



Entomopathogenic Nematodes as Biological Control Agents in Sustainable Agriculture

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ABSTRACT

Insect pests that harm major crops like sugarcane, cotton, soybeans, vegetables, and horticulture plantations severely limit agricultural productivity in Maharashtra, one of India's leading agrarian states. Serious issues include insect resistance, resurgence, pesticide residues, soil degradation, and negative effects on non-target creatures and human health have arisen as a result of the state's long-term and careless use of chemical pesticides. In this regard, entomopathogenic nematodes (EPNs), which are members of the families Steinernematidae and Heterorhabditidae, provide an environmentally safe and sustainable substitute for controlling insect pests. As a result of their special mutualistic relationship with symbiotic bacteria (*Xenorhabdus* and *Photorhabdus*), entomopathogenic nematodes may quickly infect and kill a variety of cryptic and soil-dwelling insect pests. Target-specific action, little environmental persistence, lack of hazardous residues, and compatibility with integrated pest management (IPM) and organic farming systems are some of the benefits that EPNs offer over conventional pesticides. Under laboratory and field conditions, its application has shown encouraging results in controlling important pests that are common in the agroclimatic regions of Maharashtra. However, the efficacy of EPNs is affected by environmental factors such as soil moisture, temperature, and UV radiation, which may restrict their consistency under field circumstances, in contrast to chemical pesticides that provide instantaneous and broad-spectrum effects. Their field performance and persistence have been greatly enhanced by recent developments in formulation technologies, mass production, and the use of native EPN isolates tailored to the soil and climate of Maharashtra. This study examines the relative efficacy of chemical pesticides and entomopathogenic nematodes, highlighting the potential of EPNs as biological control agents in sustainable agriculture. Incorporating EPNs into Maharashtra's pest control plans is a practical way to lessen reliance on chemical pesticides while fostering soil health, environmental safety, and long-term agricultural sustainability.

Keywords: Biocontrol, EPN, Beneficial nematodes, Entomopathogenic nematode

INTRODUCTION

Agriculture in Maharashtra is a key of the state's economy with the development of irrigated farming through then period to energy received through huge infrastructure projects and speed water supply. Sugarcane, cotton, soybean, pulses, vegetables and several other

horticultural crops are produced in the state. Agriproduct in the Maharashtra State is put to an axiomatic limit by insect pests that result in a tremendous loss of yield every year. Soil-borne and cryptic pest insects like root grubs, cutworms, borers and bollworm are the most destructive to field and horticultural crop plants. An effective pest control it is then fundamental in order to maintain the yields of crops and to secure an income for integrated farmers. Nematodes are colourless micro-organism a roundworm, bears property of schemes of higher organisms except circulatory or respiratory systems (Sikandar et al., 2019, 2020b). For many decades, use of chemical pesticides was the only weapon for the state when it came to tackling insect pests in Maharashtra. Chemical pesticides are effective in rapid and broad-spectrum control over insect pest, but the long-term and indiscriminate usage has caused to numbers of ecological and agronomic problems. Resistance has already been reported in some insect species to various chemical classes, which resulted in lack of control and increased amount of pesticide applications. Pest resurgence and the onset of secondary pests have also made it more difficult to control crop damage. Similarly, the buildup of pesticide residues in soil, water and agricultural commodities has led to environmental pollution, loss of biodiversity and health hazards. Beneficial organisms, including pollinators, natural enemies and soil microflora are often negatively impacted on, which results in ecological disturbances and persistent soil quality deterioration.

In the past years, sustainable agriculture and a more integrated pest management (IPM) approach has become increasingly important in crop protection to reduce chemical inputs. As an alternative, biological control agents are becoming more and more considered, as they provide a lister and specific pest control. Prominent among them are entomopathogenic nematodes (EPNs) because of their broad-spectrum biocontrol potential against a variety of soilborne insect pests. EPNs, The EPNs (Entomopathogenic nematodes) are Obligate parasites or facultative parasites on harmful insects. They have been recorded on all continents global except Antarctica (Abate et al., 2017). The EPNs has higher fundamental wide host range and can easily find their suitable host. Biological control is a safe way to control pests and pathogens (Sikandar et al., 2020a). Their important role in the field of biological control (Davari

and Parker, 2018; Trdan et al., 2020). which comprise the families *Steinernematidae* and *Heterorhabditidae*, are obligate insect parasites and have mutualistic relationships with bacteria strains maintained in two separate genera, *Xenorhabdus* spp. They show different form of nature be like parasitic and the predaceous, as well as free-living and have a higher association from useful to harmful (Ferris et al., 2012).

