica* | 457 | 26,353 | Flask | Rotary shaker | Ehlers *et al.* (2000). |
| *H. zealandica* | 41.1 | 2,607 | Flask | Orbital shaker | Ferreira and Malan (2014). |
| *H. megidis* | 71.47 | 44,000 | Bioreactor | Paddle impeller | Kim *et al.* (2014). |
| *H. heliothidis* | 20 | DMC* | Flask | Orbital shaker | Pace *et al.* (1986). |
| *S. carpocapsae* | 252 | 15,714 | Bioreactor | Pneumatic | Chavarría-Hernández *et al.* (2011). |
| *S. feltiae* | 225 | 7,857 | Flask | Orbital shaker | Leite *et al.* (2016). |
| *S. riobravis* | NR | DMC* | NR** | NR | Shapiro and McCoy (2000). |
| *S. colombiense* | 53 | 4,990 | Flask | Orbital shaker | Pérez-Campos *et al.* (2018). |
| *S. scapterisci* | NR | DMC* | NR** | NR** | Grewal *et al.* (1999). |
| *S. jeffreyense* | 121 | 8,560 | Flask | Orbital shaker | Dunn *et al*. (2019) |
| *S. yirgalemense* | 75 | 4,733 | Flask | Orbital shaker | Ferreira *et al.* (2016) |
| *S. bibionis* | 70 | DMC* | Flask | Orbital shaker | Pace *et al.* (1986) |

Abbreviations: DMC, data missing to calculate; IJ, infective juvenile; NR**, not reported.

that 14 nematode species were amenable to submerged liquid culture for commercial-scale production. However, the majority of the studies were carried out in flasks of different capacities under lab-scale conditions.

**Post-production down-stream processing**

Conventionally, post-production downstream processing includes several stages or steps such as separation of the live entities, cleaning, harvesting, concentrating, reconstituting into formulations with specific concentrations, packaging, labelling and storage.

**Harvesting and separation**

Like any other liquid fermentation process, the mature culture after the EPN production is a heterogeneous admixture of spent liquid medium, unspent media, waste by-products, nematode wastes, stages of nematodes and the dead. Therefore, it is important to address the post-production processes for better harvest of healthy, robust and viable IJs and also minimize or eliminate the contamination for developing authentic formulations, reliable shelf life and commercial use.

For harvesting IJs from insect cadavers during the *in vivo* production process, Gaugler *et al*. (2002) described an improved method termed, LOTEK. Instead of making the IJs migrate into a water reservoir, the LOTEK design utilizes an improvised recovery technique that involves rinsing the cadavers with a mist of water. LOTEK involves five sequential stages, inoculation, conditioning, harvesting, separation and clean-up. Except for separation, all steps take place in a reusable holding tray that makes it easier to handle the insect cadavers. The harvesting unit is designed from a perforated 20-gauge aluminium (30% open area) sheet with perforations (1.6 mm in diam.) to retain hosts while permitting passage of IJs when the cadavers are placed over the sheet. The cadaver holding tray (30 x 26 x 4 cm) can hold 500 insects, but the manufacturer can modify the size and density of the tray. A food dispenser with a calibrator can also be used to fill the holding trays with hosts.

The separation and recovery processes during *in vitro* culture have been worked out widely including settling of the nematodes in columns of water, mild centrifugation (Surrey & Davies, 1996), filtration etc., which have a recoverability of 60-70%, and a significant level of losses. The major hurdles for separation based on physical properties, are near-water specific gravity of nematodes, low settling rates and turbid liquid media wastes, etc. (Young *et al.*, 2002). Separation of IJs from bulk fermentation systems still poses a serious drawback specific to EPN among all microbial BCA. Further, some studies included the addition of anti-microbials to minimize the microbial contaminants. In modern-day agriculture, the additives need to be non-toxic, non-contaminant and specific.

