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Review Article

EFFECT OF ADJUVANTS ON THE EFFICACY OF ENTOMOPATHOGENIC NEMATODES

Gitanjali Devi*
Department of Nematology, College of Agriculture, Assam Agricultural University, Jorhat, 785013, Assam, India
*Corresponding Author: Email - gitanjali.devi@aau.ac.in

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Abstract: Entomopathogenic nematodes are one of the potential biocontrol agents against a wide range of insect pest. The entomopathogenic nematodes are mostly adapted to subterranean environments. After application to the above ground parts of plants, nematodes face the risk of desiccation and/or exposure to ultraviolet radiation. In order to enhance efficacy of EPNs following aboveground applications, addition of adjuvants to the spray suspension have been recommended. This review elaborated various adjuvants and their effects on entomopathogenic nematodes.

Keywords: *Entomopathogenic nematodes(EPNs), Adjuvants, Anti-desiccants, Bioefficacy, Insect pests*

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Introduction

In animal phyla, Nematoda is living in a wide range of habitats. Among these, entomopathogenic nematodes (EPNs) are obligate parasites of a range of insect pests. They have been using as biological control agents for combating many economically important insects' pests. Entomopathogenic nematodes in the families Steinernematidae and Heterorhabditidae utilize symbiotic bacteria (Xenorhabdus for Steinernema and Photorhabdus for Heterorhabditis) to infect and kill insect hosts. Entomopathogenic nematodes augmentation results in prey suppression, reduced plant damage and positive effects on crop yield. The main attributes of using nematodes for pest control are- they possess a short life - cycle and wide host range; they are environmentally safe and can be easily multiplied. However, entomopathogenic nematodes are adapted to subterranean environments. The invasion process of different nematode species into various insect pests can differ, where it needs support to enable their mobility. When passive uptake is the main form of entrance into the host, then prolonging survival of the nematodes becomes a priority. After application, nematodes face the risk of desiccation and/or exposure to ultraviolet radiation. Infective juveniles of *S. carpocapsae* Agriotos exposed for 7 minutes to short UV radiation (254 nm) were unable to infect larvae of *G. mellonela*. Exposure to direct sunlight reduced pathogenicity from 6.9% to 94.95% at 30 and 60 minutes of exposure. Therefore, soil application of EPN should be either in early morning or after sunset. Dauer juveniles can be entrapped in a formulation that prevents them from emigrating and preserves their energy resources [1-3]. To improve EPN survival, several formulations or application techniques have been developed to ensure a satisfactory protection of the nematodes [4,5]. In order to enhance environmental persistence of EPNs following aboveground applications, addition of adjuvants, i.e., surfactants and anti-desiccants have been recommended [6 - 9]. Without adjuvants nematodes settle in the tank mix of backpack sprayers causing uneven distribution. Generally, surfactants are used as adjuvants for EPN application [10]. Surfactants are substance that increases the spreading and penetrating properties of a liquid by lowering its surface tension. Chemical adjuvants can decrease the surface tension of a liquid and increase the liquid's dispersion properties and rate of absorbance into a hydrophobic matrix [11]. Through the addition of certain additives such as gels and adjuvants, that helps partial prevention of the rapid desiccation of nematodes [12].

Use of Entomopathogenic Nematodes Against Foliar Pests

The use of entomopathogenic nematodes has been reported against various foliar pests including the *Heliothis armigera* [13], Egyptian cotton leafworm, *Spodoptera littoralis* [6], diamondback moth, *Plutella xylostella* [8,14-17], Diaprepes root weevil, *Diaprepes abbreviates* [18] , the sweet potato whitefly, *Bemisia tabaci* [7], the African white rice stem borer(*Maliarphase paratella*) [19], the vine mealybug, *Planococcus ficus* [20,21], banana weevil *Cosmopolites sordidus* [22], false codling moth of citrus (*Thaumatotibia leucotreta*)[23] and the red palm weevil (*Rhynchophorus ferrugineus*)[24].

Adjuvants Used for Formulation of Entomopathogenic Nematodes

Various formulations have been tested to enhance environmental persistence of EPNs following aboveground applications including addition of spray adjuvants (both wetting agents or surfactant and anti-desiccants). Formulations that maintains moisture and enables survival of EPN IJs until they can infect overwintering larvae would significantly improve their utility for protection of fruit crops. The impacts of adjuvant on sedimentation of dauer juveniles in the spraying suspension, runoff from the leaf surface and host invasion were demonstrated by many workers.

