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Use of entomopathogenic nematodes in recent trends: A review

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Abstract
Entomopathogenic nematodes (EPNs) in the families Steinernematidae and Heterorhabditidae are facultative obligate parasites of soil inhibiting insects. EPNs are being widely researched as promising biocontrol agents for a wide range of agricultural pests. It is known that strains of EPNs isolated from different geographical regions differ in their attributes, such as host-finding ability, host range, infectivity, reproduction, and environmental stress tolerance. The use of EPNs as bio-pesticides against insect pests has filled quickly in recent years. The greater part of the bio-pesticides require days or weeks to kill the insect pest however EPNs kill the insect within 24-48 hours because of septicemia. EPNs are profoundly destructive, killing their hosts rapidly and can be cultured easily *in vivo* or *in vitro*. Besides, no hardships to apply EPNs as they are easily splashed using standard equipment and can be joined with practically all substance control compounds. A precise knowledge of these factors is therefore an essential pre-requisite for devising successful strategies to use these nematodes in biological control programmes.

Keywords: Entomopathogenic nematodes, insect pest, *in vivo*, *in vitro*, bio-pesticides

Introduction
Nematodes related with insects, referred to as entomophilic, entomogenous or entomopathogenic are known to parasitise and kill the insects. The term 'Entomophilic nematodes' incorporates all connections of insects and nematodes going from phoresis to parasitism and pathogenesis. Parasitic associations with insects have been depicted from 23 nematode families. Seven of these families contain species that have potential for natural control of insects (Koppenhofer and Kaya, 2001). Beneficial nematodes assault soil borne insect pests, yet are not harmful to humans, animals, plants, or earthworms, and can therefore be used as biological control organisms (Denno *et al.*, 2008) [27]. Subsequently, it is important to foster harmless to the ecosystem choices to control soil pests, such as entomopathogenic nematodes (EPNs). EPNs are grouped into two genera namely *Steinernema* and *Heterorhabditis*. They are mutualistically connected with microscopic organisms having a place with the genera *Xenorhabdus* and *Photorhabdus* are free-residing bacterial-feeder nematodes and belong to order Rhabditida (Burnell and Stock, 2000) [20]. The EPNs are potential and most encouraging biological agents for the control of various insect pests of different crops, those are eco amicable and cost effective (Ali *et al.*, 2005) [3]. Different species and isolates exhibit considerable variation in their host range, reproduction and conditions for survival, life span, delayed development, fast mortality (Bedding, 1990; Laznik and Trdan, 2012) [9, 74].

Distribution: Entomopathogenic nematodes are pervasive and broadly circulated under tropical, subtropical and temperate conditions. They have been isolated from each occupied mainland from a wide scope of environmentally assorted soil living spaces including developed fields, timberland, prairies, deserts and even sea shores. (Griffin *et al.*, 1990; Hominick, 2002) [48, 59], and in 2010, they have been accounted for to be isolated from 49 nations all over the planet (San-Blas, 2013; Stock, 2015) [85]. Dissemination studies were led all through the world using insect baiting technique (Bedding and Akhurst, 1975) [10] and recorded by Kaya (1990). The pace of EPN species discovery has been expanding extensively (Adams and Nguyen, 2002; Lewis and Clarke, 2012) [75, 1]. Since the time that first EPN species was accounted in 1923 (Steiner, 1923; Poinar, 1990) [3, 84] in excess of 85 EPN species have been depicted (Lewis and Clarke, 2012) [75] and greater part of described species have been accounted for in the beyond 10 years. *Aplectana kraussei* later to become *Steinernema krausei* (Travassos, 1927) found parasitizing the sawfly, *Cephalcia* (*Lyda*) species (Steiner, 1923) [3] is the principal record of an EPN.

This was before long followed by Glaser finding and Steiner (1929) depicting, *Neoaplectana glaseri* n.g., n.sp. from the Japanese beetle, *Popillia japonica*. Presently, over 80 species of *Steinernema* and 20 species of *Heterorhabditis* have been described (NCBI, 2015).