Their interaction is classified into four basic groups including to facultative parasitism, obligate parasitism, necromenic and phoretic (Askary et al., 2018). This mutualistic association allows them to infect and eliminate hosts insects quickly, usually within 24–72 hours.

Entomopathogenic nematodes (EPNs) are regarded as safe for non-target organisms, including beneficial insects, and are non-toxic to humans (Dutka et al., 2015; Akhurst and Smith, 2002). They are environmentally compatible and can be applied using conventional pesticide equipment (Shapiro-Ilan et al., 2006). The first entomopathogenic nematode was described by Steiner in 1923 (Dillman, 2013). Although nematodes from more than twenty families are known to parasitize insects, only a limited number show strong potential for biological control (Shapiro-Ilan et al., 2012). Among them, the families *Steinernematidae* and *Heterorhabditidae* are the most widely utilized in pest management programs (Bal & Grewal, 2017). Species of the genera *Steinernema* and *Heterorhabditis* are extensively used in plant protection (Laznik & Trdan, 2014). While members of the genus *Osccheius* also exhibit entomopathogenic properties, the former two families have received greater commercial attention (Dillman et al., 2012b). The effectiveness of these nematodes is largely attributed to their symbiotic association with bacteria of the genera *Photorhabdus* and *Xenorhabdus*, which facilitate rapid host mortality and enable control of coleopteran, dipteran, and lepidopteran pests (Mohan, 2015). Based on host-searching strategy, EPNs are categorized as cruisers or ambushers. Cruiser species such as *Heterorhabditis bacteriophora* and *Steinernema glaseri* actively move through the soil in search of hosts, whereas ambusher species like *Steinernema carpocapsae* remain near the soil surface and attack passing insects (Mohan, 2015). Host detection is mediated by cues such as carbon dioxide, vibrations, and other chemical signals (Lortkipanidze et al., 2016). After penetrating the host, nematodes

typically cause death within one to four days, depending on host-parasite interactions (De Brida *et al.*, 2017). Because a significant proportion of insect life stages occur in the soil, EPNs are particularly effective against subterranean pests (Radová, 2010). They have demonstrated the ability to suppress numerous economically important insect species (Lacey & Georgis, 2012), and substantial progress has been made in their research, production, and field application over the past decade (Lacey *et al.*, 2015).

2. Entomopathogenic Nematodes: Biology and Mode of Action: A crucial aspect of EPN biology is the behaviour of seeking hosts. Infectious juveniles locate insect hosts by responding to numerous chemical signals such as carbon dioxide, waste products from insects, and volatile compounds released by plant roots during herbivore damage. EPNs employ different foraging methods based on the species. To capture passing insects, some species, such as *Steinernema carpocapsae*, employ an "ambusher" strategy, remaining near the soil's surface. Others, including *Heterorhabditis bacteriophora*, employ a "cruiser" strategy, actively traversing the soil profile to locate stationary hosts like white grubs and larvae that consume roots. These adaptive behaviours enhance their effectiveness against various insect pests.

Entomopathogenic nematodes (EPNs) are tiny roundworms residing in the soil that function as compulsory parasites of insects. They are widely acknowledged as outstanding biological control agents in sustainable farming. These organisms are mainly categorized into the families *Steinernematidae* and *Heterorhabditidae*, encompassing the genera *Steinernema* and *Heterorhabditis*, respectively. These nematodes occur naturally in diverse soil ecosystems across temperate and tropical regions, and they play an essential role in regulating insect populations. Their capacity to actively pursue, invade, and eliminate insect hosts renders them particularly valuable in managing soil-dwelling and hidden pests (Susurluk and Ehlers, 2008). Entomopathogenic nematodes possess an egg, along with four juvenile stages and one adult stage. Their life cycle is closely associated with that of their insect hosts (Onstad *et al.*, 2006).

