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ENTOMOPATHOGENIC NEMATODES AGAINST FOLIAR PESTS

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ABSTRACT

The Entomopathogenic nematodes (EPNs) are obligate insect parasites and are being used as a biocontrol agent against a wide range of insect pests. Although EPNs are effecting in reducing the population of soil-dwelling life stages of insects, above-ground life stages is generally not effective. EPN application is influenced by environmental parameters such as temperature, moisture, exposure to ultraviolet light, and application method. Available information on the above-ground application of entomoapthogenic nematodes is brought into context in terms of the control of insect pests.

Keywords: Entomopathogenic nematodes (EPNs), biological control, foliar insect pest, temperature, moisture, UV radiation, application technology.

1. INTRODUCTION

Insect pests cause heavy losses to agricultural and horticultural crop. To limit insect pest infestation, farmers generally apply chemical insecticides. There is increasing concern in reducing pesticide inputs because of the risk they pose to humans and the environment as well as increased resistance in pest populations. Management of insect pests by biological control is an alternative approach that results in no risk to the environment. Among the different agents of biological control, entomopathogenic nematodes (EPNs) are one of them.

The EPNs of the families Steinernematidae and Heterorhabditidae are obligate insect parasites that have been successfully used against a wide range of insect pests (Kaya and Gaugler,1993; Georgis et al.2006).These nematodes have evolved a mutualistic association with bacteria in the genera *Xenorhabdus* and *Photorhabdus*. *Xenorhabdus* is associated with *Steinernema* and confined to a specific vesicle within the intestine of the infective juveniles (Bird and Akhurst, 1983). *Photorhabdus* is associated with *Heterorhaditis* and is carried in the intestine (Silva et al.2002). Nematode locate their potential host by following insect cues (Lewis et al.2006). After IJs locate the insect, they infect the host through anus, mouth, spiracles or by penetrating the cuticle. Once IJs enter the host, they shed their outer cuticle and begin ingesting hemolymph, which triggers the release of symbionts by defecation or regurgitation .The developing nematodes then consume the bacteria and liquefied host tissues. The nematodes-bacteria complex kills the host within 24 to 72 hrs. The EPNs have advantages such as movement ability, broad host range, virulence, an ability to kill hosts quickly, high reproductive potential, easy mass rearing and application, and safety to vertebrates, plants, and other non target organisms (Kaya and Gaugler, 1993). Although EPNs offer an environmentally safe alternative to chemical insecticides, they are relatively expensive, have a shorter shelf life and their efficacy are influenced by environmental factors (Georgis et al.2006). Since EPNs are soil-dwelling organisms, most research focused on controlling the soil-dwelling life stages of insects. Foliar application of EPNs is generally not effective in reducing insect pest populations (Begley, 1990; Arthurs et al. 2004). The exposure of EPN on foliage to extreme temperature (Kaya,1977; Molyneux,1985; Grewal et al.1994; Fitters et al.2001), ultraviolet light (Gaugler and Boush,1978; Gaugler *et al*.,1992), rapid fluctuation in moisture that causes desiccation (Simons and Poinar,1973;Womersley,1990;Glazer,1992;Baur et al.1995) reduces their potential as biocontrol agents.Therefore, commercialization of entomopathogenic nematodes for foliar pests has been rare and largely unsuccessful (Begley, 1990; Baur *et al*.,1997,1997a; Grewal and Georgis, 1999). Entomopathogenic nematodes have been tested in a range of above ground habitats that include boreholes and galleries in stems or wood, leaf mines, curled leaves, and growing points of plants (Cross et al.1999).

Application technology

In order to achieve good control of foliar pests application technology has good effects. Due the widening knowledge on EPNs, applications are becoming feasible against some foliar pests (Laznik et al. 2011; Lanzoni et al. 2014). In an analysis with *S. carpocapsae*, Arthurs et al. (2004) observed that nematode treatment efficacy depended on the insect’s target habitat (bore holes > cryptic foliage > exposed foliage) and trial location (greenhouse > field). For foliar application, issues relating to formulation, application equipment, and control strategies have been combined to allow the enhancement of nematode efficacy in insect control programme.

