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Review Article

A REVIEW ON ROLE OF ENTOMOPATHOGENIC NEMATODES IN INTEGRATED PEST MANAGEMENT

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ABSTRACT

Entomopathogenic nematodes (EPNs), which are microbial pathogens cum insect pest's biocontrol agents, have been used successfully in agricultural systems. They may be easily cultivated *in vivo* or *in vitro* and are extremely pathogenic, quickly killing their hosts. Due to their widespread production in liquid media, their production costs have recently decreased dramatically, while still being safe for the environment and non-target vertebrates. Additionally, there are no challenges in using EPNs because they can be coupled with practically all chemical control chemicals and are simple to spray using ordinary equipment. EPNs are frequently used to control economically significant insect pests in a variety of farming systems, including nurseries, greenhouses, turf grass, and fruit orchards. Only in the early 1980's did EPNs start to be used for biocontrol, and this needed a gradual advancement of both science and technology. The commercialization of nematode-based insect pest management was greatly aided by the nematode's mass production. This review paper discusses different species of EPNs, its Mass production and utilization in Integrated Pest Management program.

Keywords: Efficacy, Entomopathogenic, Nematodes, *Heterorhabditidae, Steinernematidae*.

INTRODUCTION

Nematodes are spherical, uncolored, segmented worms without appendages. They could be predatory, parasitic, or free-living. Numerous parasite organisms cause serious illnesses in people, plants, and animals (Pal *et al*., 2008). All interactions between nematodes and insects, such as phoresis, parasitism, and pathogenicity, are referred to as "Entomophilic nematodes" (Shinde *et al*., 2000). Entomogenous nematodes are those that are associated with insects either obligately or facultatively as parasites (Stock, 2019). Entomogenous nematodes can cause their hosts to become sterile, have less fecundity, lifespan, and flight activity, develop slowly or exhibit various morphological, physiological, or behavioural abnormalities, and, in rare situations, die quickly (Grewal and Georgis, 1999). For 23 nematode families, parasitic relationships with insects have been identified (Hussaini, 2002). There are species in seven of these families that may be used to biologically control insects. Entomopathogenic Nematodes (EPNs) may parasitize dangerous insects either obligately or facultative (Campbell and Lewis, 2002). Except for Antarctica, they have been observed on all continents. They are given a lot of thought in the area of biological control. There are 23 families of nematodes that have been identified as insect parasites, but only seven families feature species that are particularly effective at controlling insects. Pest biological control has utilised Heterorhabditiae and Steinernematidae more frequently and successfully (Ganguly *et al*., 2006).

Nematodes appear to have evolved to fit into nearly every possible niche, including a variety of parasitic environments. There are a huge variety of parasitic nematodes, and some of them have gotten along with the bacteria that cause insect diseases (Ali *et al*., 2008). Together, the worm and bacterium bring about death. These worms are known as entomopathogenic nematodes or EPNs for short. The nematodes essentially function as miniature Typhoid Marys that deliver their insect-pathogenic bacteria cargo. The nematodes seek for potential hosts, assault them, and then release their toxic payload into the nutrient-rich hemolymph. Die infected insect hosts. The nematodes develop while feeding on the bacterial and insect tissues, and the bacteria quickly

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multiply. When the host cadaver's supply of resources runs short, nematodes associated with dangerous germs emerge and search for new hosts to infect (Dillman *et al*., 2012).

Habitat

Only soil-based creatures are steinernematid nematodes. In a wide range of physiologically distinct soil habitats, such as cultivated fields, forests, grasslands, deserts, and even ocean beaches, they have been discovered in isolation. Every continent where people live has them. During surveys, entomopathogenic nematodes are found in 2-45% of the sites sampled (Harry, 1991).

Distribution

Asia, Africa, North, Central, and South America, the Caribbean, Oceania, and Europe.

Mechanism

The immunomodulatory and toxic properties of parasitic nematodes are mostly attributable to the excretory and secretory (ES) products they secrete during infection. In addition, the contagious larvae start spewing forth a complex protein mixture. After being extracted and thoroughly analysed, researchers found that this mixture comprised 472 unique proteins, many of which are proteases. Approximately 3,500 bacterial cells are required to kill a normal insect with *Xenorhabdus nematophilus*. But each *S. carpocapsae* only contains 20–200 *X. nematophilus* cells, which is far less than the deadly amount (Kulkarni *et al*., 2013).

