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BIOLOGY AND USE OF ENTOMOPATHOGENIC NEMATODES IN INSECT PESTS BIOCONTROL, A GENERIC VIEW

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ABSTRACT. The development of resistance to synthetic insecticides is one of the driving forces for changes in insect pest management. Governments regulatory bodies are in favour of environmentally safe chemicals with low toxicity, short-term persistence, and limited effects on non-target organisms as predominantly requirements for pesticides registration. Biological control can be considered as a powerful tool and one of the most important alternative control measure providing environmentally safe and sustainable plant protection. The success of biological control will depend on understanding the adaptation and establishment of applied biological control agents in agricultural ecosystems. Microbial pathogens and arthropod biocontrol agents, entomopathogenic nematodes (EPNs) have been successfully used in agricultural systems. They are highly virulent, killing their hosts quickly and can be cultured easily *in vivo* or *in vitro.* They are safe for non-target vertebrates and for the environment, and production costs have been significantly reduced in recent times as they are mass produced in liquid media. Moreover, no difficulties to apply EPNs as they are easily sprayed using standard equipment and can be combined with almost all chemical control compounds. EPNs are widely used to control economically important insect pests in different farming systems: from fruit orchards, cranberry bogs and turf grass to nurseries and greenhouses. The use of EPNs for biocontrol began only in early 1980s and involved a step-by-step scientific and technical development. Mass production of the nematodes played a key role in the commercially development of insect pests control with nematodes.

Keywords: efficacy; formation; entomopathogenic nematodes; Steinernematidae; Heterorhabditidae.

Brief history

EPNs have been well known since 1923, when Steiner (1923) identified the species *Aplectana kraussei*. Later, Glaser and Fox (1930) identified a nematode infecting grubs of the Japanese beetle, *Popillia*

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japonica at the Tavistock Golf Course near Haddonfield, New Jersey, USA. This nematode was described by Steiner as *Neoaplectana* (=*Steinernema*) *glaseri* (Rhyabditida: Steinernematidae) from Belgium as a natural pathogen of *Hoplia philanthus* (Coleoptera: Scarabaeidae) (Steiner, 1929). Later, Glaser and Fox (1930) and his colleagues propagated sufficient amounts of the species for field trials. The species was applied in the 30s in 73 different field plots to control *Popillia japonica*. A new species of entomopathogenic nematode, *Heterorhabditis bacteriophora*, was described in 1975, as a new species as well as a member of new genus, and family (Heterorhabditidae) of Rh abditida (Poinar, 1975). The family is very similar to the family Steinernematidae. In the last three decades, many EPNs have been carried out in different habitats all over the world, revealing hundreds of new isolates and many new species (Hominick, 2002). Currently, over 80 species of *Steinernema* and 20 species of *Heterorhabditis* have been described (NCBI, 2015).

Biology of EPNs

Three unique attributes of *Steinernema* and *Heterorhabditis* nematodes make them interesting model system for application in biological control. First, they form a complex nematode-bacterium mutualistic symbiosis. The bacteria are carried in the body of nematodes and released into hosts (Poinar, 1990). Second, they are insect pathogens with a very broad host spectrum that includes the majority of insect orders. Third, they can be cultured either *in vivo* or *in vitro* on a large scale. Even though the two groups of nematodes can infect, kill and emerge as a new generation from insects in a similar way, their life cycles are different.

The life cycle of the entomopathogenic nematodes (EPNs) *Steinernema* and *Heterorhabditis* is subdivided into the so-called larvae stages. The infective juvenile (IJ)/ or (dauer) represents the only stage of the nematode outside of their insect host. At this stage, the nematode is a non-feeding and soil-dwelling larvae, encased in a double cuticle with closed mouth and anus, and able to survive for long-terms in the soil. IJs of the family Heterorhabditiae use the so-called cruiser strategy to search actively in the soil for suitable insect larvae. Nematodes of the family Steinernematidae adopted the ambusher strategy, waiting passively near the soil surface for prey to cross their way.

After an insect is sensed, the nematode sheds its outer cuticle to uncover mouth and anus, enters the insect through natural openings like anus, mouth and spiracles and migrates to the insect blood cavity (Griffin and Boemare, 2005). In comparison to *Steinernema*, *Heterorhabditis* is able to penetrate directly through the thin intersegmental areas of the insect integument by using a dorsal tooth (Griffin and Boemare, 2005).