**Formulation of EPN**

In general, a formulation is composed of an active ingredient, a carrier and additives in predefined proportions

and specific purposes to maintain the biological viability, virulence, intended physiological/functional activity, physical consistency and chemical properties. Further, an optimal formulation should also possess consistently high quality, maintain a long shelf life, and be easy to handle, transport and field deliver. Infective juveniles of EPN from *in vitro* and *in vivo* production systems are formulated as gels, granules, WP, aqueous suspensions etc. Additives in a formulation comprise absorbents, adsorbents, emulsifiers, surfactants, thickeners, humectants, dispersants, antimicrobials, and UV-ray protectors (Grewal, 2002*).* Table 9 shows some of the formulations developed for different nematode species in the forms of gel, granules, wettable powders, cadavers, and aqueous suspensions. Several reviews highlighted the strides made in the EPN formulation and application technologies (Grewal 2002; Shapiro-Ilan *et al.,* 2006, 2012).

The quality of the final formulation is influenced by different materials, and the methods used to develop the formulations with EPN (Han *et al*., 1992, Shapiro-Ilan *et al.* 2006, 2012). Grewal (1998, 2000a, 2000b, 2002) periodically reviewed and identified priority areas to improve the EPN formulation process and further revealed that the use of both water-dispersible granules and calcium alginate capsules resulted in increased EPN survival time from 7 days to 180 days. Also, EPNs that were formulated and applied as insect cadavers were more effective in controlling pests than those that were applied in an aqueous solution (Shapiro-Ilan *et al.,* 2001, 2003).

**Storage of EPN**

The ingenious way to store IJs is in aqueous suspensions. After harvesting IJs from *in vivo* or *in vitro* cultures, they can be stored in tissue culture flasks in a flat position at 5-7 mm depth of water layer. Considerably higher concentrations can be stored with sufficiently aerated with an aquarium pump. Lindegren *et al.* (1979, 1993) described another method to store the IJs of the *S. carpocapsae* Mexican strain in wool configurations, consisting of mats of intertwined IJs. Finney and Jean (1981) defined a new method and package that allows for the storage and transportation of nematode eggs, and IJs within a host cadaver.

Yukawa *et al.* (1985) described and patented two improved methods for the storage for transport of nematodes wherein an adsorbent was added to a cream that had infective juvenile entomopathogenic nematodes and was stored under conditions that prevented microbial growth. In another invention, a cream of infective juvenile EPN was stored under substantially anaerobic conditions.

Biosys GmbH, an international operating company, first established in Germany in *1986* described the methods and materials for inducing anhydrobiosis in nematode IJs and then maintaining and storing them in an anhydrobiotic state. Suitable containers were also disclosed. Biosys (1993) disclosed an invention relating to formulating and packaging IJs into pseudoplastic layers for prolonged storage and convenient dispensation whenever it is desired to be used. Shahina *et al.,* (2011) disclosed methods for prolonged storage of IJs of *Steinernema* and *Heterorhabditis* in surfactant solution or antimicrobial solution on sterilized polyurethane foam which allowed the nematodes sustaining infectivity of at least 50% for six months at 10-15$^{o}$C.

Guangdong Entomological Institute (2001) devised methods for cleaning EPN and defined the composition of the material to prepare IJs-based liquid, granule or wettable powder formulations, and absorbents for metabolic $CO_2$ and/or ammonia. This helped in storing EPN for long periods and transporting it for long distances.

In general, the ideal temperature for storage of EPN formulations ranges between 6 and 20°C for survival times of 6–12 months for *Steinernema* spp. and 3–6 months for *Heterorhabditis* spp. However, the refrigeration requirements increase costs and hinder normal transport systems.

**Field application and delivery systems**

One of the most daunting tasks for wide-scale use and adoption after the tasks of developing formulations with a formidable shelf life is the field-level delivery systems as close to the pest as possible. Ideally, the applications should be simple to operate, economical and direct to deliver as close to the pest as possible. As discussed elsewhere, the EPN is formulated in aqueous, powder, granular, gel, paste and direct cadaver forms. The targeted pest could be in simple niches like lawns, open fields, complex canopies, closely planted crops, etc. The delivery systems can be direct application, through irrigation systems or any specialised application devices (Shapiro-Ilan *et al.,* 2006; Toepfer *et al.,* 2010). Shapiro-Ilan *et al.* (2001, 2003) and Gumus *et al.* (2015) described methods to deliver the EPN-laden cadavers to the field.