Para-aminobenzoic acid, an ingredient in many commercial sunscreen lotions, was found to be an effective radiation protectant for the nematode *S. carpocapsae*. This material produced a three-to-four-fold increase in the period over which nematode pathogenicity and reproduction could occur [25]. In a microplot experiment, foliage application of *S. carpocapsae* Mexican (250 IJs/ml) mixed with Folicote (6% wt/wt) resulted in a 61% reduction in the persistence of *S.littoralis* larvae on cotton plants. Damage to the foliage was reduced by 46% compared with the control. Substantial reduction (76%) of *E. insulana* larvae was achieved by application of nematode with Folicote (6% wt/wt)[6]. The fluorescent brightener Tinopal LPW is commonly used in soaps, detergents, bleaches, and fabric softeners. It absorbs ultraviolet energy and converts it to visible light. Tinopal LPW at 1% concentration appeared act as a very effective radiation protectant and provided complete protection for nematodes for up to 8 hours in the laboratory and 4 hours in full sunlight. Moreover, the material appeared to be harmless to nematode [26]. Nematode susceptibility to ultraviolet light can be overcome with tannic acid or stilbene brightener [26-28].

The combination of TX7719 with Blankophor BBH increased *S. carpocapsae* and *S.riobravis* persistence on watercress leaves and efficacy against *P.xyllostella* under field condition [14]. A reduced nematode *S. feltiae* desiccation rate at the foliar surface with a survival time of 16 hours was shown in field situations when using polymer. The placement and survival of nematodes is improved and an enhanced control of cryptic insect pests was found. Enhancing nematode survival using cross-linked polyacrylamide and the benefits of anti-desiccants combined with surfactant-polymer formulation was studied by Piggot *et al.*, [29]. With lower concentration of EPNs and better formulation like polymeric formulation and quality application equipment the foliar pests such as *Spodoptera exigua* (Hübner) and Lyriomyza can be managed. Navon *et al.*,[30] developed an edible alginate nematode gel formulation for the control of *Spodoptera littoralis* and *Helicoverpa armigera*.