It is worth to mention that steinernematid and heterorhabditid nematodes are associated with the symbiotic bacteria *Photorhabdus* and *Xenorhabdus* (Jagdale *et al.*, 2009). The bacteria are gram-negative, with facultative anaerobic rods in the family Enterobacteriaceae, and are found within the intestine of the infective juvenile (IJ) nematode (Forst and Clarke, 2002). An IJ carries between 0 and 2000 cells of its symbiont bacterium in the anterior part of the intestine (Spiridonov *et al.*, 1991; Endo and Nickle, 1994). *Xenorhabdus* occurs naturally in a special intestinal vesicle of *Steinernema* IJs (Bird and Akhurst, 1983), while *Photorhabdus* is distributed in the foregut and midgut of *Heterorhabditis* IJs (Boemare *et al.*, 1996).

The relationship between the nematode and the symbiotic bacterium is a type of symbiosis, where both benefit from the association. The nematode provides protected shelter for the symbiotic bacteria and carries the bacteria into the host. After entering the host, the nematode penetrates through the gut wall, and regurgitates symbiotic bacteria into the insect hemocoel. Nematode and bacteria overcome the insect immune system and the host insect is killed within 48 hours post-infection (Adams and Nguyen, 2002). The bacteria break down the host tissues, and provide food sources for the nematode, which feeds and multiplies on bacterial cells and degrading host tissues. During the process, the bacteria provide the nematode, and themselves a protected niche by producing antibiotics, that suppress the competition from other microorganisms (Kondo and Ishibashi, 1986). Due to the different symbiotic bacteria associated with EPN, heterorhabditid nematodes turn the host cadaver red, purple, orange, yellow, brown or sometimes green, whereas steinernematid nematodes turn the insect cadaver tan, ochre, gray or dark gray.

The first stage after entering the insect is the so-called recovery phase (J3). Triggered by a unknown food signal, the nematodes exit the infective stage in a developmental step that is known as recovery and transform into the fourth stage (J4), causing a toxicogenesis by releasing an immunosuppressive factor, that inhibits antimicrobial peptides, produced by the insect. J4 stages nematodes develop into egg lying female or male adults in the insect cadaver and hereby run through four juvenile stages (J1 - J4) and the adult stage has up to three generations (Kaya and Gaugler, 1993). After reproduction and depletion of all nutrients, a high nematode population density triggers the nematode development into IJs again. In the case of *Steinernema*, IJs become colonized by bacteria *via* one or two founder bacterial cells. Finally, dependent on the size of the insect prey, up to several hundred thousand individuals emerge from the empty carcass.

The life cycle of *Heterorhabditis* is similar to that of Steinernematids except for the fact that the IJs always develop into self-reproducing hermaphrodites (Poinar, 1990). Strauch *et al*. (1994) observed that offspring of the first generation hermaphrodites can either develop into amphimictic adults or into automictic hermaphrodite, both can occur simultaneously. The development into amphimictic adults is induced by favourable nutritional conditions, whereas the development of hermaphrodites is induced by low concentrations of nutrient. The life cycle is completed in a few days and thousands of new IJs emerge, searching for new hosts. The cycle from entry of IJs into a host until emergence of new IJs is dependent on temperature and varies for different species and strains.

Recently, other nematode species have been shown to use pathogenic bacteria to parasitize insect hosts. Two *Oscheius* (=Heterorhabditoides) species, *O. chongmingensis* and *O. carolinensis*, and *Caenorhabditis briggsae* have been identified as potential insect pathogens by baiting soil for nematodes using insect larvae as prey, a common approach used for finding EPNs (Nguyen and Hunt, 2007). All of these have been found to associate with insect pathogenic bacteria of the genus Serratia, while *O. carolinensis* may have additional associates (Torres-Barragen *et al*., 2011). *O. chongmingensis* and *C. briggsae* require their bacterial partners to cause host death, to grow and reproduce within killed insects, and emerging dauer juveniles are associated with the vectored pathogen (Ye *et al*., 2010). On going studies suggest that these species are entomopathogenic nematodes, though their classification as entomopathogens has been contested both semantically and conceptually in the literature and scientific meetings (e.g., Nov. 2010 NemaSym NSF RCN meeting and the Jul. 2011 Society of Nematologists meeting) (Rae and Sommer, 2011; Stock *et al*., 2011).

Production and formulation

EPNs are currently mass-produced by different methods either *in vivo* or *in vitro* (Shapiro-Ilan and Gaugler, 2002). *In vivo* production is considered the most appropriate technology for growers cooperatives and for developing countries, where labor is less expensive (Gaugler and Han, 2002). In addition, it is a simple process of culturing specific EPNs in live insect hosts, which requires less capital and technical expertise. *In vivo* production system is based on the White trap (White, 1929), which take advantage of the IJ's natural migration away from host cadaver upon emergence. The most common insect host used for *in vivo* production is the last instar of the greater wax moth *Galleria melonella* (L.) (Lepidoptera: Pyralidae). Producing the greater wax moth in mass has many complications, including the production of cocoons and the extreme fragility of nematode-infected larvae. The yellow