Zhu *et al.* (2011) developed a method for delivering desiccated nematode-infected cadavers into the soil using a modified crop seed planter on a small scale. The system primarily consisted of a metering unit, an air pressure source, a cadaver scraper, a custom-designed cadaver container, tension adjustment devices, a double disk soil opener, a discharge tube, a packer wheel, and a press-drive wheel. At a constant rate, the metering unit intermittently discharged a cadaver into the discharge tube. A narrow slot measuring 7.5 cm deep was created by the double disk opener for the

**Table 9.** Formulations developed for different nematode species

| Formulation | Nematode | Feature | References |
|---|---|---|---|
| Aqueous | *H. bacteriophora, H. indica, S carpocapsae. S. glaseri, S. feltiae, S. abbasi, S. riobrave* | A thin layer of clean water with 1% formalin. | |
| polyurethane sponges | *H. bacteriophora, H. indica, a carpocapsae* | 5-25x$10^5$ IJs per sponge @500–1000 IJs/$cm^2$. | Chen and Glazer (2005) |
| Sponge sheets | | 1-3 months @ 5-10°C. | Grewal (2002) |
| Gels with activated carbon powder | | | Yukawa and Pitt (1985) |
| Polyacrylamide gel | | | Bedding and Butler (1994) and Bedding *et al.* (2000) |
| Calcium alginate sheets dispensed on plastic screens | | | Georgis (1990) |
| Encapsulated in a matrix of macro-gels | *S. carpocapsae* | Hydrogenated vegetable oil paste containing mono- and diglycer-ides. | Chang and Gehert (1991) |
| Hydrogenated oil and acrylamide | *S. carpocapsae* | 80 per cent survival for 35 days at 24–35°C. | Chang and Gehert (1992) |
| Alginate capsules | *S. feltiae* | 99.8 per cent survival after 6 months at 23-25°C and 100 per cent relative humidity. | Chen and Glazer (2005) |
| Alginate capsules | *H. indica* | population density and storage was 10°C, up to 1000 IJs per capsule and 90 days of storage. | Goud *et al.* (2010) |
| Calcium alginate | *H. bacteriophora and S. car-pocapsae* | with survival values higher than 50% after 40 days. | Hussein and Abdel-Aty (2012) |
| Encapsulated in hygroscopic at-tapulgite clay | *S. bibionis, S. glaseri, H. heliothidis* | survival time of 8 weeks at 23°C. | Bedding (1988) |
| Edible-to-insects calcium alginate gel and yeast extract as a phagostimulant | *S. riobravis, S. carpocapsae* | 1000 IJs/g. | Navon *et al.* (1998) |
| Modified alginate capsules with excessive Ca2+ | *S. carpocapsae, S. feltiae, S. riobravis* | | Kim *et al.* (2015) |
| Pellets | | it is consisting of a mixture of alfalfa meal, wheat flour, wheat bran, corn oil, and water. | Capinera and Hibbard (1987) |
| Wheat flour granules (Pasta) | *S. carpocapsae* | 6 weeks of storage at 21°C. | Connick *et al.* (1993); Nickle *et al.*, 1994 |
| Wettable powders | *S. feltiae* | | |
| Hygroscopic attapulgite clay – sand-wich method | *S. feltiae, S. bibionis, S. gla-seri, H. heliothidis* | 8 weeks at 23°C. | Bedding (1988) |
| Granules with diatomaceous earth, hydroxy ethyl cellulose, amorphous silica, fumed hydrophobic silica, lignosulfonate, starch, gelatinised starch, and pre-gelled attapulgite clay | *S. carpocapsae, S. feltiae, S. scapterisci, S. riobravis.* | 90% survival after storage for 6 weeks at 25°C. | Silver *et al.* (1995) |
| Dispersible granules (WDG) | *S. carpocapsae, S. feltiae, S. riobravis* | 80% and infectivity greater than 60% after 5 months of storage at 25°C. | Grewal (2000) |
| Infected cadavers | *H. bacteriophora, H. indica, S. capocapsae.* | | Shapiro-Ilan *et al.* (2001, 2008); Shapiro *et al.* (2003); Bruck *et al.* (2005); Del Valle (2008a, b); Lacey *et al.* (2010); Raja *et al.* (2015) |
| nfected cadavers coated with kaolin-starch mixture. | *H. bacteriophora, H. indica, S. capocapsae* | | Ansari *et al.* (2009) |
| Infected cadavers coated with unfla-voured gelatin. | *H. bacteriophora, H. indica, S. capocapsae.* | | Del Valle *et al.* (2009) |

placement of the discharged cadavers. The packer wheel then covered the cadavers with loose and slightly moist soil. The metering unit with modified double bean plates has delivery accuracy between 79% and 94% at 500-Pa air pressure. A slower travel speed and fewer cells on the metering plate improved the accuracy of delivery and delivered the cadavers at a rate of 1.6, 3.3, or 6.6 cadavers/m length in the soil.