Nonetheless, the third-stage infectious juvenile (IJ) represents the most crucial phase for biological control. The infectious juvenile represents a non-

feeding, developmentally paused phase that has adapted uniquely to thrive in soil habitats. It can actively search for insect hosts and is tough against environmental obstacles. To be pathogenic and effectively reproduce within the host, the nematode needs to have symbiotic bacteria present in its gut during this phase (De Siqueira Sabino *et al.*, 2014). EPNs offer several benefits compared to traditional pesticides, such as environmental protection, no harmful residues, minimal effects on non-target species, and alignment with IPM and organic farming practices. Nonetheless, environmental factors such as soil moisture, temperature, and ultraviolet light can all influence their performance in the field. Recent progress in mass production techniques, novel formulations, and the identification of indigenous EPN isolates that match local soil and climatic conditions have enhanced their effectiveness and market viability. Infectious juveniles penetrate the insect's body via natural openings such as the mouth, spiracles, or anus once they locate an appropriate host. Through the use of a specialized dorsal tooth, penetration may occasionally occur directly through the insect's cuticle, particularly with *Heterorhabditis* species. The nematodes discharge their symbiotic bacteria *Xenorhabdus* spp. for *Steinernema* and *Photorhabdus* spp. for *Heterorhabditis*—once they enter the insect's hemocoel, or body cavity. (Grewal *et al.*, 2005). A Entomopathogenic nematodes maintain a highly specific mutualistic association with symbiotic bacteria that is crucial for successful host infection (Divya & Sankar, 2009). These bacteria rely entirely on the nematode for transmission between insect hosts and for protection from the host immune system (Lewis & Clarke, 2012). Once released into the insect hemocoel, the bacteria multiply rapidly, causing host death within 24–48 hours. The nematodes then feed on the proliferating bacteria and degraded host tissues, enabling them to develop and complete their life cycle (Adams & Nguyen, 2002).

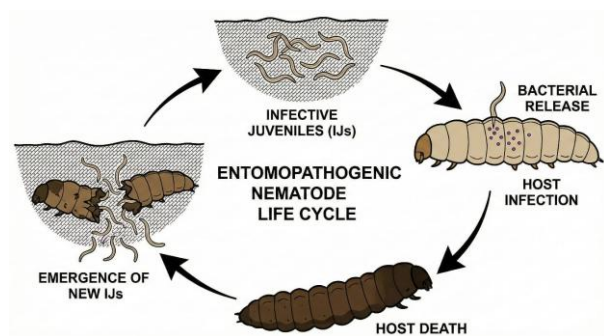


Figure 1 Life cycle of EPN (Grewal, P. S., *et al* (2005).

Environmental factors greatly affect the success and longevity of entomopathogenic nematodes. As they move by depending on the thin layers of water present between soil particles, sufficient soil moisture is essential. A significant aspect is temperature; the majority of species are most active in the range of 20°C to 30°C. Their vitality can be reduced by excessive temperatures and UV exposure, particularly in surface applications. The texture and concentration of organic matter in the soil also affect their spread and contagiousness. The infective juvenile stage of EPNs actively searches for insect hosts in the soil. When nematodes penetrate the host body through natural openings or delicate cuticular areas, they inject symbiotic bacteria into the bloodstream. The bacteria multiply, produce toxins, and lead to septicaemia, ultimately causing the host's death. The nematodes then reproduce inside the corpse, dispersing a fresh generation of infectious juveniles into the ground. This biological process renders EPNs perfect for managing soil-borne pests such as white grubs, root borers, and cutworms, prevalent in Maharashtra's diverse agroclimatic regions. The nematodes feed on the dissolved tissues of the host and proliferating bacteria upon the host's death. Inside the insect body, they develop into adults and reproduce. Depending on the host's size and the environmental conditions, one or multiple generations may be completed within it. Numerous new infectious juveniles ultimately develop, reconnect with their symbiotic bacteria, and penetrate the soil in search of new hosts once the resources within the carcass have been depleted. In appropriate conditions, their ability to self-replicate enhances their resilience and effectiveness. Inside the insect haemolymph, the bacteria that are released multiply rapidly and produce various toxins, enzymes, and antimicrobial agents. These substances lower the insect's immune response, inhibit the development of competing microbes, and cause septicaemia and toxicosis. The infected bug typically perishes within 24 to 72 hours consequently. Due to the nematode-bacterium complex's capability to induce quick death, EPNs act as swiftly as certain chemical pesticides while remaining safe for the environment.