mealworm, *Tenebrio molitor* (L.) (Coleoptera: Tenebrionidae), is an alternative host for *in vivo* nematode production, which does not produce cocoons and retains structural integrity, while infected by nematodes. Mealworms have the additional advantage of being produced commercially in large quantities in many countries around the world. Scientists of the U.S. Department of Agriculture, Agricultural Research Service have developed improved systems to rear, separate, infect, and pack mealworm for production and distribution of EPNs. The structural integrity of nematode-infected mealworm cadavers has enabled the development of mechanized methods for packing, thereby reducing labor costs. Technologies developed by ARS have been implemented in a small company in the U.S. Nematodes produced in vivo using these technologies have been proven effective against the citrus weevil (*Diaprepes abbreviatus*) and the small hive beetle (*Aethina tumida*) and may be effective against other important insect pests. Methods to produce mealworms in mass do not require the use of sophisticated technology and can be implemented in less industrialized countries. Production of biological control agents can be difficult in countries where access to technology is limited. This reduces opportunities for application of biological control strategies in developing countries. Technologies developed by ARS scientists for production of nematodes using mealworms have the potential to be implemented in such countries.

The most important requirement for successful and economically reasonable usage of EPNs in crop protection is large scale production at low cost within a short process time (Ehlers, 2001). This can only be achieved under well-defined liquid culture conditions and successful management of nematode population dynamics (Ehlers, 2001). Nowadays, EPNs are produced for commercial purposes by several companies in large liquid fermentation tanks which range from 50,000 up to 100,000 liter fermentation system (Grewal *et al*., 2005).

In vitro culturing of EPNs is based on introducing nematodes to a pure culture of their symbiont in a nutritive medium. A liquid medium is mixed with foam, autoclaved, and then inoculated with bacteria, followed by the nematodes. Nematodes are then harvested within 2-5 weeks (Bedding, 1981) by placing the foam onto sieves immersed in water. Media include various ingredients including peptone, yeast extract, eggs, soy flour, and lard (Han *et al*., 1993) Nematodes can be stored and formulated in different ways including the use of polyurethane sponge, water-dispersible granules, vermiculite, alginate gels and baits.

Formulated EPNs can be stored for 2 to 7 months depending on the nematode species and storage media and conditions. Unlike other microbial control agents (fungi, bacteria and virus) EPNs do not have

a fully dormant resting stage and they will use their limited energy during storage. The quality of the nematode product can be determined by nematode virulence and viability assays, age and the ratio of viable to non-viable nematodes (Grewal *et al*., 2005; Mahmoud *et al*., 2006).

Application methods

EPNs can be applied with nearly all agronomic or horticultural ground equipments, including pressurized sprayers, mist blowers, and electrostatic sprayers, or as aerial sprays (Georgis, 1990; Shapiro-Ilan *et al*., 2006a). The application equipment used depends on the cropping system, and in each case there are a variety of handling considerations, including volume, agitation, nozzle type, pressure and recycling time, system of environmental conditions, and spray distribution pattern (Lara *et al*., 2008).

It is important to ensure adequate agitation during application. For small plot applications, hand held equipment or back-pack sprayers may be appropriate. When nematodes are applied to larger plots, a suitable spraying apparatus, such as a boom sprayer, should be considered. Applicators could also be using other methods, such as through microjet irrigation systems, subsurface injection or baits (Wright *el al*., 2005; Lara *et al*., 2008). Various formulations for entomopathogenic nematodes may be used for applying EPNs in aqueous suspension, including activated charcoal, alginate and polyacrylamide gels, clay, peat, polyurethane sponge, vermiculite, and water dispersible granules (WDG).

Enhanced efficacy in EPN applications can be facilitated through improved formulation. Substantial progress has been made in recent years in developing EPN formulations, particularly for aboveground applications, such as mixing EPNs with a surfactant and polymer (Schroer and Ehlers, 2005). Improved efficacy may also be achieved by relying on leaf flooding with the addition of surfactants to increase leaf coverage (Williams and Walters, 2000; Head *et al.,* 2004). Additionally, *S. carpocapsae* applications for control of the lesser peach tree borer, *Synanthedon pictipes*, were greatly improved by a follow-up application of a sprayable gel, the gel is commonly used for protecting structures from fire (Shapiro-Ilan *et al*., 2010). *S. carpocapsae* caused high levels of suppression (98% efficacy in a preventative treatment) in case of the red palm weevil, *Rhynchophorus ferrugineus*, when applied in a chitosan formulation (Llacer *et al.*, 2009). Furthermore, Lacey *et al*. (2010) mentioned that when EPN applied with the sprayable fire-gel or wood flour foam as a protecting agent for controlling the codling moth in apple tree trunks, *Cydia pomonella* (L.), treatments were enhanced and improved.