Amongst the most popular EPN delivery systems through irrigation, is the sprinkler system. Hayes *et al.* (1999) observed that the sprinkler equipment did not affect the infectivity of *S. carpocapsae* on *G. mellonella* larvae.

Nilsson *et al.* (1999) found that the viability of *S. feltiae* (Filipjev) was unaffected when a backpack sprayer and a high-pressure sprayer were employed for field delivery. Similarly, Georgis (1992) did not record adverse effects for *Steinernema* spp. and *H. bacteriophora* after flow through several different pumps, nozzle types, and strainers. Fife *et al.* (2003) reported differences in viability and infectivity among EPN species concerning pressure differential treatments. They recommended 1380 kPa (200 psi) for *H. megidis* and 2000 kPa (290 psi) for *S. carpocapsae* (Weiser) Wouts *et al.*, 1981 and *H. bacteriophora*. Garcia *et al.* (2005) pointed out that *S. glaseri* (Steiner) Wouts *et al.* (1981) kept its viability under pressure of 1379 MPa. In general, the aforementioned figures suggest that low-pressure equipment does not affect the viability and infectivity of IJ. As well, it is obvious that each nematode species/strain might have its own recommended pressure.

Further, the delivery of EPN through drip irrigation was found to be prospective for operational reasons. The distribution pattern of EPN applied by drip irrigation was evaluated by injecting small volumes of four nematode suspensions into drip irrigation lines (Wennemann *et al.*, 2003). The nematodes were evenly distributed along the drip lines with a recovery ranging from 42 to 92%. Drip irrigation lines have the potential to deliver EPN efficiently into pest habitats.

In another study, four different irrigation drippers (in-line short path, in-line long path, in-line cylindrical and on-line button) were observed to have significantly different effects on EPN discharge ratio. Therefore, optimizing drip irrigation systems for EPN applications depending on the crop and nematode species is essential.

Raja *et al.* (2015) reported that the four different methods of applying the IJs (IJs) of EPN to the soil, including nematode-infected cadavers, subsurface injection, spraying, and drip irrigation were effective with no significant differences.

## COST OF PRODUCTION AND THE NEED FOR TECHNOLOGIES FOR SCALE-UP PRODUCTION-SUPPLY CHAIN

The costs of production of EPN products differ widely between the *in vivo* and *in vitro* production systems; the production media *in vitro*/on the insect host used *in vivo*; among the EPN species; the scale of production, and between the country to country. Very few systematic attempts on the costs of production for EPN have been reported so far.

Nguyen *et al.* (2002) reported that the expenditure on diet/nutrition varied between 6.76 to 26.63 USD per billion IJs for the EPN strains cultured on *T. molitor* larvae and from 3.54 to 7.81 USD per billion IJs for nematode strains cultured on *G. mellonella* larvae which shows culturing cost in terms of food expenditure altered between host insect larvae and nematode strains. The full cost for a nematode product of 2.5 $\times 10^9$ IJs/ha, produced through *in vivo* mass culturing, of the most efficient nematode strain, H-KT3987, was 191.3 USD, slightly cheaper than 199.4 USD for the same nematode product produced through *in vitro* mass culturing.