Desiccation can be reduced by using adjuvants or antidesiccants (e.g. surfactants, wetting agents, oils) or humectants (e.g. Barricade $^{(R)}$ fire gel) in the spray solution (Baur et *al*.1997a; Noosidum et al.2016). Attempts to reduce moisture stress initially relied upon leaf flooding, together with the addition of surfactants /additives to increase leaf coverage (Head et al. 2004; De Waal et al. 2013; Van Niekerk & Malan,2014). These additives are useful when nematodes are applied to waxy and glaborous leaves (Baur et al.1995; Dito et al. 2016). Baur al.(1995,1997a) demonstrated that additives generally improved EPN persistence and efficacy, but the improvement was probably not sufficient to increase the feasibility of foliar applications of EPNs against *Plutella xylostella*. Schroer et al. (2005a) proved that a mixture of a surfactant and a polymer thickening agent in the spray suspension reduces the mobility of larvae of *P.xylostella*, and thereby increases control results with *S. carpocapsae*. The use of a wetting agent seems to be essential to spread the EPN into the growing points of plants. This can be done through injection or direct spray applications (Bedding, 1990; Unruh and Lacey, 2001;Shapiro-Ilan et al. 2006; Jung, 2008). Nickle and Shapiro (1992) demonstrated effective protection of *S.carpocapsae* from sunlight using fluorescent brightener viz., Tinopal, Ujala, Ranipal. EPNs applied to foliage must be protected from high temperature and UV radiation with evening and early morning applications (Lello et al.1996), but maintaining high humidity(>80% RH) and free water on leaf surfaces has been more difficult to achieve. Pre and post application humidity is essential for nematode movement, persistence, and infection (Odendaal et al.2016a).

Desiccation survival of IJs varies markedly between species and isolates of entomopathogenic nematodes (Glazer, 1992), and with foliar application of IJs, there is inevitable exposure to increasing evaporative and osmotically driven water loss (Piggott et al. 2000). Most nematode species do not infect hosts at temperature exceeding 32^0C and differ in desiccation and UV tolerance. Ambushers tend to use a desiccation tolerance strategy, whereas cruisers use a desiccation avoidance strategy. *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 that are encountered on the surface soil when they descend from foliage.

It is well known that EPNs are best effective in controlling younger developmental stages, as they can enter into the host much easier when successive developmental stages appear (Lebeck et al. 1993; Trdan et al. 2009).Foliar applications on the other hand, which are directed against adults and young nymphs of thrips, proved more promising (Tomalak et al. 2005). Table 1 displays an overview of the EPNs application and their success against a wide range of above - ground pests.

The quantity of infective juveniles for application in the field varies according to the crop, target insect, developmental stage of insect, formulation, and application. Research on *S.carpocapsae* and *S.feltiae* demonstrated their potential for control of the leafminer, *Liriomuza trifolii* (Hara et al.1993; Sher et al.2000), *Liriomyza huidobrensis* (Williams and Walters,2000), and *Tuta absoluta* (Batalla-Carrera et al.2010) and other leafminer species. Entomopathogenice nematodes including *S. carpocapase* and *S. feltiae* when applied at the rate of 5.3 X 10^8 nematodes/ha can cause over 64% mortality of leafminers but need at least 92% relative humidity. Less than 50% of targeted populations were controlled with high concentrations of EPNs (Somvanshi et al.2006). EPNs can only stay infective for a limited period on foliage (Brusselman et al. 2012) therefore, repeated applications are necessary to follow new generations. Application volume vary with crop, target insect, insect behavior, formulation, and plant architecture (vanTol et al.2004). Trdan et al. (2007) observed good control of western flower thrips (*Frankliniella occidentalis*) in greenhouse chrysanthemums by weekly applications of specially formulated *S. feltiae* UK76 at 2.5 billion IJs/ha with 1000 liter water and with a suitable wetting agent based on polymeric material. However, it has been demonstrated that nematodes can be applied against foliar pests such as *Spodoptera exigua* (Hübner) and *Lyriomyza* with a low-nematode rate and better placement of nematodes using polymeric formulations and adapted application equipment (Piggott et al. 2000).