In the same context, efficacy of EPN applications can also be enhanced through improved

application equipment or approaches. Despite well-established procedures, equipment used for entomopathogen application can be improved further, e.g. optimizing spray systems (e.g. nozzles, pumps, spray distribution) for enhancing pathogen survival and dispersion (Shapiro-Ilan *et al*., 2006a). Bait formulations can enhance EPN persistence and reduce the quantity of microbial agents required per unit area (Grewal *et al*., 2005); though limited thus far, conceivably, baits can be developed further for wide applications. Another novel application approach that has gained attention is delivery of EPNs in their infected host cadavers (Shapiro-Ilan *et al*., 2010b).

Another most stricking observation is the fact that application of EPNs in capsules, prepared from several compounds, including polysaccharide extracted from the algae, *Laminaria* spp. (Hiltpold *et al*., 2012) are easy to apply in the field. From these capsules entomopathogenic nematodes can easily break through, and successfully infect insect pests, such as *Diabrotica virgifera virgifera*. In addition, these nematode-filled capsules can attract insect pests in the field if they are coated with insect food stimulant or attractants (Hiltpold *et al*., 2012).

Application of cadavers may be facilitated through formulations that have been developed to protect cadavers from rupture and improve handling process (Shapiro-Ilan *et al*., 2010b), and development of mechanized equipment for field distribution (Zhu *et al*., 2011). The period of six to ten days between infection and application on soil of *Galleria mellonella* cadavers resulted in higher emergence of IJs and was thus recommended when using the cadaver application approach (Shapiro-Ilan *et al*., 2010). Lately, Deol *et al*. (2011) stated that nematodes applied in host cadavers were effective and persistent when added to bags of potting media for subsequent distribution to target pest sites.

Use of EPNs in biocontrol

Biological control

There are three strategies of biological control: classical, augmentative, and conservation control (Bale *et al*., 2008). Classical control involves importing and releasing the parasitoid or predator of an exotic pest that has become established in a new region. The parasitoid or predator is expected also to establish itself in its new environment, so that no further releases are necessary. Augmentative control can be divided into two sub categories: inundative release, i.e. the application of large numbers of the control organism against a pest, and seasonal inoculative release, in which the control organism is released once in a season and is expected to produce progeny that will continue to control the pest throughout the growing season. Conservation biocontrol refers to a whole set of measures that can be taken to favour the population build-up of indigenous natural enemies of

(native) pests (e.g. creating refuges and providing alternative food for natural enemies).

The use of EPNs in biocontrol has a long history. Early uses going back to the 1930s were geared towards classical biological control, as in the case of the introduction of *S. glaseri* to control the Japanese beetle *Popilla japonica* in the USA. EPNs re-emerged as potential biocontrol agents in the 1960s and 70s, with research mainly focusing on *Neoaplectana =(Steinernema) carpocapsae* (Pye and Burman, 1978). By the 80s, large scale production of EPN in bioreactors was being actively researched (Bedding, 1981; Gaugler, 1981). Several EPN species are now produced commercially and available in a formulation suitable for short-term storage. Since IJs can now be produced relatively cheap in large numbers, the preferred method of application is inundative, i.e. short-term application of large numbers of nematodes to create a direct impact on the pest population (Shapiro-Ilan *et al*., 2006a).

The vast majority of applied research has focused on their potential as inundatively applied to augment biological control agents (Grewal *et al*., 2005). These can be considered as good candidates for integrated pest management and sustainable agriculture due to a variety of attributes. In addition, some species can recycle and persist in the environment; they may have direct and/or indirect effects on populations of plant parasitic nematodes and plant pathogens; can play an indirect role in improving soil quality; and are compatible with a wide range of chemical and biological pesticides used in IPM programs. This paper will focus on some selected review on the successful use of EPNs in biocontrol and *Table 1* show current use of *Steinernema* and *Heterorhabditis* nematodes, as biological control organisms (Shapiro-Ilan and Gaugler, 2010) and modified by (Mahmoud 2014a; Mahmoud and Osman, 2014).

In Florida citrus groves, augmentation of EPN is considered one of the most effective ways of reducing populations of the Diaprepes root weevil, *Diaprepes abbreviatus* (L.), and growers have been applying commercially produced EPN in their groves to control root weevils for many years (Shapiro-Ilan *et al*., 2005).

The carpenter worm, *Prionoxytus robiniae*, are completely suppressed in commercial fig orchards by *Neoaplectana* (=*Steinernema*) *carpocapsae* (Lindegren and Barnette, 1982). Similarly, this nematode killed 85-90 percent of the larvae of the *Zeuzera pyrina*, a pest of fruit trees in Italy (Deseo and Docci, 1985). In addition, *Steinernema riobrave* caused 92.8 to 94.7% mortalities in *Zeuzera pyrina* larvae, when used at the concentration of 3000 IJs and 5000 IJs in the field experiment in Egypt (Shamseldean *et al*., 2009). The large scale use of *Steinernema* spp. has been developed to control wood borers in the family Sessidae. More

than 90 percent mortality of borer, *Synanthedon tipuliformis* has been obtained on currants with the application of *S. bibionis* (Miller and Bedding, 1982).