An in-house study on the expenses incurred towards production, formulation and packaging of *H. indica, H. bacteriophora, S. carpocapsae, S feltiae* and *S. abbasi* on *G. mellonella* larvae arrived at an indicative cost of Rs.120-150 or 1.48-1.85 USD per Kg WP pack (Nagesh *et al*., 2023). The costs of *in vivo* production were variable among these EPN species on *G. mellonella* as the yields per larva were variable, especially concerning *S. glaseri* and *S. riobrave*. The costs were primarily based on recurring expenses that included insect diet ingredients, minimal wages for one person, electricity, water, consumables filter paper, talc, kaolinite, lined aluminium sachets, etc. The cost of production was calculated at a lab scale of 60-100 Kg WP formulation per production cycle of 30 days or less. The profitability of the *in vivo* production of EPN using *G. mellonella* larvae was arrived at and projected for different production capacities based on the prevailing market MRP value for WP formulations of *H. indica* by M/S. Multiplex, Bengaluru, Karnataka; M/s. Ponalab, Bengaluru, Karnataka; M/S. Khandelwal, Ichalakunji, Karanataka, M/S. Mitrakida, Pune, Maharashtra, and M/S. Nirmal Seeds, Pochora, Maharashtra, to whom the ICAR-NBAIR has licensed the technologies on a non-exclusive basis (Figure 12, Nagesh *et al*., 2017, 2023). However, the benefit-to-cost ratio is expected to be much

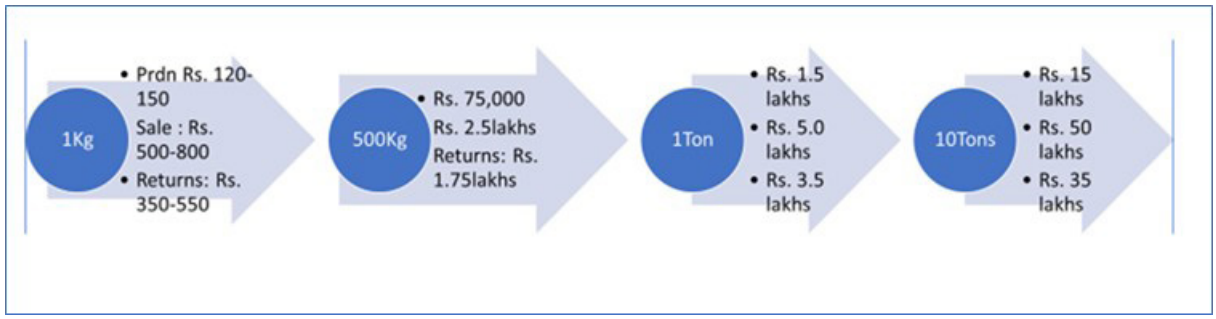



**Figure 12.** Predictable benefit to costs and profitability at different levels of production of EPN under *in vivo* conditions on *G. mellonella* larvae.

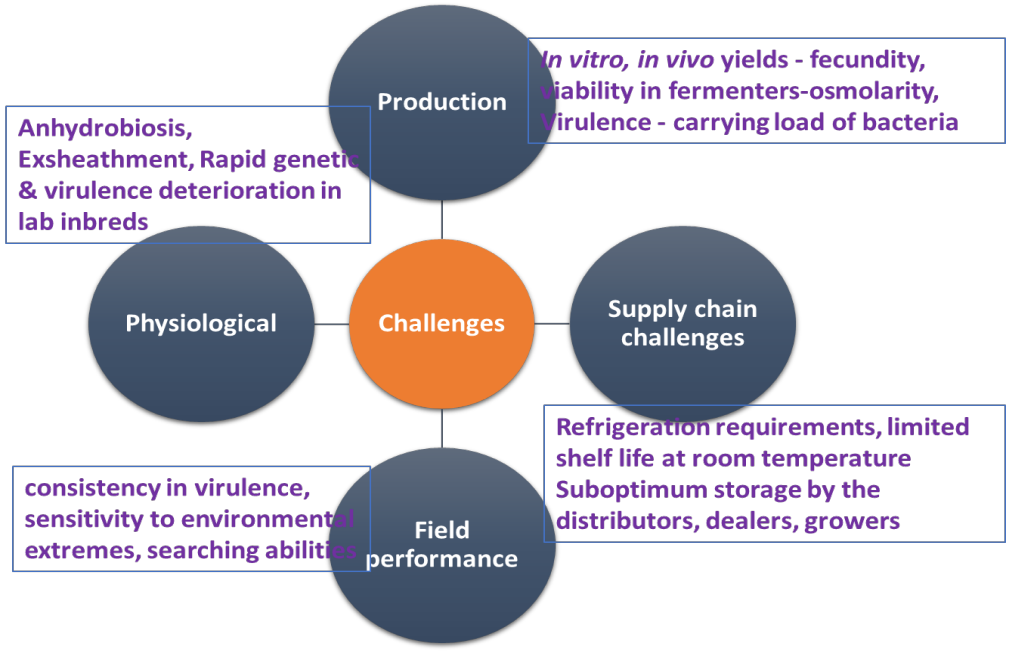



**Figure 13.** Schematic presentation of physiological challenges related to the events in EPN production-to-field use.

higher especially while working on the costs of recurring costs for bulk purchases, and scale of production.