Morphology

The only stage that is free-living and consequently environmentally tolerant is the IJ stage. The sole free-living and thus environmentally tolerant stage of *S. carpocapsae* nematodes, infective juveniles, are created and used in formulation and application. IJs of *S. carpocapsae* are between 0.4 and 0.65 mm in length, with males being between 1 and 1.7 mm long and females being between 2.8 and 5.1 mm long (Khan *et al.*, 2007). The IJs can be frozen for long-term storage in liquid nitrogen, or they can be kept in tap water or buffer for several months. Although disturbed nematodes move actively, preserved nematodes often exhibit diminished movement. Along with other ambush foragers, *S. carpocapsae* when submerged in water, they quickly return to their typical 'J'-shaped resting posture, which involves a straight body posture with a slight twist at the tail's end. In other words, nematodes may need to be induced to move first (*e.g.*, using probes, acetic acid, or moderate heat) before viability can be determined (Koppenhöfer and Kaya, 2001). Lack of movement is not always a symptom of mortality. While almost translucent nematodes are frequently active but have limited infection potential, living infective juveniles, which would still be effective in biological control, tend to have high lipid levels that give them a thick look (Pervez *et al*., 2012).

The majority of Steinernematid nematodes, including *S. carpocapsae*, kill insects by turning their tissues brown or tan; Heterorhabditids kill insects by turning their tissues red and sticky. Putrefaction-related black cadavers show that the host was not killed by entomopathogenic species. Such cadavers often include nematodes, which are free-living soil saprophytes. When exposed to insect tissue, the developmentally arrested IJ stage will become active. Changes in morphology, the restart of development, the release of symbiotic bacteria, and the release of a number of proteins assumed to be involved in active parasitism are all part of the activation process. Opening of the mouth and anus, opening of the oesophagus, expansion of the pharynx's basal bulb, and the start of pharyngeal pumping are only a few of the morphological changes connected to activation (Subramanian and Muthulakshmi, 2016).

Life Cycle

Steinernema carpocapsae is categorised as an entomopathogenic nematode, which is a specialised subgroup of insect-parasitic nematodes (Yadav, 2016). The only free-living stage of this nematode is the infective juvenile stage (IJ), which is a modified third-stage larva. It is a non-feeding, developing stage that is in search of an insect host to infect. The only other life stages that take place inside an insect host are L1, L2, L3, and adult. IJ enters the host naturally through the spiracles, mouth, anus, or in some species, the intersegmental membranes of the cuticle, before moving into the hemocoel. *Xenorhabdus nematophila* and *S. carpocapsae* are mutually related bacteria. *X. nematophila* bacteria are carried by the IJ in the receptacle, a particular part of the anterior gut, but they are discharged into the host during defecation (Yadav, 2012). Within 24 to 48 hours, the infected host typically passes away as a result of the bacteria multiplying in the insect haemolymph. Nematodes continue to feed on the host tissue and the growing germs even after the host has died. In order to breed, they evolve into males and females. The offspring grow through four juvenile phases before becoming adults. One or more generations may develop inside the host cadaver, depending on the resources available, and a huge number of infectious juveniles are finally discharged into the environment to infect further hosts and complete their lifespan (Gozel and Gozel, 2016).

Infective Stage of *Steinernema* spp.

Entomopathogenic nematodes are tender-bodied, non-segmented roundworms that are obligate or, on occasion, facultative parasites of insects. Entomopathogenic nematodes arise evidently in soil environments and discover their hosts in response to carbon dioxide, vibration, and other chemical cues. Steinernematidae and Heterorhabditidae, chiefly, have been extensively used as bio-insecticides in the treatment of insect pests. Because they are frequently non-toxic to people, very target-specific, and work with standard pesticide application equipment, entomopathogenic nematodes integrate perfectly into integrated pest management, or IPM, procedures. The Environmental Protection Agency (EPA)

no longer requires the registration of pesticides in the United States for entomopathogenic nematodes. They don't need personal protection equipment, and there are no issues with admission limitations. Insect resistance issues are also quite uncommon (Stock, 2019).