Table 1 - Current use of entomopathogenic nematodes Steinernema and Heterorhabditis nematodes as biocontrol agents of insect pests

Crops/ Targets	Insect pests		Nematodes*
	Common name	Scientific name	
Artichokes	Artichoke plume moth	*Platyptilia carduidactyla*	Sc
Vegetables	Armyworm	Lepidoptera: Noctuidae	Sc, Sf, Sr
Ornamentals	Banana moth	*Opogona sachari*	Hb, Sc
Bananas	Banana root borer	*Cosmopolites sordidus*	Sc, Sf, Sg
Turf	Billbug	*Sphenophorus* spp. (Coleoptera: Curculionidae)	Hb,Sc
Turf, vegetables	Black cutworm	*Agrotis ipsilon*	Sc
Canola	Black cutworm	*Agrotis ipsilon*	Sc, Hb
Berries, ornamentals	Black vine weevil	*Otiorhynchus sulcatus*	Hb, Hd, Hm, Hmeg, Sc, Sg
Fruit trees, ornamentals	Borer	*Synanthedon* spp. and other sesiids	Hb, Sc, Sf
Home yard, turf	Cat flea	*Ctenocephalides felis*	Sc
Citrus, ornamentals	Citrus root weevil	*Pachnaeus* spp. (Coleoptera: Curculionidae)	Sr, Hb
Pome fruit	Codling moth	*Cydia pomonella*	Sc, Sf
Canola	Diamondback moth	*Plutella xylostella*	Sc, Hb
Vegetables	Corn earworm	*Helicoverpa zea*	Sc, Sf, Sr
Vegetables	Corn rootworm	*Diabrotica* spp.	Hb, Sc
Cranberries	Cranberry girdler	*Chrysoteuchia* topiaria	Sc
Turf	Crane fly	Diptera: Tipulidae	Sc
Citrus, ornamentals	Diaprepes root weevil	*Diaprepes abbreviatus*	Hb, Sr
Mushrooms	Fungus gnat	Diptera: Sciaridae	Sf, Hb
Grapes	Grape root borer	*Vitacea polistiformis*	Hz, Hb
Iris	Iris borer	*Macronoctua onusta*	Hb, Sc
Forest plantings	Large pine weevil	*Hylobius albietis*	Hd, Sc
Vegetables, ornamentals	Leafminer	*Liriomyza* spp. (Diptera: Agromyzidae)	Sc, Sf
Turf	Mole cricket	*Scapteriscus* spp.	Sc, Sr, Sscap
Nut and fruit trees	Navel orangeworm	*Amyelois transitella*	Sc
Fruit trees	Plum curculio	*Conotrachelus nenuphar*	Sr
Stone fruit orchards	Flat-headed root borer	*Capnodis tenebrionis*	Sc, Sf
Date palm	Red palm weevil	*Rhynchophorus ferrugineus*	Sc
Turf, ornamentals	Scarab grub[3]	Coleoptera: Scarabaeidae	Hb, Sc, Sg, Ss, Hz
Ornamentals	Shore fly	*Scatella* spp.	Sc, Sf

Crops/ Targets	Insect pests		Nematodes*
	Common name	Scientific name	
Berries	Strawberry root weevil	*Otiorhynchus ovatus*	Hm
Bee hives	Small hive beetle	*Aethina tumida*	Hi, Sr
Sweet potato	Sweetpotato weevil	*Cylas formicarius*	Hb, Sc, Sf
Termites hills	Subterranean termites	*Psammotermes hypostoma*	Sc, Hb

* Nematode species are abbreviated as follows: Hb = *Heterorhabditis bacteriophora*, Hd = *H. downesi*, Hi = *H. indica*, Hm = *H. marelata*, Hmeg = *H. megidis*, Hz = *H. zealandica*, Sc = *Steinernema carpocapsae*, Sf = *S. feltiae*, Sg = *S. glaseri*, Sk = *S. kushidai*, Sr = *S. riobrave*, Sscap = *S. scapterisci*, Ss = *S. scarabaei*.