The family, Heterorhabditidae, and genus *Heterorhabditis*, was laid out by Poinar (1975b) [83] when he depicted *H. bacteriophora* found parasitising *Helicoverpa punctigera* in Australia. The number of new strains and species found will probably keep on rising adding more potential for biocontrol applications. In India, the underlying exploration with EPNs was led principally with exotic species/strains of *Steinernema carpocapsae*, *S. glaseri*, *S. feltiae* and *Heterorhabditis bacteriophora*, imported by specialists (Kaya *et al.*, 2006). By and large, these exotic EPNs yielded conflicteing outcomes in field preliminaries, most likely due to their unfortunate flexibility to the nearby agroclimatic conditions. There was worry that the presentation of exotic EPNs could likewise adversely affect on non-target organisms (Kaya *et al.*, 2006). Numerous valid species of *Steinernema* and *Heterorhabditis* have been described (e.g., Shapiro-Ilan *et al*. 2020), only 13 of these described species have been marketed till now (Koppenhöfer *et al*. 2020a) [71]. Consequently, the isolation of the local species and populations of EPNs was emphasized and considered a valuable resource, not only from a biodiversity viewpoint but also from an ecological point of view as a result of their versality and utility in natural control.

Persistence of EPNs and their host finding strategies: Soil is the most appropriate natural surroundings for focusing on or isolating EPNs. Undisturbed soils like plantations and woodlands are the normal supply for both steinernematids and heterorhabditids. A few elements including soil texture, pH, dampness, hotness, desication and ultraviolet radiation are portion of the natural variables which might effect on the adequacy of EPNs (Shapiro *et al.*, 2000). Soil pH can also influence natural EPN circulation (Kanga *et al.*, 2012) [66]. The impacts of these elements also depend on the species, strain, or natural surroundings of the EPNs. Their wide dissemination all through the world demonstrate their genetic ability to adust to different ecological burden that they experience in the soil environment (Hominick *et al*, 1996) [61]. Infective juvenile persistence is higher in sandy loam with persistence lower in clay soils. The poor persistence in clay soil is attributed to bring down oxygen fixation because of their little pore spaces and unfortunate water depleting limit. Infective juveniles can perserve in soils with pH values of 4 and 8, but persistence might decline with pH of 10. Salinity does not influence EPNs since they have been detached from soils close to the coastline (Griffin *et al.*, 1994) [49]. Nematodes created ecosysemto endure unfavourable natural circumstances. In a dormant stage, the quiescent (Glazer, 2002) [41] their digestion is brought down and a longer persistence is conceivable. However, nematodes in this condition are not microorganism and provided that the ecological circumstances are good, they retain active and able to penetrate into insects. The quiescent stage can be prompted by outrageous temperatures, oxygen lack and high salt substances (Glazer, 2002) [41].

Only EPNs in the infective juvenile stage will survive in the soil, find and enter into insect pests. Infective juveniles find their hosts in soil through two procedures-ambushing and cruising (Gaugler *et al.*, 1989) [53]. EPNs use the ambushing strategy tend to remain stationary at or close to the soil surface and find host insects by direct contact (Campbell *et al.*, 1996). An ambusher searches by standing on its tail so that greater part of its body is in the air, referred to as "nictation." The nictating nematode connect to and attacks passing insect hosts. Ambusher EPNs most successfully control insect pests that are highly mobile at the soil surface, for example, cutworms, armyworms and mole crickets.

EPNs that use the cruising technique are highly mobile and ready to move all through the soil profile. Cruisers find their host by detecting CO_2 or different volatiles delivered by the host. Cruiser EPNs are most effective against stationary and sluggish insect pests at different soil depths, like white grubs and root weevils. IJs of the family Heterorhabditiae utilized the supposed cruiser technique to search actively in the soil for appropriate insect larvae. Nematodes of the family Steinernematidae adopted the ambusher technique, waiting passively near the soil surface for prey to cross their direction. Ambusher species include *Steinernema carpocapsae* and *S. scapterisici*; cruisers include *Heterorhabditis bacteriophora* and *S. glaseri*. *S. riobrave* and *S. feltiae* do a bit of both ambushing and cruising (Campbell and Gaugler, 1997) [22].