Application method and equipment for entomopathogenic nematodes are as effective as that of chemical pesticides. Entomopathogenic nematode has been applied in very large numbers using a variety of application systems (Georgis, 1990). Considerations involved in the selection of an application system are volume, agitation system, pressure and recycling time, environmental conditions, and spray distribution pattern. IJs may be applied to foliage using common agrochemical equipment, including hand held pressurized sprayers, mist blowers, electrostatic or spinning disc systems and aircraft mounted atomizer sprayers. Factors such as droplet size and spray distribution are important considerations when applying conventional pesticides and biopesticides in the foliar environment (Matthews, 1992). For the application of IJs, spray volume influencing the amount of free water on the leaf surfaces is also important. The type of nozzles, flow rates and pressure are important factors in nematode delivery. The fan nozzles generally produced consistently smaller sized droplets compared with the full cone nozzles. In general, an increase in flow rate resulted in greater numbers of nematodes deposited per cm^2 (Akhtar et al. 2012). The proportion of droplets capable of holding an IJ increased with flow rates as a consequence of an increase in the number of larger sized droplets. Nematodes can withstand pressures up to 1000 psi (Dutky,1974).The IJs can withstand the shear forces associated with delivery through a range of nozzle types with openings as small as 50 microns diameter and high hydraulic pressures (2-5×10^3 kPa) without significant loss of viability(Georgis,1990). The viability and concentration of *S.feltiae* remained stable when sprayed with tips having 50 mesh (Nilson & Gripwall,1999). Based on the average size of *S.carpocapsae* IJ, it was calculated that the minimum droplet diameter needed to accommodate an IJ is about 178µm. Nematodes are generally applied at concentration in the range 10^2-10^4 IJs ml^{-1} until spray runs off the target area to ensure maximum coverage. Lello et al.(1996) reported that the higher output hydraulic nozzles deposited greater numbers of nematodes onto leaves and gave up to 98% mortality of *P.xylostella* on Chinese cabbage. Spinning disc nozzles, are not so effective as over 90% of the drops never had nematodes (Mason et al.1998a). The deposition of the nematodes on foliage is generally increased by the addition of adjuvants to the spray solution (Mason et al. 1998b).Although considerable effort has been expended in the identification of adjuvants that enhance nematode deposition, retension, and survival on foliage, improvements have been insufficient to recommend foliar application.

2. CONCLUSION

Expanded use of entomopathogenic nematodes in biological control can be brought about through the development of superior nematode species or strains that are more capable of suppressing the target pest. Increased understanding of entomopathogenic nematode biology and the target pest’s biology and ecology will facilitate more efficient and improved delivery to the target site (Piggott et al.2003). Besides, crop morphology and phenology must be considered in predicting whether nematodes are viable control candidates (Georgis et al. 2006). Other innovative system like the use of modern electronics such as Global Positioning Systems (GPS) combined with direct injection or sensor controlled delivery as well as weather forecasting (De Luca et al. 2015) may also offer opportunities for entomopathogenic nematodes. More effort needs to be devoted to exploit them as biocontrol agents in sustainable, biologically based integrated pest management programme.

Table 1: Application of entomopathogenic nematodes against above-ground insect pests.

Pest	Nematode	Reference
Apple sawfly (*Hoplocampa testudinea*) plum sawflies (*Hoplocampa minuta , H. flava*) Pear sawfly (*Hoplocampa brevis*)	*Heterorhabditis bacteriophora,* *Steinernema carpocapsae,* *S. feltiae* *S. feltiae, S. carpocapsae,* *H. bacteriophora* *H. bacteriophora, S. carpocapsae,* *S. feltiae*	Vincent & Bélair, 1992 Belair *et al.*,1998 Njezic &Ehlers , 2020 De Luca *et al.*,2015
Whitefly(*Bemisia tabaci*)	*S. feltiae*	Cuthbertson *et al.*,2003; 2007; Head *et al.*,2004; Qiu *et al.*,2008
Whitefly (*Trialeurodes vaporariorum)*	*S. feltiae; H. bacteriophora*	Laznik *et al.*,2011; Nastaran *et* al.,2015; Rezaei *et al.*,2015
Banana stem borer (*Odoiporus longicollis*)	*S. carpocapsae*	Peng & Han , 1996
Clear-wing moth borer,Seslid borer (*Synanthedon culiciformis,* *S.resplendens* *S. tipuliformis* *S. myopaaeformis* *S. pictipes*)	*H. bacteriophora, S. carpocapsae,* *S. feltiae,S.bibionis* *Neoaplectana bibionis* *H. bacteriophora, S.sp.* *S. carpocapsae*	Deseo & Miller.1985; Kaya &Brown, 1986; Shapiro-Ilan *et al.*,2009; Shapiro-Ilan *et al.*,2015, Shapiro-Ilan *et al.*,2016; 2016a Miller & Bedding ,1982 Parvizi, 2003 Shapiro-Ilan *et al.*,2010