The black vine weevil, *Otiorhynchus sulcatus* (Curculionidae) is one of the most important pest species of cranberries, strawberries, and nursery ornamentals in USA, Canada and Western Europe. An average of $25–70 million is spent annually in the USA and Canada to control this pest (Shapiro-Ilan *et al*., 2006b), whereas approximately $0.5–2 million is spent yearly to protect against this insect in hardy ornamental production in the Netherlands (Van Tol and Raupp, 2006). EPNs in field experiments showed that *Heterorhabditis* species are better than *Steinernema* species in controlling the larvae (Van Tol and Raupp, 2006). However, not all *Heterorhabditis* species and strains are equally effective in spring applications, compared to fall applications (Van Tol and Raupp, 2006).

Steinernema feltiae is an efficacious and economical replacement for chemical insecticides for controlling the fungs gnats (Diptera: Sciaridae) in the Netherlands, England and Germany (Jagdale *et al*., 2004). *S. feltiae*, a cold-adapted nematode, has been successfully used to control fungus gnats at temperatures ranging from 12 to 25ºC (Jagdale *et al*., 2004). Application rates of 1.0 to 1.5 x 10^6 of *S. feltiae* IJs/m^2 provide affordable and effective control of the fungus gnats, *Lycoriella* spp., that is comparable to or better than that of insecticides, commonly used in mushroom production (Jagdale *et al*., 2004). In Egypt, *S. feltiae* showed high virulence toward second and third instar larvae of *Musca domestica*, *Stomoxys calcitans*, *Lucilia sericata* and *Calliphora vicina*; however, its virulence toward fly pupae was less pronounced, particularly in manure (Mahmoud *et al*., 2007). It is also effective against the peach fruit fly, *Bactrocera zonata*. In Egypt, S. *feltiae* Cross N 33 proved to be effective against 2nd and 3rd instar larvae of *B. zonata* in Petri-dishes lined with moist filter paper (Mahmoud and Osman, 2007).

Greenhouse tests have demonstrated the potential of using nematodes as foliar treatments against the larval stages of various leafminers (Williams and Walters, 2000; Head

and Walters, 2003). In general, to achieve reliable control, optimum spray volume is essential to allow the nematodes to come in contact with the larval stages. Maintaining high relative humidity (above 90%) in the greenhouse and/or moisture on the plants for at least 6–8 h after nematode applications is critical for successful control (Williams and Walters, 2000; Arthurs *et al*., 2004). The best control of *Liriomyza trifolii* Burgress was achieved with 2-4 weekly applications of *S. carpocapsae* or *S. feltiae* at 1×10^6 IJs/m^2 against the second and the third instars larvae (Williams and Walters, 2000; LeBeck *et al.*, 1993). In Egypt, Beet armyworm *Spodoptera exigua* (Hubner), Black cutworm *Agrotis ipsilon* (Hufnagel), Silver y moth, *Phytometra gamma* L. (Lepidoptera: Noctuidae) and Diamondback moth *Plutella xylostella* L. (Lepidoptera: Plutellidae) are serious insect pests on canola (Mahmoud and Shebl, 2014). EPN, *Steinernema carpocapsae* in greenhouse experiments was highly efficient when applied in aqueous suspension 6000 IJs/ 25 ml directed to larvae on canola leaves, causing mortality of 74%, 72% and 68% to *A. ipsilon*, *P. xylostella* and *S. exigua*, respectively (Mahmoud, 2014b).

Steinernema carpocapsae is the most commonly applied species for control of foliar and other above-ground pests. Due to its ambusher host-finding strategy, they are ideal candidates for pest insects encountered on the surface soil when they descend from foliage. Belair *et al.* (2003) demonstrated that foliar applications of *S. carpocapsae* did not provide an acceptable level of control of imported cabbageworm, *Artogeia rapae* (Lepidoptera: Pieridae), under environmental conditions in Québec. On the other hand, research on *S. carpocapsae* and *S. feltiae* demonstrated their potential for control of the leafminers (Diptera: Agromyzidae): *Liriomyza trifolii* (Tomalak *et al.,* 2005), *Liriomyza huidobrensis* (Williama and Walters, 2000) and *Tuta absoluta* (Batalla *et al.*, 2010) and other leafminer species.

Codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae), a worldwide pest of apple and other pome fruit, provides an excellent example of the successful use of EPNs in cryptic habitats. After harvest, they account for 100% of the codling moth population. Control of these larvae would result in reduced emergence of adult moths the following spring. The most evaluated species for codling moth control are *S. carpocapsae, S. feltiae, H. bacteriophora,* and *H. zealandica.* The abiotic factors that have the greatest influence on their larvicidal activity against *C. pomonella* are temperature, moisture, and type of habitat (de Waal *et al.*, 2011; Navaneethan *et al.*, 2010). Application of IJs of *S. carpocapsae* or *S. feltiae* at 2.5 x 10^6 IJs/tree or 1-2.5 x 10^9/ha under optimal conditions of temperature and moisture (20-25°C, saturated humidity) can provide up to 90% reduction of overwintering larvae (Unruh and Lacey, 2001). Applications of EPNs to apple tree

trunks for control of codling moth, were improved when the treatments included the sprayable fire-gel or wood flour foam as a protective agent.