The infective juvenile stage, also known as 1st J in entomopathogenic nematodes, is the only free-living stage. The juvenile stage enters the host insect's hemocoel after penetrating it through its spiracles, mouth, anus, or, in certain species, intersegmental cuticle membranes. The bacterial genera *Photorhabdus* and *Xenorhabdus*, respectively, share a mutualistic relationship with *Heterorhabditis* and *Steinernema* (Lewis *et al.*, 1992). From their intestines, juvenile stage organisms expel cells containing symbiotic bacteria into the hemocoel. The inflamed host normally dies within 24 to 48 hours as the bacterium multiplies in the insect haemolymph. Nematodes continue to feed on the host tissue after the host has died, maturing and multiplying. The offspring nematodes grow from the fourth juvenile stage to adulthood. One or more generations may also occur in the host cadaver depending on the resources available, and a large number of infectious juveniles are then released into the environment to infect further hosts and complete their life cycle. *Steinernematid* and *Heterorhabditid* have different reproductive processes. However, in the following generation, each individual is able to produce both males and females, unlike in *Steinernematid*, when all generations are capable of producing both males and females. Infectious juvenile stages of *Heterorhabditids* grow into hermaphroditic adults. When insects are murdered by *Heterorhabditids*, their corpses are coloured red; when they are killed by *Steinernematids*, they are coloured brown or tan. The pigments produced by the monoculture of mutuality bacteria living in the hosts are indicative of the colour of the host's body (Kaya and Gaugler, 1993).

Influence of Abiotic Factors on EPN

Studies on the biological and environmental factors affecting the performance of *Steinernema innovationi* have shown that different entomopathogenic nematode species behave differently under various environmental settings. The study showed that temperature has an impact on host range, foraging behaviour, and infectivity and reproduction. Thermal activity was at its peak between 22 and 25 °C. *Galleria mellonella's* last instar had the highest infectious juvenile (IJ) yields at 22 °C (333, 014 IJs g^{-1}) and 25 °C (354, 165 IJs g^{-1}). Within 24 hours, an average of 26% of the IJs infected *G. mellonella* larvae at a depth of 15 cm. *Steinernema innovationi* IJs moved in a circular motion and elevated almost 95% of their bodies off the ground without jumping. The least susceptible hosts were *Acheta domesticus, Chilo partellus,* and *Plutella xylostella. Eldana saccharina, Sesamia calamistis, Tenebrio molitor, G. mellonella,* and *Cydia pomonella* were the only other hosts that experienced complete larval mortality. Pupal mortality was between 47 and 68%. *Agrotis ipsilon* has LC_{50} and LC_{70} values of 3 and 31 IJs $larva^{-1}$, respectively (Kaya *et al.*, 2006).

Influence of Biotic Factor on EPN

Renting the symbiotic bacteria of EPN or their metabolites or by products as control agents for arthropod pests or plant pathogens is the only other route to wider usage. EPNs will be able to lessen reliance on chemical inputs in agriculture and increase sustainability with these advancements. EPN usage has increased as a result of advancements in large-scale production and application technology. *In vivo* EPN production is the method that is most ideal for laboratory use, small-scale field testing, and niche markets. However, it is constrained by the high costs of personnel and insect media (Bhat *et al*., 2020). *In vitro* solid culture is typically seen as being midway between in vivo and liquid culture when it comes to commercial application for foreign markets. *In vitro* liquid culture is thought to be the most cost-effective method. Although liquid culture is more cost-effective than other manufacturing techniques, it also requires more capital investment and technical know-how (Kulkarni *et al*, 2016).

Mass Production Technique of EPN

Entomopathogenic nematodes (genera *Steinernema* and *Heterorhabditis*) kill insects with the useful resource of mutualistic bacteria. The nematode-bacteria complicated are mass produced for use as bio-insecticides using *in vivo* or *in vitro* techniques, *i.e.*, solid or liquid fermentation. Although *in vivo* production (culture in live insect hosts) has a short lifespan, low start-up costs, and high nematode quality as a result, it has a poor cost-effectiveness. The development of nematodes and bacteria on crumbled polyurethane foam, or "solid tradition" in vitro, results in an intermediate level of time and cost (Krishnayya and Grewal, 2002). Through advances in mechanisation and streamlining, *in vivo* manufacturing and solid culture can advance (Shapiro-Ilan and Dolinski, 2015). The most cost-effective production method is *in vitro* liquid life, but it also has the highest start-up costs and a lower nematode exceptional rate. Through advancements in media development, nematode restoration, and bioreactor design, liquid culture can advance. Nematode storage and application can be facilitated by a number of formulations. EPN's function in pest management Researchers from all around the world have explored the ability of EPNs to act as retailers of insecticidal agents against a wide variety of insect species (Koppenhöfer and Kaya, 2001). They have been employed with varying degrees of success against insect pests in particular settings. Using entomopathogenic nematodes as biological control agent, a great deal of progress has been made to fight against pests that live in the soil or in secretive (Divya and Sankar, 2009).