Mortality of *B.tabaci* on tomato and verbena was significantly increased by addition of either Triton X-100 or Agral to the spray suspension of *S. feltiae* (10000 IJs/ml).The use of TritonX-100 raised the mortality level to 63 and 37% on tomato and verbena, respectively, while 50 and 27% mortality followed the use of Agral on the two hosts. There were no phytotoxic effects by those adjuvants [7]. Head *et al.* [7] found that the addition of either of the two surfactants, Agral® and Triton X-100®, to formulations of *S. feltiae* significantly increased the latter's efficacy against the foliage-dwelling life stages of the tobacco whitefly, *Bemicia tabaci* on tomato and verbena plants, with no adverse effects occurring on EPNs or in terms of host plant phytotoxicity. Additives were tested to improve nematode performance, *S. carpocapsae* against *P. xylostella*. TritonX-100(0.3%) caused phytotoxic effects. The addition of xanthan gum or potassium alginate resulted in a two-fold increase of insect mortality at 80% relative humidity and a five-fold increase at 60% RH. Mixtures of 0.3% xanthan or alginate with 0.3% surfactants further improved efficacy. Using a mixture of 0.3% xanthan or 0.3% alginate with 0.3% surfactant, the LT50 was reduced to <25h [15]. Additives were tested to improve nematode performance, *S. carpocapsae* against *P. xylostella*. TritonX-100(0.3%) caused phytotoxic effects. The addition of xanthan gum or potassium alginate resulted in a two-fold increase of insect mortality at 80% relative humidity and a five-fold increase at 60% RH. Mixtures of 0.3% xanthan or alginate with 0.3% surfactants further improved efficacy. Using a mixture of 0.3% xanthan or 0.3% alginate with 0.3% surfactant, the LT50 was reduced to <25h [8,15,16,31]. A mixture of 0.3% Rimulgan (surfactant) combined with 0.2-0.3% xanthan (thickener- galacto-polysaccharides, polymer) with *S. carpocapsae* provided efficacy against larvae of *Plutella xylostella* on cabbage foliage. Compared to water, the the formulation reduced larval mobility >90% at 80% relative humidity and >70% at 60% relative humidity and at the same time improved conditions for nematode invasion [16]. The effect of a wetting agent (Silwet L77 at 0.05%) or a humectants (Stockosorb at 0.2%, also known as Sta-Moist, a crosslinked potassium polyacrylate/ polyacrylamide copolymer), on the infectivity of *S. feltiae* was observed. *S. feltiae* (106 IJs/tree) were most effective for control of sentinel CM larvae cocooned in cardboard strips (80% mortality) and logs (34-47%) when combined with a wetting agent (Silwet L77) or a humectant (Stockosorb) and the trees were misted for 4h post-treatment. In the absence of post-application wetting, the addition of either adjuvant (Silwet and Stockosorb) to IJs alsoincreased larval mortality in strips, although it did not significantly improve nematode efficacy on logs. Both Silwet and Stockosorb individually improved activity of *S. feltiae* for control of cocooned CM.The combined use of wetting agent and humectant further improved efficacy of *S. feltiae* [32]. The efficacy of *S. feltiae* UK 76 at 2.5 billion infective juvenile per ha, formulated with polymeric based material with 1000 litre water showed good control of the foliar pest *Frankliniella occidentalis*. Fire retardant gel, Barricade® II, comprises a mixture of superabsorbent polymer, suspended in vegetable oil and an emulsifier and thickener. The ratio of gel to water at the time of application is 1:100. wood flour foam: 20 g hard wood fiber,6 g Celvol® A 125 (polyvinyl alcohol), 37.5 g Amioca starch (waxy corn starch), 9 g Foamcell® A-100,60 g wood flour, 653 ml deionized water. Mulched field plots treated with *S. carpocapsae* or *S. feltiae* infected *G. mellonella* ,followed by an application of wood flour foam as an anti-desiccant resulted in 56 and 86% mortality respectively of CM larvae. Aqueous suspensions of *S. carpocapsae* IJs applied to cardboard bands on apple tree trunks followed by water, fire retardant gel or wood flour foam resulted in 11,35, and 85% mortalities respectively whereas *S. feltiae* resulted in 20,19, and 97% respective mortalities of codling moth [32,33]. A significant effect was recorded for *S. feltiae* in the surfactant-polymer-formulation (SPF) (0.3% Rimulgan® and 0.3% Xanthan) supporting the results in the bark piece assay. Mortality of codling moth larvae reached to 32% at 80% RH [34]. In the field *H. bacteriophora* RS107 and *H. bacteriophora* RS57, applied with sorbitol as an adjuvant reached 70.2 and 61.1% larval mortality of Brazilian apple leafroller *Bonagotasa lubricola*, respectively [35]. Shapiro-Ilan *et al.