Life cycle: Entomopathogenic nematodes (EPNs) have six life stages: The egg, four Juvenile stage (J1, J2, J3, and J4), and an adult. The non-feeding, free-living, third stage IJ infects the insect host in the soil environment. EPNs are a nematode-bacterium complex. In this affiliation, the nematode is reliant upon the bacterium for rapidly killing its insect hosts, establish a reasonable climate for its development by delivering antibiotics that stifle contending microorganisms and changing the host tissues into a food source. An IJ carries between 0 and 2000 cells of its symbiont bacterium in the anterior part of the intestine (Spiridonov *et al.*, 1991; Sturhan and Kreimeier, 1992; Endo and Nickle, 1994). *Xenorhabdus* happens normally in a special intestinal vesicle of *Steinernema* IJs (Bird and Akhurst, 1983) [1], while *Photorhabdus* is circulated in the foregut and midgut of *Heterorhabditis* IJs (Boemare *et al.*, 1996) [13]. Typical for the symbionts for two genera is the phenomenon of phase variation, two extremes that are the primer and the secondary phase (Akhurst, 1980) [2]. Middle phases have been accounted by Gerritsen and Smits, 1997. The primary phase is detached from the IJ or infected insects, whereas the secondary phase occurs either after *in vitro* subculturing or *in vivo*, when the EPN emigrate from the dead insects (Grunder, 1997) [50]. Infection with under 10 bacterial cells of the bacteria into the host insect haemocoel can be adequate to kill a defenceless insect, for example, *Galleria mellonella* or *Manduca sexta* L. (Forst *et al.*, 1997; Ffrench Constant and Bowen, 2000) [31, 33]. EPNs produce resistant suppressive compounds to stop the insect host immune system to protect their mutualistic bacteria. Consequently, EPNs have surface coat proteins and other surface parts to stifle the host's immune response as well as annihilate the host hemocytes (Maizels *et al.*, 2004; Li *et al.*, 2007). Different studies show that the cuticle of *S. feltiae* assists in the inactivation of the prophenoloxidase pathway which prevents the melanization interaction (Brivio *et al.*, 2002) [16]. Moreover, *S. feltiae* protects its associated bacteria by sequestering opsonization factors from the insect hemolymph resulting in reduced phagocytosis by the host hemocytes in *G. mellonella* (Brivio *et al.*, 2010) [15]. At last, EPNs are known to create incapacitating exotoxins and cytotoxic and proteolytic extracellular compounds that unfavorably influence the host and upgrades survival and

reproduction of the nematode and bacteria.
The bacterium requires nematode for protection from the outer climate, entrance into the host haemocoel. In steinernematid IJs, *Xenorhabdus* bacterial cells are housed in the foremost gastrointestinal caecum (a vesicle in the anterior part of the intestine), while in heterorhabditid IJs, *Photorhabdus* cells are found throughout in the intestinal tract. The life cycle begins, when the IJs move towards the host and enter into the insect body by mean of the mouth, anus, spiracles, or direct penetration through the cuticle. It delivers the symbiotic bacteria, which then, multiply quickly in the hemocoel. Usually the insect dies within 48 - 72 hours (Shairra, 2009). While moving through the soil, IJs of genus *Steinernema* lose their J2 cuticle; IJs of the genus *Heterorhabditis* retain the J2 cuticle until they enter a host. IJs can survive extended periods in the soil while waiting for the opportunity to infect a host.
Nematode development and reproduction rely on conditions led out in the dead insect by the bacterium. The bacterium further contributes anti-immune proteins to help the nematode in overcoming host protections, and anti-microbials that stifle colonization of the dead insect by competing secondary invaders. The life cycle of *Heterorhabditis* is like that of steinernematids with the exception of the way that the IJs generally form into self-reproducing hermaphrodites (Poinar, 1990) [84]. Strauch *et al*., (1994) [95] observed that offspring of the first-generation hermaphrodites can either form into amphimictic adults or into automictic hermaphrodite; both can happen simultaneously. The development into amphimictic adults is induced by favourable nutritional circumstances, through the development of hermaphrodites is indcited by low concentrations of supplement. The life cycle is completed in a few days and large number of new IJs emerge in searching for new hosts. The cycle from entry of IJs into a host until rise of new IJs is dependent on temperature and varies for various species and strains. It generally takes about 6-18 days at temperatures ranging from 18 to 28 °C in *G. mellonella* (Poinar, 1990; Nguyen and Smart, 1992) [84]. Alternately, the bacterium lacks invasive powers and is dependent upon the nematode to find and penetrate favorabe hosts.