## PHYSIOLOGICAL AND BIOLOGICAL CHALLENGES RELATED TO THE EPN FOR THEIR UTILIZATION

The physiological and biological challenges unique to EPN production that need research innovations and technologies are summarised and presented in a schema (Figure 13). The daunting tasks from production through post-production to supply chain and field use are categorised under four main categories. The physiological challenges related to the production are primarily the fecundity of the nematode species and realization of yield potentials under *in vivo* and *in vitro* conditions; retention of viability and virulence of EPN independent of the production systems; osmotic and pH tolerance; improvement of carrying capacity of the bacterial load *in vitro* production and ex-sheathment during submerged culture (Glaser & Salame, 2000; Ferreira & Malan, 2014; Lunau *et al.*, 1993). During the *in vitro* production process, longer retention of the primary phase of the monoxenic bacterium in media is most desired for better production of robust and healthy nematode juveniles Individual and interactive biology of both the nematode species and their respective bacterial symbionts are vital for the production systems. In the case of EPN species that are amphimictic in reproduction, the challenge is to effectively sustain the mating of male and female juveniles and minimize the premature *endotokia matricida* in agitating media under *in vitro* conditions, and bioreactor parameters ($DO_2$ concentration, pH, temperature, agitation, etc.) are some of the other challenges in mass production (Tumialis *et al.*, 2021; Ehlers, 2001; Gil *et al.*, 2002; Ehlers, 1996; Ehlers *et al.,* 1992, Zervos *et al.,* 1991).

The physiological traits that have a bearing on post-production processing, formulation and shelf life are, the robust IJs inherently enriched with glycerol/fat body;

robust juveniles with better anhydrobiotic sustenance, and better genetic stock for virulence and fitness traits. (Strauch *et al.*, 2004; Ferreira & Malan, 2014; Lunau *et al.*, 1993). The physiological features that need attention from the point of formulation, field applications and field survival are temperature and desiccation tolerance (Perry *et al.,* 2012; Spence *et al.*, 2011; Zhu *et al.*, 2011); desiccation, temperature, osmotic and pH tolerance for developing novel formulations (Mukuka *et al.*, 2010) and host searching abilities.

## THE WAY FORWARD – INNOVATIONS NEEDED FOR THE PARADIGM SHIFT IN THE PROMOTION OF EPN IN IPM AND SUSTAINABLE PLANT HEALTH MANAGEMENT

In India and other developing countries, the current EPN production and supply chain is in its infancy, thriving as a cottage industry on *G. mellonella/T. molitor* and very rarely on *C. cephalonica*. Due to the wide gap between demand and availability of EPN, the products/formulations that are available in the market are either exorbitantly priced, unregulated and or spurious to meet the requirement and due to the lack of appropriate technological innovations from production to the final supply at the farm.

Some of the interventions and innovations made in recent times could resolve this paradoxical demand-supply scenario to some extent. Most significantly, the innovation related to the WP formulation of ICAR-NBAIR, Bengaluru, to improve the shelf life, which was granted an Indian patent, is noteworthy in the efforts made for the promotion of EPN for commercial use in PPP mode. The WP formulations evolved by Nagesh *et al.* (2010) (Indian Patent No. 295748/3490/CHE/2010), for *H. indica* ICAR-NBAIR EN Hi101 and *H. bacteriophora* ICAR-NBAIR EN Hb105, were significant break-through innovations in terms of their shelf-life up to 10-12 months. This innovation momentously facilitated the availability of a tangible EPN product in the commercial market and was promoted through the license of technology to 27 firms since 2010 which led to the production and supply of 1800 metric tonnes of EPN WP formulations, with a ground coverage of about 40,000 ha.