* [5] observed 70-100% suppression of the lesser peachtree borer (*Synanthedon pictipes*) when application of aqueous suspension of *S. carpocapsae* IJs were followed by application of a sprayable fire gel Barricade® gel. Shapiro-Ilan *et al.*, [36] observed that the formulation of *S. carpocapsae* with 2% Barricade® fire gel gave control of *S. pictipes* equivalent to the application of chlorpyrifos. The requirement of a dual application reduces the ease of handling and attractiveness of the approach to growers. Ear wax remover, Oleyl-polypeptide facilitate, increase their penetration and reduce the time of penetration of *S. carpocapsae* against adults and nymphs of the mealybugs, *Ferrisia virgata* and consequently protect them from the heat and ultraviolet radiation. The efficiency of EPN increased by mixing with wax remover oleyl-polypeptide against both nymphs and adults of striped mealybug [37]. *S. carpocapsae* mixed with Super Misrona oil and Oleyl-polypeptide gave high percent reduction grape mealybug, *Planococcusficus* after the 1st, 2nd and 3rd weeks before and after pruning [38]. A viability test of *S. feltiae* and *S. carpocapsae* suspended in different solutions of adjuvants showed that all selected alcohol ethoxylates and an alkyl polysaccharide have an immobilizing effect on the selected nematode spp. Xanthan gum proved to be the only adjuvant in a broad selection, capable of delaying sedimentation of EPNs in suspension. When xanthum gum (0.3g/l) was added to the suspension, no signs of sedimentation of EPNs were noticed after 20 minutes with both *EPN* spp. [39]. The IJs of *S. carpocapsae* BA2 and *H. bacteriophora* BA1 were formulated with calcium alginate adjuvants and sprayed at rate of 2 X 104 IJs/plant against *P. xyllostella* infesting watercress causing 64.4% and 79.8% reduction respectively [40]. The infectivity of *S. carpocapsae*plus 0.25% and 0.5% Barricade® (a fire retardant gel) against third-instar *S.litura* and *P. xylostella* was evaluated by spraying 2500 IJs /15 ml/plant and assessing mortality after 72 h and mortality rate 66.0% and 61.55 respectively and significantly less leaf damage (11.0-11.1%). Barricade® could protect EPNs from rapid desiccation for at least 3h following application [41]. Suspension of nematodes, *S. feltiae* against larvae of Colorado potato beetle on potato foliage in 1% agar gel was shown to be efficacious in both laboratory and greenhouse tests for extension of the nematodes survival. The agar formulation enhanced nematode survival by providing a suitable environment thereby delaying drying and increasing the possibility for nematodes to invade their host on the foliage [2,42]. Adjuvants, such as the polyacrylate gel Barricade®, improve adhesion of spray droplets and provide a protective medium to EPNs [5,36,43]. Combination of Barricade (polymer gel; 1%) and Scanmask (*S. feltiae*) resulted in highest yield on canolaat high feeding pressure of flea beetle, *Phyllotreta cruciferae*[44]. *H.zealandica* SF41, were applied at a concentration of 50 IJs/cm^2, Zeba at 3g/L water [starch-g-poly (2-propenamide-co-2-propenoic acid)] potassium salt , Nu-Film-P® (poly-1-p-menthene, spreader/sticker) at 0.6 ml/L water for controlling diapausing codling moth, *Cydia pomonella* on pear tree trunk. The use of the super absorbent polymer Zeba formulation, with the nematodes, improved the level of control obtained at 60% and 80% RH in the laboratory and that it also enhanced the survival and infection-ability of the nematodes in the field [45]. Van Niekerk and Malan [9] performed a similar bioassay, assessing the mortality of citrus mealy bug *Planococcus citri*, post-application of *H. zealandica* and *S. yirgalemense* in suspension with distilled water, xanthan gum or Zeba®. They found that the addition of Zeba® caused a significant increase in the mortality of *P. citri*, improving the *H. zealandica* -induced mortality by 22% and the *S. yirgalemense* mortality by 27% at 80% relative humidity. Xanthan gum, at a concentration of 0.2%, was highly effective at retarding sedimentation, with 72% of the initial nematode number still in suspension after 1 h. Adding Penterra, Silwet L-77, Sunspray11N, or Syl-Tac to solutions containing EPNs *H. bacteriophora*, *S. feltiae*, and *S. riobrave* resulted in higher wheat stem sawfly, *Cephus cinctu*s larval