Mass production of EPNs: Entomopathogenic nematode-bacteria complexes can be mass produced as biopesticides utilizing both *in vivo* (inside hosts) and *in vitro* (outside hosts) strategies respectively, (Shapiro-Ilan and Gaugler, 2002) [87].

***In vivo* culture:** *In vivo* culturing methods of EPNs have been reported by various authors (Woodring and Kaya, 1988; Flanders *et al.*, 1996; Kaya and stock, 1997; Shapiro-Ilan *et al.*, 2002a) [104, 32]. Most *in vivo* production methods are depended on the White trap system (White, 1927) [103], which is the norm for laboratory scale culturing. Thus, *in vivo* production is generally a two-layered framework depending on a progression of shelves and plates. The size of White traps as well as the amount can be expanded based on need (Shapiro-Ilan *et al.*, 2012) [92]. The methodology comprises of inoculation, harvest, concentration, and (if needed) decontamination. Insect hosts are inoculated on a dish or plate fixed with absorbent paper or another substrate helpful for nematode infection like soil or plaster of Paris. After approximately 2-5 days, infected insects are moved to the White traps; if infections are permitted to progress too long before move the opportunity of the cadaver rupturing and harm to reproductive nematode stages is expanded (Shapiro-Ilan *et al.*, 2001). White traps comprise of a dish or plate on which the cadavers rest surrounded by water, which is contained by a large arena. As IJs emerge they move to the surrounding water trap where they are collected. *In vivo* production yields differ greatly among insect hosts and nematode species. Because of high susceptibility to most nematodes, wide availability, ease in rearing, and the ability to produce high yields, the most common insect host used for laboratory and commercial EPN culture is G. *mellonella* (Woodring and Kaya, 1988) [104]. *In vivo* production yields depend on nematode dosage and host density (Zervos *et al.*, 1991; Boff *et al.*, 2000; Shapiro-Ilan *et al.*, 2002a) [108, 87, 14]. A dosage that is too low results in low host mortality and a dosage that is too high may result in failed infections due to competition with secondary invaders (Woodring and Kaya, 1988) [104]. Thus, intermediate dosages can be utilized to high yield (Boff *et al.*, 2000) [14]. Yield is generally inversely proportional to nematode size (Grewal *et al.*, 1994; Shapiro and Gaugler, 2002) [1, 87]. The choice of host species and nematode for *in vivo* production should ultimately rest on nematode yield per cost of insect and the suitability of the nematode for the pest target (Blinova and Ivanova, 1987; Shapiro-Ilan *et al.*, 2002a) [87].
Environmental factors including ideal temperature, and maintaining sufficient air circulation, and moisture can influence yield (Burman and Pye, 1980; Woodring and Kaya, 1988; Friedman, 1990; Grewal *et al.*, 1994) [104, 34, 19]. Infection effectiveness and yield can also be impacted by inoculation strategy. *In vivo* can be achieved by pipetting or showering nematodes onto a substrate, drenching of insects in a nematode suspension, or (in some cases) applying the nematodes to the insect's food. Drenching of hosts is generally more time efficient but requires more nematodes than other methods (Shapiro-Ilan *et al.*, 2002a) [87].

***In vitro* solid culture *m*ethod:** *In vitro* culturing of EPNs is based on introducing nematodes to a pure culture of their symbiont in a nutritive medium. In prior work, to create monoxenic cultures surface sterilized nematodes were added to a lawn of the bacterial symbionts (Akhurst, 1980; Wouts, 1981) [2, 105]. However, Lunau *et al*. (1993) [77] proposed that surface sterilization of IJs is lacking to lay out monxenicity because defiling bacteria survive beneath the nematode's cuticle. Thus, a superior strategy has been created where axenic nematode eggs are put on a pure culture of the symbiont (Lunau *et al.*, 1993) [77].
Solid culture was first achieved in two-dimensional arenas e.g, petri dishes, utilizing different media (Hara *et al.*, 1981; Wouts, 1981) [53, 105]. Hence, *in vitro* solid culture progressed extensively with the development of a three-dimensional rearing system including nematode culture on crumbled polyether polyurethane foam (Bedding, 1981) [6]. A liquid medium is mixed with foam, autoclaved, and afterward inoculated with bacteria followed by the nematodes. Nematodes are then harvested within 2-5 weeks (Bedding, 1981; Bedding, 1984) [6-7] by placing the foam onto sieves submerged in water. Media for this approach was initially animal product based (e.g., pork kidney or chicken offal) however was subsequently improved and may include different ingredients including peptone, yeast extract, eggs, soy flour, and lard (Han *et al.*, 1992). 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 (Gauglar and Han, 2002) [36].