Strawberry crown moth (*Synanthedon bibionipennis*)	*S.carpocapsae, H.bacteriophora*	Bruck *et al*.,2008
Citrus mealybug (*Planococcus citri*, *P. ficus*)	*H. bacteriophora, H. zealandica,* *S. yirgalemense*	van Niekerk & Malan, 2012; 2015 ; Le Vieux and Malan,2013
Codling moth (*Cydia pomonella*)	*S.carpocapsae, S.feltiae, S.kraussei, S.riobrave, H. zealandica, H.marelatus, H.bacteriophora,S.yirgalemense*	Kaya *et al*.,1984; Lacey & Unruh,1998; Lacey & Chauvin,1999; Unruh, & Lacey,2001; Lacey *et al*., 2005,2006; Navaneethan *et al*.,2010; Odendaal *et al*.,2016; 2016a; de Waal *et al*.,2018
Fruit flies (*Drosophila suzukii*)	*S. kraussei, H. bacteriophora,* *S. carpocapsae, S. feltiae*	Kepenekci *et al*.,2015; Garriga *et al*.,2018; Cuthbertson & Audsley 2016
Cucurbit fly(*Dacus ciliates*)	*H. bacteriophora* *S. carpocapsae*	Kamali *et al*.,2013
Goat moth (*Cossus cossus*)	*S. carpocapsae, S. weiseri*	Gumus *et al*.,2015
Litchi longhorn beetle (*Aristobia testudo*)	*S. carpocapsae*	Han *et al*.,1996
Litchi stem borer (*Arbela dea*)	*S. carpocapsae*	Saleh,2017
Navel orangeworm (*Amyelois transitella*)	*S. carpocapsae, S. feltiae*	Siegel *et al*.,2004,2006
Oriental fruit moth	*H. bacteriophora, S. carpocapsae,*	Riga *et al*.,2006;

(*Grapholita molesta* (=*Cydia molesta*))	*S. feltiae, S. rarum, S. riobrave,* *H. marelatus*	Negrisoli *et al.*,2013.
Palm borer (*Paysandisia archon*)	*S. carpocapsae*	Nardi *et al.*,2009
Plum curculio (*Conotrachelus nenuphar*)	*S. feltiae, S. riobrave ,* *S. carpocapsae*	Lacey & Georgis ,2012 Belair *et al.*,1998; Pereault *et al.*,2009
Red longicorn beetle (*Aromia bungii*)	*S. carpocapsae*	Saleh, 2017; Liu *et al.*,1997
Red palm weevil (*Rhynchophorus ferrugineus*)	*H. bacteriophora, H. ferruginophorus,* *H. indicia, S. abbasi, S. carpocapsae, S. glaseri*	Dembilio *et al.*,2010; Wakil *et al.*,2017; Yasin *et al.*,2017
Western flower thrips (*Frankliniella occidentalis*)	*S.feltiae, H. bacteriophora, H. indica,* *S. arenarium, S. bicornutum,* *S. carpocapsae, S. feltiae,* *Thripinema nicklewoodi*	Belay *et al.*,2005;Buitenhuis & Shipp,2005; North *et al.*,2006; Ebssa *et al.*,2001; Ebssa *et al.*,2004;2004a; Wardlow *et al.*,2001 Arthurs & Heinz, 2006; Trdan *et al.*,2007
artichoke plume moth (*Platyptilia carduidactyla*)	*Neoaplectana carpocapsae*	Barp & Kaya ,1984
Wheat stem sawfly (*Cephus cinctus*)	*H. indica,S.kraussei, S. feltiae*	Portman *et al.*,2016
Legume pod borer (*Helicoverpa armigera*)	*S.carpocapsae, H.indica,*	Prabhuraj *et al.*,2004; 2005; 2008; Abid *et al.*,2014; Hussain *et*

		al.,2014
Tomato leaf miner (*Tuta absoluta*)	*S.affine, S.carpocapsae, S.feltiae,* *H.bacteriophora*	Gozel & Kasap,2015 Batalla-Carrera *et al*.,2010
Cabbage worm (*Artogeia rapae*)	*S.carpocapsae*	Belair *et al*., 2003.
Onion thrips(*Thrips tabaci)*	*H.indicus, S. feltiae, S.carpocapsae, H.bacteriophora*	Al-Siyabi *et al*.,2006; Khajehali & Poorjavad, 2014
(*Thrips palmi*)	*S. feltiae*	Cuthbertson *et al*.,2007
Leaf miner(Liriomyza trifolii) South American leafminer (*Liriomyza huidobrensis,* *L.bryoniae,* *Chromatomyia syngenesiae*)	*S.carpocapsae*(All) ,*S.feltiae*(MG-14), *S.carpocapsae, S. feltiae*	Harris *et al*.,1990; Hara *et al*.,1993; Olthof &Broadbent , 1992;Broadbent & Olthof, 1995;Williams & Macdonald, 1995;Head *et al*.,2000; Williams & Walters,2000; Head & Walters, 2003
Diamondback moth (*Plutella xylostella*)	*S.carpocapsae* (All strain, PDBC,JMU), *S.riobravis,* *S.carpocapsae* BA2, *H. bacteriophora*BA1 *H.indica*(PDBC) *Steinernema* sp.95, *S.weiseri, H.indica* *Rhabditis blumi* *H. bacteriophora* HNI0100	Baur *et al*.,1995; Baur *et al*.,1997; Baur *et al*.,1997a; Schroer & Ehlers, 2005b; Schroer *et al*.,2005;Gupta *et al*.,2011 Hussein *et al*.,2015 Johnson *et al*.,2008; Park *et al*.,2012; Saenz Aponte *et al*.,2020