The invasive mole cricket, *Scapteriscus vicinus* (Orthoptera: Gryllotalpidae), from South America, is a serious pest of lawn and turf in the Southern United States. Successful classical biological control of the cricket with *Steinernema scapterisci*, an EPN collected in the putative center of origin of the cricket in Uruguay, is documented by (Parkman and Smart, 1996; Parkman *et al*., 1996). The nematode was successfully established after introduction of *S. scapterisci*-infested cadavers and applications in small plots at a rate equivalent to 2 x 10^9 IJs/ha (Parkman and Smart, 1996; Parkman *et al*., 1996). In addition, *S. scapterisci* was auto-dispersed by infected mole crickets to create new foci of infection (Parkman *et al*., 1993). Due to the territoriality of *S. vicinus*, Parkman and Frank (1992) developed a unique method of treatment using sound traps to attract and infect the crickets. Three years after the initial introduction of *S. scapterisci*, mole cricket populations at release sites were reduced by up to 98%. Application of *S. scapterisci* to untreated sites and augmentative applications have been facilitated by commercial production of the nematode (Grewal *et al*., 2001).

Cutworms (Lepidoptera: Noctuidae) (*Agrotis, Amathes, Noctua, Peridroma*, *Prodenia* spp.) are leaf, bud, and stem feeders and some species feed on roots. They spend some or all of their feeding stages in contact with the soil. Many species overwinter as penultimate or last instar larvae or pupae in the soil or under fallen leaves and other debris at the soil surface. During their feeding or resting activity on the surface of the soil they are good targets for ambusher EPNs when soil moisture is sufficient for IJ survival and infectivity. Although several studies have demonstrated good control of cutworms in crops and turf (Mahmoud, 2014b; West and Vrain, 1997; Capinera *et al*., 1988; Ebssa and Koppenhofer, 2012), they are not yet implemented on a large scale.

In some Mediterranean countries, EPNs are applied through drip-irrigation in sweet peppers against soil-dwelling stages of Thrips (*Frankliniella occidentalis*) with approximately 100.000 infective juveniles (IJs) m^{-1} every two weeks in Spain (Ehlers, 2011). This foliar application also controls the tomato leaf miner, *Tuta absoluta* as EPN enter into the mines and kill the larval stages. This pest is an important one of tomato crops in South America and it has recently been introduced to the Mediterranean area including Egypt. Susceptibility of *T. absoluta* larvae and pupae to the entomopathogenic nematode (EPNs) was determined under both laboratory and field condition (Batalla *et al.,* 2010; Shamseldean *et al.*, 2014). Field efficacy of *H. bacteriophora* varied along 3 consecutive years from around 60% in years 2011 and 2012

to 80% mortality in 2013. Whereas, *S. monticolum* efficacy mounted to 58 to 61% in years 2011 and 2012, respectively, and reached a maximum of 67% in 2013 (Shamseldean *et al.*, 2014). The Flat-headed Root borer *Capnodis tenebrionis* (Linné) (Coleoptera: Buprestidae) is a major pest in Mediterranean stone fruit orchards (peaches, plums, apricots, cherries etc). It attacks roots and stem. In *Prunus* orchards, the larvae and adults of *C. tenebrionis* have been successfully controlled on > 3.000 ha in Spain by applications of 1 million IJ m^{-1} of *Steinernema carpocapsae* or *S. feltiae* in spring and autumn (Ehlers, 2011). Recently, application of nemastar® is effective against all stages including adults after pupation. It is applied through drip irrigation, drench or soil injection in April/ May and September/October, when soil humidity is high. The dose rate is 1-3 million nematodes per tree applied in at least 30 liters of water. For best results irrigate before and after application. Efficacy of nemastar has been successfully used on many thousand hectares in Spain. In field trials in apricot trees the efficacy ranged from 75 - 90%, independent from the application method. For long term suppression of the pest it is necessary to apply three years in a row (Martinez *et al.*, 2008). This application is currently introduced also into Italy and Greece. Trials in Spain against the recently introduced invasive Red Palm Weevil *Rhynchophorus ferrugineus* (Coleoptera, Curculionidae) revealed that the addition of Chitosan to the IJ suspension can significantly increase nematode efficacy. IJs of *S. carpocapsae* survive approximately one month inside the palm tree trunk. Monthly applications of 1-5 million IJs per tree are necessary to kill the larvae and protect the tree against new invasions. Installation of a tube system into the tree canopy can ease application and reduce costs of the treatment. After successful introduction of EPN plus Chitosan against the Red Palm Weevil in Spain, the method is now introduced into Greece and Italy. The combined use of EPN and Chitosan is patented in Europe by the Spanish company Idebio (Ehlers, 2011).