Factors affecting yield for *in vitro* solid culture: Nematode inoculum rate (IJs per unit of media) can influence yield in a few nematode strains yet not others (Han *et al.*, 1992; Wang and Bedding, 1998). Culture time is inversely related to temperature and optimized for maximum yield on a species or strain basis (Dunphy and Webster, 1989; Han *et al.*, 1992) [28]. Expanding inoculum size can increase nematode growth and reduced culture time (Han *et al.*, 1992). Longer culture times can give more significant yields yet nematode mortality may also increase with time (Han *et al.*, 1992, 1993).
Media composition can have considerable effects on yield in solid culture. Expanding the lipid quantity and quality leads to increases in nematode yield (Dunphy and Webster, 1989; Han *et al.*, 1992) [28]. Lipid components mirroring the nematode's natural host composition are generally appropriate (Abu Hatab and Gaugler. 2001) [54]. Different media ingredients that might effect on nematode yield include proteins and salts (Dunphy and Webster, 1989) [28].

***In vitro* liquid culture method:** The development of monoxenic liquid culture of EPNs faces the contradicting difficulties of providing sufficient oxygen while preventing excessive shearing of nematodes (Pace *et al*., 1986; Buecher and Popiel, 1989; Friedman *et al.*, 1989) [18, 82, 34]. Generally, in liquid culture, symbiotic bacteria are first presented followed by the nematodes (Buecher and Popiel, 1989; Surrey and Davies, 1996; Strauch and Ehlers, 2000) [18, 98, 100]. Different elements for liquid culture media have been accounted for including soy flour, yeast extract, canola oil, corn oil, thistle oil, egg yolk, casein peptone, milk powder, liver extract and cholesterol (Surrey and Davies, 1996; Ehlers *et al.*, 2000; Yoo *et al.*, 2000) [100, 106]. Culture times vary depending on media and species, and might be up to three weeks (Surrey and Davies, 1996; Charvarria-Hernandez and de la Torre, 2001) [100] however numerous species can reach at greatest IJ production in about fourteen days or less (Friedman, 1990; Ehlers *et al.*, 2000; Neves *et al.*, 2001; Strauch and Ehlers, 2000; Yoo *et al.*, 2000) [34, 98, 79, 106]. Once the culture is completed, nematodes can be collected from media through centrifugation (Surrey and Davies, 1996) [100].

Factors affecting yield for *in vitro* liquid culture: Both steinernematids and heterorhabditids share the requirements of sufficient aeration (without shearing). Otherwise, the procedures for augmenting yield of the two genera in liquid culture differ because of their life cycles and reproductive biology. Steinernematids (except one species) occur only as males and females and are capable of mating in liquid culture (Strauch *et al.*, 1994) [95] accordingly, expansion of mating is paramount and can be accomplished through bioreactor design and regulation of aeration (Neves *et al.*, 2001) [79]. Maximization of mating, however, is not applicable for heterorhabditid production in liquid culture because the first generation is exclusively hermaphrodites and, albeit ensuing ages contain amphimictic forms, they cannot mate in liquid culture (Strauch *et al.*, 1994) [95]. Thus, maximizing heterorhabditid yields in liquid culture relies upon the level of recuperation. While levels of heterorhabditids recuperation *in vivo* tend to be 100% (Strauch and Ehlers, 1998) [97], recuperation in liquid culture might go from 0-85% (Ehlers *et al.*, 2000; Jessen *et al.*, 2000; Yoo *et al.*, 2000) [65, 106, 98]. Recuperation can be impacted by nutritional factors, aeration, CO_2, lipid content, and temperature (Ehlers *et al.*, 2000; Yoo *et al.*, 2000) [98, 106]. Yield from liquid culture may also be impacted by different variables including media, nematode inoculum, and nematode species (Han, 1996; Ehlers *et al.*, 2000) [51, 98]. The central component of the liquid culture media is lipid source and amount (Abu-Hatab *et al.*, 1998; Yoo *et al.*, 2000) [106]. Different supplements that have been accounted to influence yield positively include the substance of glucose (Jeffke *et al.*, 2000) and yeast extract substance (Chavarria- Hernandez and de la Torre, 2001). Generally nematode yield is inversely proportional to the size of the species (Ehlers *et al.*, 2000) [98].
Each approach has its benefits and impediments relative to production cost, specialized expertise required, economy of scale, and product quality, and each approach has the potential to be improved. Following production, a variety of formulation and application options are also available (Grewal, 2002) [72].