Brinjal shoot and fruit borer (*Leucinodes orbonalis*)	*S.carpocapsae* PDBC EN 6.11 *S.carpocapsae*	Hussaini *et al*.,2002 Visalakshy *et al*.,2009
Carob moth(*Ectomyelois ceratoniae)*	*S.feltiae*,*S.carpocapsae*,*H.bacteriophora*	Memari *et al*.,2016
Cabbage maggot(*Delia radicum*)	*H. bacteriophora* Oswego,*S.carpocapsae* NY001,*S.feltiae*,*S.riobravus*	Schroeder *et al*.,1996; Beck *et al*.,2013
Cabbage moth(*Mamestra brassicae)*	*S.carpocapsae*	Beck *et al*.,2014
Filbertworm(*Cydia latiferreana*)	*S.carpocapsae*	Chambers *et al*.,2010
Parahypopta caestrum	*S.feltiae*, *H.bacteriophora*	Salpiggidis *et al*.,2008
Oblique banded leafroller (*Choristoneura rosaceana)*	*S.carpocapsae* All strain, *S.riobrave*335, *S.feltiae* UK, *S.glaseri* 326	Belair *et al*.,1999
European corn borer (*Ostrinia nubilalis*)	*S.carpocapsae* All strain, Mexican strain, *H.bacteriophora* HP88	Ben-Yakir *et al*.,1998
Colorado potato beetle (*Leptinotarsa decemlineata)*	*H.marelata*, *S.carpocapsae*, *S.feltiae*, *H.bacteriophora*, *H.megidis*	MacVean *et al*.,1982;Nickle *et al*.,1994;Armer *et al*., 2004; Trdan *et al*.,2009; Adel &Hussein ,2010; Laznik *et al*.,2010
Corn earworm(*Heliothis zea*)	*Neoaplectana carpocapsae DD136*	Bong & Sikorowski,1983; Bong,1986
Heliothis armigera	*S. feltiae* (Mexican,Pye strain),	Glazer & Navon, 1990
Spodoptera frugiperda *Spodoptera litura*	*H. indica IBCB-n5,* *Steinernema* spIBCB-n6 *S.carpocapsae*	García *et al*.,2008. Sezhian *et al*.,1996

	H. indica,S.glaseri	Umamaheswari *et al*.,2006
web-spinning larch sawfly (*Cephalcia lariciphila)*	*S. feltiae*	Georgis & Hague, 1991
Earias insulana, *Heliothis armigera,* *Spodoptera littoralis*	*S.carpocapsae*	Glazer *et al*.,1992
pink bollworm (*Pectinophora gossypiella)*	*S.riobravis*	Gouge *et al*.,1996
Caribbean fruit fly (*Anastrepha suspense*)	*H.bacteriophora*	Heve *et al*.,2018
Paddy cutworm (*Pseudaletia separate,* *Cirphis compta*)	*Neoaplectana carpocapsae* DD-136	Israel *et al*.,1969
Carpenterworm	*S. feltiae*	Lindegren *et al*.,1981; Lindegren & Barnett 1982
Western poplar clearwing (*Paranthrene robiniae*)	*Neoaplectana carpocapsae* All strain	Kaya&Lindegren,1983
Banana weevil (*Cosmopolites sordidus*)	*S. carpocapsae* All and NC513 *S. karii,H.indica*	Treverrow *et al*.,1991; Waturu *et al*.,1998
Alfalfa snout beetle (*Otiorhynchus ligustici*)	*H.bacteriophora*'Oswego' ,'NC'	Shields *et al*.,1999
Bradysia odoriphaga *B.coprophila*	*S. feltiae* SF-SN	Jagdale *et al*.,2004; Wu *et al*.,2017
Fall webworm(*Hyphantria cunea*)	*S. feltiae*	Yamanaka *et al*.,1986

Squash vine borer, *Melittia cucurbitae*	*S. carpocapsae* All, *S. feltiae* SN, *S.riobrave* TX	Canhilal & Carner, 2006

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