mortality than solutions made with water alone. Field tests showed that sprays containing *S. feltiae* added to 0.1% Penterra increased WSS mortality up to 29.1% [46]. *S. carpocapsae* in 1% protective gel caused higher host mortality 60%. UV protection provided by titanium dioxide (TD) and octyl methoxycinnamate (OMC) with 1% protective gel solution formulation resulted in higher host mortality (43 and 25%). The gel at low concentration protects EPNs and addition of TD enhanced the protective properties of the formulation [43]. The combination of adjuvants, Nu-Film-P ® (poly-1-p-menthene,spreader/sticker) and Zeba® resulted in significantly more infective juveniles (30) being deposited per 4 cm^2 leaf disc than with either the control (14.8). In a glasshouse trial, the combination of S. yirgalemense, Zeba® and Nu-Film-P® resulted 88% control of *P. ficus* on leaf discs hung on potted vines, as compared with individual treatment. This study demonstrates the potential of a combination of *S. yirgalemense* with adjuvants to give significant control of *P. ficuson* grapevine foliage, compared with using EPNs alone [47].

The compatibility of some vegetable oils with the nematode has been reported. The evaporation rate of vegetable oil is slower as compared to water and by mixing with EPN before application will improve their efficacy by delaying desiccation. Alves *et al*.,[48] prepared an Aureo® vegetable oil emulsion containing 2000 IJs *Heterorhabditis* sp.CB40. That product did not affect the viability(87.4%) or infectivity(78%) of nematodes and results were highlighted the possibility of joint use of this product and the nematode against *Hedypathes betulinus*. Neem as pure oil at the field recommended concentration (5-10ml L-1) had no effect on the viability and virulence of *S. feltiae* up to 120 h incubation and can be safely tank mixed. However, the neem formulation, Nimbecidine and neem oil when mixed with a bactericidal soap(commonly used as a surfactant with neem oil) caused 13-25% mortality of *S. feltiae*. The toxic effect was due to the soap that alone caused about 24% mortality. Neem oil, Nimbecidine, soap had no adverse effect on nematode virulence[49]. Mangoud *et al.*[20] studied the effect of different natural control agents (Biofly, NeemAzal and Super Mesrona oil) comparing with entomopathogenic nematodes on the grape mealybug, *P. ficus* and its predator *Cryptolaemus montrouzieri* (Mulsant) under laboratory conditions. Mustard oil had the highest nematicidal activity against entomopathogenic nematodes, *S. carpocapsae* GSN-1 and *H.*sp. Gyeongsan. Mustard oil at 20 ppm, 3 days after treatment resulted in 69.0% and 100% mortality of *S. carpocapsae* and *H.*sp. [50]. Essential oil like Clove, garlic, lemongrass, peppermint and cinnamon oil do possess noxious effects on beneficial nematodes like *P. hermaphrodita*, *S. feltiae* and *H. bacteriophora*, but Spearmint, Rosemary, Eucalyptus (all at 1% concentration), are less toxic to entomopathogenic nematodes, *H. bacteriophora* and *S. feltiae* [51]. Activity of *S. colombiense* when mixed with two plant-based oils (coconut and olive oils), maintained at different temperatures and times. Infective juvenile survival was higher in coconut than olive oil and water mixtures up to 7 days at 4°C. Conversely, olive oil supported higher larval mortality than coconut oil at 4 to 20°C and 14 days. Similarly, the number of days needed to kill insect larvae increased at extreme temperatures (4 and 24°C) after 14 days [52].

However, Richter and Fuxa [53] observed no effect when adding the surfactant Triton X-100 to *S. carpocapsae* applications targeting the fall armyworm, *Spodoptera frugiperda*. Similarly there was no effect of a organo-silicone surfactant (Kinetic) on pecan weevil, *Curculio caryae*, suppression with entomopathogenic nematodes *Heterorhabditis megidis* (UK211) and *Steinernema carpocapsae* (All), *S. carpocapsae* (Agriotos), *S. carpocapsae* (Mexican), and *S. riobrave* (355) [54].

Conclusion

Nematode survival mechanisms are tolerance of heat and cold, desiccation, osmotic stress, hypoxia, and energy reserves in storage or in field. The function of the formulation is not only to prolong nematode survival and rapid dispersion of IJs in spray suspension but to provide environmental conditions which enable rapid invasion of the nematodes. When assessing adjuvants for use in EPN suspensions going forward, attention must be paid to the qualities of each constituent and how they interact. The time of application to the aboveground location of insect, and nematode dosage applied (both concentration and use of consecutive applications 3-4 days apart) are important point to be considered. The adjuvants work well in some studies and not others suggests that one adjuvant will not be best for all pest/plant complexes and different adjuvants need to be screened for each pest/plant complex. It is important to continually improve options for different growing systems and insect pest behavior [4,55]. A cost/benefit analysis of the various formulation options is also needed. The development of low volume sprays application technology to optimize the cost-effectiveness of foliar treatment. Greater emphasis should be placed on the selection of nematode species/strains which are adapted to local temperatures and have a high level of efficacy against the target pest. In colder regions cold adapted species such as *S. feltiae* could be applied. It has been demonstrated that the Pye and Mexican strains of *S. carpocapsae* are more desiccation tolerant than All strain [13]. Shapiro-Ilan *et al.* [56] generated several novel hybrid and bacterial transfer strains using DD136 and the Italian strain as parent strains. The novel strains exhibited superior heat and desiccation tolerance compared with the Italian strain, but virulence levels were not compromised (virulence in the original DD136 strain was inferior). Genetically improved nematode isolates should be an option [57]. Manipulation of the habitat where the nematodes will be applied to favour IJ survival and infectivity can offer a practical solution [58]. Although manipulation of macro environment (weather) is impossible, maintaining the micro climate (substrate humidity) may give success in the control of insect pest.

Application of research: This review elaborated various adjuvants and their effects on entomopathogenic nematodes

Research Category: Nematology

Abbreviations: EPN: Entomopathogenic nematode.

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****Principal Investigator or Chairperson of research: Dr Gitanjali Devi**
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