EPN formulation and application
EPN formulation is a process of the transformation of living entities into a product that can be applied by practical methods. Active nematodes are immobilized to prevent the depletion of their lipid and glycogen reserves. Generally, the components of the formulations are: an active ingredient, a carrier and additives. Active ingredients in the formulations are EPNs whereas the carriers used are solids, liquids, gels, and cadavers. The additives are various substances with different functions, like sponges, adsorbents, emulsifiers, surfactants, thickeners, humectants, dispersants, antimicrobials, and UV-ray protectors (Grewal 2002) [72]. The main purpose of the additives used in the formulations has been to increase the survival and maintain the virulence of the EPNs. Different formulations have been developed in order to preserve the integrity of stored EPNs, to facilitate their storage application. These formulations include activated charcoal, alginate and polyacrylamide gels, baits, clay, paste, peat, polyurethane sponge, vermiculite and water-dispersible granules. Successful storage under refrigeration conditions ranges from one-seven months relying upon the EPN species. Temperatures as low as 2-5 ˚C diminishes their metabolic activity and consequently further develop their shelf life, however, some species like Heterorhabditis indica and Steinernema riobrave do not store well at temperatures underneath 10˚C (Strauch, *et al*., 2000) [98].
Aqueous suspension: The most well-known EPN formulation is an aqueous suspension. It has been used mainly for storage, transportation, and application (Chen & Glazer 2005) [25]. Storage temperatures between 4 and 15 °C have produced survival times of 6-12 months for Steinernema spp. and 3–6 months for Heterorhabditis spp. (Hazir *et al*. 2003) [56]. However, there are many factors that affect their survival time: sedimentation, high oxygen demand, decreased response of some species at low temperatures, susceptibility to microbial contamination, special storage conditions and appropriate concentration for each species (Grewal & Peters 2005) [46]. Also, the refrigeration requirements increase costs, hinder transport (Grewal 2002) and include the utilization of application equipment with specific requirements (Toepfer *et al*., 2010; Brusselman *et al*., 2012; Lacey & Georgis, 2012; Beck *et al*., 2014; Shapiro-Ilan *et al*., 2015) [17, 101, 90, 73].

Synthetic sponges: The formulation in polyurethane sponges is achieved by applying an aqueous suspension of 500–1000 IJs/cm2, which results in an amount of 5–25 million IJs per sponge, which is subsequently placed in a plastic bag for storage. The EPNs formulated in sponges achieve a survival time of 1–3 months at 5–10 °C (Grewal, 2002) and for their release, the sponges are dipped in a bowl with water. This formulation is not appropriate for mass dissemination because it needs refrigeration for storage, the release of the EPNs is time and labour demanding and great amounts of sponge waste are generated (Cruz-Martínez, *et al*., 2017) [78]