3. Major Agricultural Pests in Maharashtra and Scope for EPN Application

Maharashtra encompasses diverse agroclimatic regions that support the cultivation of sugarcane, cotton, soybean, vegetables, and various horticultural

crops. These cropping systems are frequently affected by serious insect pests that cause substantial economic losses. In sugarcane, early shoot borers and white grubs are major constraints, while cotton production is challenged by pests such as pink bollworm and American bollworm. Soybean fields commonly suffer from stem flies and defoliating insects, and vegetable crops are often damaged by cutworms and fruit borers. Many of these pests spend a significant portion of their life cycle in the soil or remain concealed within plant tissues, reducing the effectiveness of conventional insecticide applications (Lacey and Georgis, 2012). Entomopathogenic nematodes (EPNs) offer a promising alternative for managing such hidden and soil-dwelling pests. Their infective juveniles actively move through the soil profile in search of suitable hosts and can penetrate and eliminate insects residing below ground (Kaya & Gaugler, 1993). Species belonging to the genera *Steinernema* and *Heterorhabditis* have demonstrated high pathogenicity against white grubs, cutworms, and other subterranean larvae (Shapiro-Ilan *et al.*, 2017). Incorporating EPNs into pest management strategies in Maharashtra could enhance biological suppression of key pests while reducing reliance on synthetic pesticides, thereby supporting environmentally sustainable agricultural production systems (Campos-Herrera, 2015).

4. Comparative Analysis: Chemical Pesticides vs. Entomopathogenic Nematodes

Synthetic insecticides are widely used because they provide rapid knockdown of pest populations. However, their repeated and indiscriminate application has resulted in widespread resistance development, contamination of soil and water resources, and adverse effects on non-target organisms, including natural enemies and pollinators (Kaya and Gaugler, 1993; Lacey *et al.*, 2015). In addition, chemical residues may persist in agricultural produce and the surrounding environment, raising ecological and public health concerns.

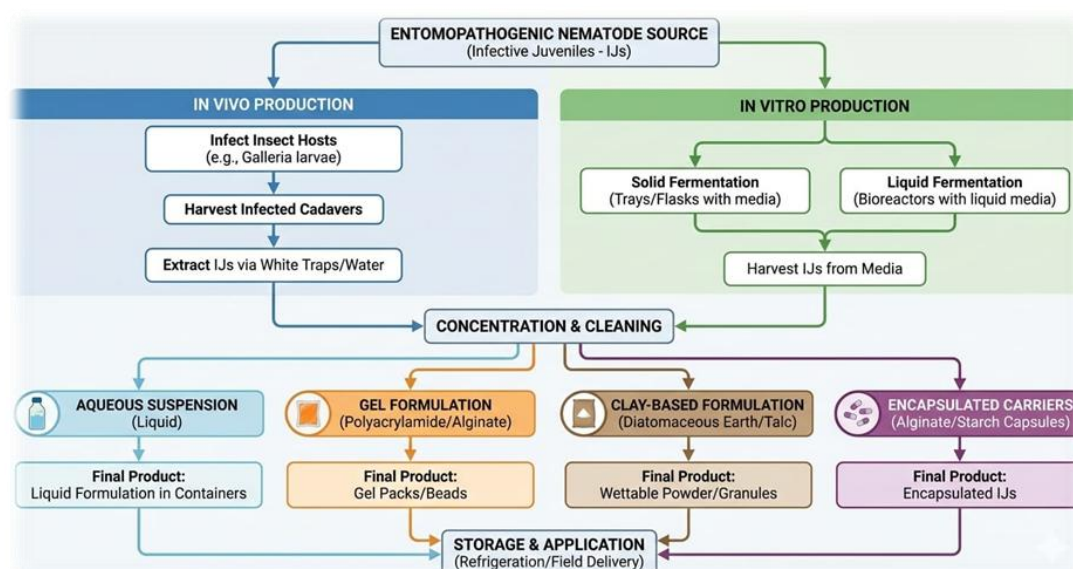
In contrast, entomopathogenic nematodes (EPNs) offer a biologically based alternative that is environmentally compatible and relatively specific to insect hosts. Once applied, infective juveniles actively locate suitable hosts and typically cause mortality within 24–72 hours through their symbiotic bacterial association (Shapiro-Ilan *et al.*, 2017). Unlike chemical pesticides, EPNs do not leave toxic residues and are considered

safe for non-target organisms when properly applied (Campos-Herrera, 2015). The risk of resistance development against EPNs is comparatively low due to their complex mode of action involving both nematode invasion and bacterial pathogenicity (Kaya & Gaugler, 1993). Furthermore, EPNs are compatible with Integrated Pest Management (IPM) programs and can be used alongside cultural practices, biological agents, and selective pesticides (Ehlers, 2001).