Technological innovations are the need of the hour for the transformation of the upcoming EPN industry to a self-reliant, self-sufficient and profitable enterprise and accomplish better uptake of EPN individually or in IPM. Importantly, there are wide gaps in the EPN industry between the West and developing countries. EPN research is mid-way with inherent challenges related to production, post-production processing, quality, shelf-life, delivery systems and tangible advisories. The infrastructural changes needed include a strong supply chain network, warehousing, market intelligence, industry-friendly policies and registration procedures. Innovations play a crucial role in the paradigm shift. There is an urgency to develop custom-made proprietary technologies for the venture models to be successful in a shorter time that matches the market development. The technologies range from simpler *in vivo* to advanced monoxenic EPN in flasks and fermentation. The formulation, storage, delivery, economy-to-scale and benefit-to-cost present challenges unique to EPN, not encountered with microbial or a chemical pesticide, and therefore, offer opportunities for innovations.

Most production technologies and commercial formulations available in the market are restricted to a few species of EPN. For instance, in the USA and Canada, *H. bacteriophora, S. carpocapsae, S. feltiae, S. riobravis* and *S. glaseri* are available as products in different states; in EU, *H. indica, H. bacteriophora, S. carpocapsae, S. feltiae;* while in Asia-Pacific, there are *H. indica, H. bacteriophora, S. carpocapsae* and *S. feltiae*, available. In India, *H. indica,* is the only commercially formulated product, although products containing *S. carpocapsae* and *S. glaseri* are also encountered. Further, the number of targeted pests and crops is mostly restricted to soil pests, although some aerial and cryptic pests are also targeted. There is a vast scope for utilization of several species of EPN and their isolates with specific traits against several crop-based pest complexes, for developing as commercial products. This gives a better chance for EPN diversity to be utilized locally or regionally.

EPN production through *in vivo* systems essentially requires a paradigm shift in innovations in

- Innovations needed in bioreactors and Controlled Production Systems (CPS): Currently the media and fermentation conditions harmonized for 6-8 nematode spp. Many other EPN species that are very effective but urgently need to be developed into commercial products are many. *In vitro,* production technologies are IP-protected and not available for investors.

- Innovations needed for Downstream processing: Currently, white trap extraction for *in vivo* and centrifugation/floatation for *in vitro* extraction, separation and decontamination are only available. Lack of easy and harmonized SOPs for separation, extraction, harvesting and decontamination - high mortality of IJs, large portions of unspent media and contamination. Downstream processing is invariably dependent on large quantities of water, which requires technologies to rationalize the WU. Techniques related to floatation, filtration, centrifugation and concentration and their combinations are open to innovations.

- Innovations needed in formulations, packing and dispensing: Shelf life of 18 months at NTP; development of newer formulations - gel, liquid and paste for each nematode species ready for commercialization; automated bulk formulation, machinery and exploration of novel additives with better properties.

- Innovations needed for field delivery systems: Nozzle volume, time cut-off controls; Drip and micro-irrigation systems; Drone assisted delivery systems for cryptic pests and Mechanized and tractor-mounted delivery systems.

- Innovations in genetic improvement: Genetic enhancement can increase the efficiency and survival of EPN. Improved strains of EPN may have various useful characteristics, including environmental tolerance, pathogenicity, and reproductive ability. Natural selection, breeding and biotechnological approaches to improve the traits such as mating capability, fecundity, shorter lifecycles, tolerance to fluctuations in osmolarity, desiccation and temperatures, and bioaccumulation of fat and fatty acids, are the innovative approaches to be adopted.

## SUMMARY

An upsurge of scientific and economic interest in EPN as tangible BCA in IPM and crop health is now envisaged in EPN primarily due to the advancements in the mass-production and formulation technologies of these nematodes, besides the identification of several effective isolates/strains, and the policies to minimize the use of chemical insecticides for the management of insect pests. Entomopathogenic nematodes are now produced and marketed by a handful number of companies, at low investment, low supply and low profitability which needs a transformation to continuing commercial manufacturing. It is anticipated that the next generation of inter-disciplinary innovations in understanding invertebrate behaviour, physiology, and biology; adoption of systems modelling; cost-effective fermentation and formulation techniques; manufacturing and design engineering; molecular and biotechnological applications for improving the fitness attributes would achieve the plausible pesticide-free agriculture and realization of SDG goals while achieving the immediate farm requirement of EPN as reliable, cost-effective and tangible biological control agents in IPM for better crop health management.

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