Clay and powder

Bedding (1988) [8] encapsulated S. feltiae, Steinernema bibionis, Steinernema glaseri, and Heterorhabditis heliothidis in a hygroscopic attapulgite clay formulation with survival time of 8 weeks at 23 °C. The formulation was called a "sandwich" type because the EPNs are stored between two layers of clay. Products with this formulation were sold, but soon were discontinued due to poor storage stability, clogging of the spray nozzles, and a low nematode-clay proportion (Grewal 2002). Silver *et al*. (1995) [93] encapsulated the EPNs S. carpocapsae, S. feltiae, Steinernema scapterisci, and Steinernema riobravis in granules with diatomaceous earth, hydroxyethylcellulose, amorphous silica, fumed hydrophobic silica, lignosulfonate, starch, pregelatinised starch, and pregelled attapulgite clay achieving 90% survival after storage for 6 weeks at 25 °C. An increase of the survival time of S. carpocapsae was achieved with the dispersible granules (WG) developed by Grewal (2000a) [72] with a survival rate greater than 80% and infectivity greater than 60% after 5 months of storage at 25 °C. Recently, Matadamas-Ortiz *et al*. (2014) [78] encapsulated S. glaseri in diatomaceous earth pellets (Celite 209) employing the downward vertical flow of hygroscopic clays in converging hoppers, with storage conditions at room temperature and ambient moisture and reported 56% survival after 14.1 days. They proposed the mechanisation of the pellet production process. Finally, Cortés-Martínez *et al*. (2016) [26] evaluated the impact of moisture evaporation from the diatomaceous earth pellets on the survival time and infectivity of S. glaseri stored at room temperature and high relative humidity. Also, they evaluated a moisture transfer model by diffusion and evaporation to depict the temporal behaviour of moisture content in diatomaceous earth pellets. It was observed that deficiency of moisture content by evaporation was related to the decrease of storage stability of the IJs in diatomaceous earth pellets.

Gels: Yukawa and Pitt (1985) [107] depicted a system for nematode storage and transport. The EPNs were homogeneously blended with absorbent materials, for example, activated carbon powder, to form a cream, but this formulation has introduced the disadvantages of significant expense, unpleasant handling and low stability at room temperature (Grewal 1998) [43]. Subsequently, Bedding *et al*. (2000) developed an aggregate utilizing polyacrylamide, where the EPNs were partially desiccated, but the survival time at room temperature was low and had difficulties to dissolve (Grewal 2002) [44]. On the other hand, Georgis (1990) [38] reached a significant improvement of this formulation through the use of calcium alginate sheets disseminated on plastic screens for EPN storage. Chang and Gehert (1991) [23] encapsulated Steinernema carpocapsae in a matrix of microgels, a partially hydrogenated vegetable oil paste containing mono- and diglycerides, which essentially prolonged the feasibility of the EPNs. Alginate capsules represented an important advance in the EPN formulation. Chen and Glazer (2005) [25] encapsulated Steinernema feltiae in this material, which was exposed to osmotic treatment before formulation, with 99.8% survival after 6 months at 23 °C and 100% relative humidity. Goud *et al*. (2010) [42] encapsulated Heterorhabditis indica with different concentrations of EPNs and at different temperatures. In the research, the best combination of temperature, population density, and storage was 10 °C, up to 1000 IJs per capsule and 90 days of storage. Hussein and Abdel-Aty (2012) [62] encapsulated nematodes of Heterorhabditis bacteriophora and S. carpocapsae in calcium alginate with a survival rate higher than 50% after 40 days. In general, with alginate capsules, survival times up to 6 months at 25 °C have been reached (Chen & Glazer 2005) [25].

The efficacy of pest management and control relies upon the strategies utilized for the application of EPNs and these techniques incorporate the utilization of spray equipment and several irrigations and pumping systems (Shapiro-Ilan *et al*., 2006) [88]. Techniques and supplies used to apply specific biological control agents influence and furthermore increment the possibilities of appropriate contact between the nematode and the insect (Jagdale *et al*., 2009) [63]. They can be applied with most agronomic equipment like hand or ground sprayers, mist blowers etc. (Georgis *et al*., 1995) [39]. They can also be applied through water systems using microjet, drip, sprinklers (Cabnillas and Raulston 1996) [21]. They are usually applied at rates of 25IJs/cm2 of the treated area and the rates can either be expanded or diminished relying upon the nematode species and target pests (Shapiro-Ilan and Gaugler 2002) [1].

Table 1: Expected storability of some commercially available formulations of entomopathogenic nematodes (adapted from Grewal 1999b).