Although their field performance may vary depending on soil moisture, temperature, and formulation quality, advancements in strain selection and improved delivery systems have enhanced their reliability under diverse agroecological conditions (Shapiro-Ilan *et al.*, 2017). Adoption of EPN-based biocontrol strategies can therefore reduce reliance on synthetic insecticides and contribute to long-term sustainable agricultural production.

Table 1: Key Differences Between Chemical Pesticides and Entomopathogenic Nematodes

Parameter	Chemical Pesticides	Entomopathogenic Nematodes
Speed of action	Immediate	24–72 hours
Spectrum	Broad	Target-specific
Residue	Persistent	No toxic residues
Resistance risk	High	Low
Environmental impact	Often harmful	Environmentally safe
Compatibility with IPM	Limited	Highly compatible



Grewal, P. S., *et al* (2005).

Figure 2 Mass Production, Formulation, and Application of Entomopathogenic Nematodes

5. Factors Affecting EPN Efficacy

The effectiveness of entomopathogenic nematodes (EPNs) in controlling insect pests is influenced by several biotic and abiotic factors. Among these, soil moisture is particularly critical because infective juveniles depend on thin water films within soil pores to move and locate hosts; insufficient moisture can restrict their mobility and reduce survival (Kaya & Gaugler, 1993; Shapiro-Ilan *et al.*, 2017). Soil texture also affects nematode dispersal and infection efficiency. Sandy or well-aerated soils generally facilitate movement, whereas heavy clay or compacted

soils may limit penetration and host contact (Campos-Herrera, 2015). Temperature is another major determinant of performance, as most EPN species exhibit optimal activity between 20°C and 30°C; exposure to extreme heat or low temperatures can impair infectivity and longevity (Grewal, Ehlers, & Shapiro-Ilan, 2005). Additionally, ultraviolet (UV) radiation from direct sunlight can rapidly reduce nematode viability during surface applications, thereby limiting their persistence in exposed environments (Lacey & Georgis, 2012).

Living factors like the host species, developmental stage, and population density also influence the success of infection. Nematodes demonstrate the highest efficacy against vulnerable larval or pupal stages, while adult forms of certain pests can exhibit resistance. The health of symbiotic bacteria is essential, since toxins produced by these bacteria lead to the death of the host. Ultimately, farming methods such as irrigation, soil improvements, and the application of chemical pesticides can either boost or hinder EPN effectiveness. Grasping these elements is crucial for enhancing field implementations and ensuring reliable pest management.

6. Advances in EPN Technology

6.1 Mass Production Techniques

The mass production of entomopathogenic nematodes has greatly enhanced their accessibility and cost-effectiveness for agricultural applications. Production occurs via *in vivo* techniques, where nematodes develop inside live insect hosts, and through *in vitro* approaches, utilizing artificial media in either liquid or solid fermentation systems. *In vitro* liquid culture enables large-scale production in regulated environments, lowering expenses and enhancing uniformity. Cultivating on solid substrates, typically involving grains or organic matter, is effective for generating infective juveniles with elevated survival rates. Improvements in automation, sterilization, and refined nutrient media have enhanced yield, virulence, and longevity.

6.2 Formulation and Storage

Formulation technology improves the survival of nematodes and their effectiveness in the field. Typical formulations consist of water-based suspensions, gels, foams, and clay-derived carriers, which shield nematodes from drying out, UV exposure, and high temperatures. Microencapsulation methods are being investigated to prolong shelf-life and facilitate slow release in the soil. Correct storage at low temperatures (4–10°C) maintains nematode viability for weeks to months. Improvements in formulation guarantee reliable field performance and simplicity of application for farmers.

6.3 Use of Native Strains

Studies indicate that EPN strains tailored to local conditions exhibit superior performance in specific regional soil and climate environments. The isolation

and characterization of indigenous strains from Maharashtra soils have produced nematodes exhibiting increased tolerance to temperature changes, soil pH variations, and drought stress. Utilizing native strains mitigates environmental adaptation challenges and enhances the reliability of pest management.

6.4 Integration with Other Pest Management Strategies

Current developments emphasize the incorporation of EPNs within Integrated Pest Management (IPM) strategies. EPNs may be utilized in conjunction with pheromone traps, biopesticides, and selective chemical pesticides without negative interactions. Compatibility investigations guarantee synergistic outcomes, minimizing total chemical usage. The timing of application and management of irrigation additionally improve nematode efficacy.