Compatibility of EPNs with pesticides

Since the nematodes are applied in crops that receive varying agricultural inputs, such as fertilizers and chemical products applied on the leaves; some products may reduce the survival and infectivity of these nematodes (Grewal *et al.,* 2001). In integrated pest control, selective insecticides are used together with biological control agents, and they may influence the activity of these organisms (Alves *et al.*, 1998). It has thus become very important to learn more about which insecticides help the nematodes in integrated control and, in consequence, reduce the establishment of populations with genes that confer resistance to a control agent (Hoy, 1995). Thus, it is vital to evaluate critically the

compatibility of insecticides and entomopathogenic nematodes, aiming to introduce these organisms into integrated pest management (IPM). Many insecticides, nematicides, fungicides and acaricides have been tested to determine their compatibility with EPN (Zimmerman and Cranshaw, 1990). Results from these studies are variable, depending on the type of chemical and nematode species studied Koppenhöfer and Grewal, 2005). For example, the insecticide carbaryl (1-naphthyl methylcarbamate) showed a positive compatibility with *Steinernema carpocapsae* and *Steinernema feltiae* (Das and Divakumar, 1987), which indicates that these EPN species can tolerate the exposure to carbaryl. In contrast, the same insecticide showed a negative compatibility with the EPN *Heterorhabditis bacteriophora* (Zimmerman and Cranshaw, 1990).

The combination of EPNs and other control agents has proved to be synergistic and produces higher mortality than either agent alone. For instance, Koppenhöfer and Kaya (1997) showed additive and synergistic interaction between EPNs and *Bacillus thuringiensis* for scarab grub control. Mahmoud and Pomazkov (2004) and Mahmoud (2007) stated that the combined use of botanical insecticides based on azadirachtin with the entomopathogenic nematode *S. feltiae* might offer an integrated approach to increase the efficacy of control of the peach fruit fly, *B. zonata* and the onion maggot, *Delia antiqua.* Koppenhöfer and Fuzy (2008) demonstrated a synergistic effect between the neonicotinoid insecticide, imidacloprid and EPNs. Also, Mahmoud *et al*. (2016) showed synergistic effect between Imidacloprid, Thiamethoxam, NeemAzal, Neemix and *S. carpocapsae* when applied against the Black cutworms, *Agrotis ipsilon*. Patil *et al*. (2015) mentioned that the combinations of imidacloprid and nematodes, *Heterorhabditis indica,* had a strong synergistic effect on mortality of early and late 3rd instars of coconut white grub, *Leucopholis coniophora* at different concentrations of imidacloprid. Combinations of imidacloprid and entomopathogenic nematodes may provide a powerful and economically feasible curative control in white grub management in coconut. However, Cappaert and Koppenhöfer (2003) observed antagonistic effect of a combination of between imidacloprid and *S. scarabaei* for the control of the European chafer, *Rhizotrogus majalis* (Scarabaeidae). Despite the demonstrated synergistic effect of the combined use of EPNs and other control methods, this strategy has yet to be used on a practical basis for control of scarab larvae. Incompatibility between nematodes and agrochemicals can be overcome by applying nematodes at intervals in between chemical applications, depending on the persistence of the chemical applied (Capinera *et al*., 1988).

Conclusion and future prospects

Entomopathogenic nematodes (EPNs) possess many advantages as a viable tool for pest control. They are safe on non-target organisms, but can be highly effective to their target hosts. They can be applied with standard spray equipment, are as easy to use as conventional insecticides, and are compatible with many chemical insecticides. In addition, most of these nematode agents can be mass-produced *in vitro*. Noticeably, all these advantageous characteristics have triggered the rapid development and commercialization of nematodes.

High costs to manufacturers and end-users, short shelf-life, and unstable field efficacies are among the major disadvantages that limit the development and large-scale application of nematode products. Despite the cost savings from registration exemption it is still expensive to produce and market insecticidal nematodes, given the high costs and techniques involved in mass-production and formulation, and the low market share of clientele.

Further advancements are expected in view of the current efforts for improving mass-production techniques and lowering the manufacturing costs and in developing more advanced carriers and techniques in formulation to widen the IJs shelf-life. Moreover, genetic improvement may be considered as a novel venue that would help increasing nematode performance and efficacies in the field.

Since the symbiotic bacteria *Photorhabdus* and *Xenorhabdus* are highly insecticidal against certain groups of insect pests, the potential of insecticidal toxins isolated from these bacteria as novel insecticidal proteins for insect control is also under investigation. Overall, the future use of EPNs is promising, given all the advantages they possess, as well as the increasing demand for any virulent microbial pathogen to help mitigate the environment and resistance pressure of synthetic chemical insecticides.

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