Formulation	Nematode Species	Room Temperature	Refrigerated
Liquid concentrate	*S. carpocapsae*	5–6 days	12–15 days
	S. riobravis	3–4 days	7–9 days
Sponge	*S. carpocapsae*	0	2–3 months
	H. bacteriophora	0	1–2 months
Vermiculite	*S. feltiae*	0	4–5 months
	H. megidis	0	2–3 months
Alginate gels	*S. carpocapsae*	3–4 months	6–9 months
	S. feltiae	1/2–1 month	4–5 months
Flowable gels	*S. carpocapsae*	1-1 1 /2 months	3–5 months
	S. glaseri	0	1–1 1 /2 months
Water dispersible granules	*S. carpocapsae*	4–5 months	9–12 months
	S. feltiae	1 1 /2–2 months	5–7 months
	S. riobravis	2–3 months	4–5 months
Wettable powder	*S. carpocapsae*	2 1 /2–3 1 /2 months	6–8 months

	S. feltiae	2–3 months	5–6 months
	H. megidis	2–3 months	4–5 months
Nematode wool	*H. bacteriophora*	21 days	Unknown

Compatibility of EPNs with chemicals and fertilizers

The utilization of EPNs as biocontrol agents requires information on the occurrence, species diversity, biology, environment, conveyance and insect host range of local EPN species. The application of local EPNs as insect biological control agents has a superior advantage over exotic EPNs due to the fact that local species are better adapted to local ecological conditions (Ehlers, 2001) [29]. Non-native EPNs may target helpful insect populations and may displace nearby EPNs. In addition, non-native EPNs may not be powerful against nearby insect pests. Infective juveniles of EPNs have been accounted to be able to withstand about 2-24 hours exposure to many chemical and biological insecticides, as well as fungicides, herbicides, nematicides, fertilizers and growth factors. Hence, they can be blended and applied at the time on the field offering a cost-effective approach to pest management. Alumai and Grewal, (2004) [4], noted that the concentration of chemicals to which the EPNs are blended relies upon the volume of the combination to be utilized. EPNs have been found to be compatible with various chemical herbicides, fungicides and insecticides (Koppenhofer and Grewal, 2005) [46]. Koppenhofer, *et al*., (2000) [72] noted that most EPNs are compatible with chemical pesticides with the interaction ranging from synergism to augmentation. The nematode-bacteria complexes are generally compatible with chemical fertilizers and composted manure but fresh manure on the other hand had a detrimental effect unfavourable impact on them. Conclusion: Progress in developing large-scale production and application technology has prompted to the expanded utilization of EPNs. For research centre use, limited scope field-testing, and niche markets, *in vivo* EPNs production is the appropriate strategy requiring the most un-capital expense and the minimal measure of specialized skill for a start-up but is hindered by the expenses of work and insect media. When it comes to commercial use for global business sectors, *in vitro* liquid culture is viewed as the most expense effective interaction while *in vitro* solid culture is generally viewed as moderate between *in vivo* and liquid culture. Although liquid culture offers increased cost-effectiveness compared with other production strategies, it also requests more capital investment and a more elevated level of specialized skill. Enhancement in productivity and versatility by producing the insect hosts "in-house" and mechanizing the process diminish labour, enabling *in vivo* production to play an expanded role in pest management programs. Similar to *in vivo* production, technical improvements will expand the efficiency of *in vitro* solid production, however, neither one of the methodologies might reach the scale-up potential of liquid culture technology. These nematode-bacteria complexes have emerged as great choices as biological control agents attracting worldwide interest. This is for the most part because of their wellbeing to the climate, high reproducibility, excellent host-seeking ability, quick at killing host, their safety security to non-target pests and most no instance of pest resistance and resurgence has been reported with EPNs. Recently, improvements of nematode formulation, application equipment or approaches, and strain improvement have been made to upgrade EPN application efficacy. Extra exploration toward bringing down product costs, expanding product availability, enhancing ease-of-use, and improving efficacy and carryover effect will stimulate the broad utilization of EPNs in biocontrol. With these advances, EPNs will effectively decrease chemical insecticide inputs and add to the adjustment of crop yields and the climate.

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