7. Integration of EPNs into Integrated Pest Management (IPM)

Entomopathogenic nematodes (EPNs) can be successfully integrated into IPM strategies as a biological substitute for chemical pesticides. They aim at soil-dwelling and hidden insect pests while protecting beneficial species, allowing compatibility with various control strategies like biopesticides, pheromone traps, and cultural techniques. Correct timing of application, management of soil moisture, and choosing locally suitable strains improve their efficacy. Incorporating EPNs into IPM decreases the use of chemical pesticides, enhances environmental safety, and fosters sustainable agricultural practices.

8. Challenges and Limitations

Entomopathogenic nematodes (EPNs) encounter several biological and operational constraints that may limit their widespread use in sustainable agriculture. Their performance is highly influenced by environmental conditions such as soil moisture, temperature, pH, and soil structure, which directly affect survival, movement, and infectivity (Kaya & Gaugler, 1993; Shapiro-Ilan *et al.*, 2017). Both excessively dry and waterlogged soils can impair host-seeking efficiency, while extreme temperatures may reduce activity or cause mortality. In addition, exposure to ultraviolet (UV) radiation during surface applications significantly decreases nematode viability, restricting their effectiveness in open-field environments (Lacey & Georgis, 2012). Biological

limitations also affect their practical application. EPNs are most effective against larval and pupal stages of insects, particularly soil-dwelling species, whereas adult insects and those with heavily sclerotized exoskeletons are generally less susceptible (Grewal *et al.*, 2005). Differences in host susceptibility among pest species may lead to variable control outcomes, especially in cropping systems where multiple pests coexist. Another important constraint is their limited shelf life and specific storage requirements. Despite advances in formulation technologies, EPNs must be stored under controlled conditions—typically at low temperatures with adequate humidity—to maintain infectivity (Ehlers, 2001). These storage demands can create logistical and economic challenges, particularly in regions lacking reliable cold-chain infrastructure. Collectively, these environmental, biological, and storage-related factors underscore the need for improved formulations, optimized delivery systems, and better field management practices to enhance the consistency and large-scale applicability of EPN-based biocontrol strategies.

9 Future Prospects

A further limitation of entomopathogenic nematodes (EPNs) is their comparatively short shelf life and dependence on controlled storage conditions. Even with improvements in formulation technologies, EPNs must be stored at low temperatures and appropriate humidity levels to preserve their viability and infectivity (Ehlers, 2001; Grewal *et al.*, 2005). Deviation from these conditions can significantly reduce their field performance. Such storage requirements may create logistical and economic difficulties, particularly for small-scale farmers or in areas where cold-chain infrastructure is limited. Collectively, environmental sensitivity, biological constraints, and storage challenges emphasize the importance of developing improved formulations, efficient delivery systems, and optimized field management strategies to enhance the reliability and large-scale adoption of EPN-based biocontrol programs (Shapiro-Ilan *et al.*, 2017).

10. CONCLUSION

Entomopathogenic nematodes (EPNs) are becoming recognized as efficient and eco-friendly substitutes for chemical pesticides in farming. Their distinct biology, featuring the infective juvenile phase, partnership with symbiotic bacteria, and swift pathogenicity,

enables them to effectively target soil-dwelling and hidden insect pests, which are hard to control with traditional chemicals. In contrast to synthetic pesticides, EPNs are environmentally safe, do not leave harmful residues, and align well with integrated pest management (IPM) and organic farming methods. Although environmental factors like soil moisture, temperature, and UV exposure can affect their field performance, the reliability of these organisms has been greatly enhanced by improvements in formulation technology, mass production, and the employment of locally adapted strains. Incorporating EPNs into Maharashtra's farming practices provides a viable strategy for decreasing chemical reliance, safeguarding beneficial species, and enhancing enduring soil vitality and agricultural sustainability. In general, EPNs are a valuable resource for attaining safer, efficient, and sustainable pest control in contemporary agriculture.

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Author contribution:

Mr. S. N. Salve- Corresponding author: Assisted and organized experimental information, and carried out data analysis for this research. Mr. S. R. Naphade and Dr. S. T. Naphade- Performed and contributed to the preparation and revision of the manuscript.

Conflict of interest

The authors declare no competing interests.

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