Application Methods

Nearly all agronomic or horticultural ground equipment, like as pressurised sprayers, mist blowers, and electrostatic sprayers, can be used to apply EPNs. They can also be sprayed from the air (Shapiro-Ilan and Dolinski, 2015). The equipment used for application is dependent on the cropping system. Numerous handling considerations must be made for each situation, including volume, agitation,

nozzle type, pressure, recycle time, system of environmental conditions, and spray dispersion pattern. It's important to make sure there is enough agitation during application. Applications for small plots may be appropriate for handheld or backpack sprayers. When administering nematodes to larger plots, think about utilising a boom sprayer or similar appropriate spraying apparatus. Alternatively, applicators could use micro-jet irrigation systems, subsurface injection, or baits (Liu *et al*., 2000). A range of formulations, including activated charcoal, alginate and polyacrylamide gels, clay, peat, polyurethane sponge, vermiculite, and water dispersible granules, can be used to apply EPNs in aqueous suspension (WDG). Increased efficacy in EPN applications may be possible with a better formulation. Significant progress has been achieved in recent years in the development of EPN formulations, particularly for above-ground applications such mixing EPNs with a surfactant and polymer. Using surfactants to increase leaf coverage and relying on leaf flooding can both increase effectiveness (Grewal, 2012).

A follow-up treatment of a sprayable gel, which is frequently used to protect buildings from fire, significantly improved *S. carpocapsae* applications for control of the lesser peach tree borer, *Synanthedon pictipes*. When utilised in a chitosan formulation, *S. carpocapsae* significantly suppressed the red palm weevil, *Rhynchophorus ferrugineus* with 98% efficacy in a prophylactic treatment. Furthermore, Raja *et al.* (2011) noted that treatments were strengthened and improved when EPN was used in conjunction with sprayable fire-gel or wood flour foam as a protective agent for reducing the number of *Cydia pomonella* (L.) codling moths in apple tree trunks.

CONCLUSION

EPNs might be effective biocontrol agents for insect pests, and as a result, a potential industry has emerged. However, a variety of obstacles have prevented the hiring of EPNs on a much larger scale. The right nematode species must be matched with the target pest. Additionally, biotic factors like illnesses and EPN predators, additional soil organisms, and abiotic factors like ultraviolet light, soil moisture and relative humidity, and temperature might affect how well-behaved deployed EPNs are. EPN composition, application tools or methodologies, and strain development have recently been improved to increase utility efficacy. Expanded use of EPNs in biocontrol will be encouraged by more research aimed at lowering product costs, increasing product availability, and enhancing efficacy and carryover impact. The genetic development and stabilisation of EPN lines, the advancement of method technology primarily focused on aboveground applications, and conservation biocontrol are major research fields that may be likely to produce significant results. Increased use will also result from the development of new target pests as well as from the discovery of the most recent EPN species and strains. EPN usage has increased as a result of advancements in large-scale production and application technology. In vivo EPN production is the method that is most ideal for laboratory use, small-scale field testing, and niche markets. However, it is constrained. In vitro solid culture is typically seen as being midway between in vivo and liquid culture when it comes to commercial application for foreign markets. In vitro liquid culture is thought to be the most cost-effective method. Although liquid culture is more cost-effective than other manufacturing techniques, it also requires more capital investment and technical know-how. By manufacturing the insect hosts "in-house" and automating the process, improvements in efficiency and scalability allow in vivo production to play a larger part in pest management programmes. Technical advancements will increase the efficiency of in vitro solid formation similarly to in vivo production, but neither method will be able to scale up as much as liquid culture technology.

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