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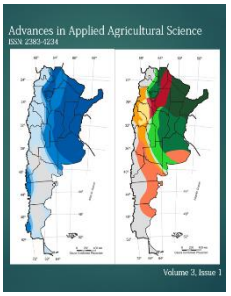

Research Article

Foliar application of entomopathogenic nematodes for controlling *Spodoptera littoralis* and *Agrotis ipsilon* (Lepidoptera: Noctuidae) on corn plants

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ABSTRACT

The cotton leaf worm *Spodoptera littoralis* and the black cut worm, *Agrotis ipsilon* (Lepidoptera: Noctuidae) are common destructive pests of many economic vegetable and field crops including corn (*Zea mays*). They attack the corn seedlings causing damage of the inner whorl leaves and cutting the plant stems above the soil surface. Both insects hide in soil from sun rays and drought or for pupation. Chemical control is used, but with low efficiency. Application of entomopathogenic nematodes (EPNs) for above ground pests is a breakthrough in the field of the bio-pesticides. This work was designed to evaluate the effect of several additives or adjuvants on the performance of two Egyptian isolates of EPNs namely, *Heterorhabditis bacteriophora* BA1 and *Steinernema carpocapsae* BA2 in laboratory, semi-field and corn field bioassays against larvae of *S. littorallis* and *A. ipsilon* infesting corn plants. Tested adjuvants had no adverse effects against nematode survival or infectivity. Some adjuvants significantly improved the performance of the tested nematodes in both semi-field and field experiments. Combinations of more than one adjuvant were more efficient than single adjuvants. Adding formulation adjuvants to sprayed nematode suspension on corn plants was more effective in case of *S. littoralis* infesting whorl corn leaves than in case of *A. ipsilon* attacking bases of corn stems.

Entomopathogenic nematodes (EPNs) are a welcome addition to the biological control arsenal (Hussein, 2004). Recently at 13% of sales of bio insecticides in industrialized countries, ENPs were already second only to *Bacillus thuringiensis* at 80% (Lisansky & Coombs, 1994). The genera *Steinernema* and *Heterorhabditis* have been successfully used to control a number of soil dwelling insect pests (Klein, 1990). In Egypt, the cotton leaf worm, *Spodoptera littoralis* Boisd (Lepidoptera: Noctuidae) and/or the black cut worm, *Agrotis ipsilon* Hufnagel (Lepidoptera: Noctuidae) were reported to be susceptible to EPNs (Saleh & Ragab, 1999; Hussein, 2004). They have also been demonstrated to control foliage and cryptic pests, although apart from a few notable exceptions laboratory and field based trials have been plagued with problems (Begley, 1990). Arthurs *et al.* (2004), analyzed data from dozens of field trials in which

EPNs were applied for control of insect pests in above ground habitats. The lowest efficacy was reported for foliar habitats.

Steinernema carpocapsae Weiser, 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. The try of Be´lair *et al*. (2003) of EPNs did not provide satisfactory results. Thanks to the advanced in the commercial production and formulation technology, EPNs are now used to apply against wide range of foliar pests (Baur *et al.*, 1997; Brusselman *et al*., 2012; Schroer & Ehlers, 2005; Beck *et al*., 2013).

Baur *et al.* (1997, 1998) successfully applied *S. carpocapsae* combined with *B. thuringiensis* on foliage of watercress (*Nasturtium officinale*) for controlling larvae of diamond back moth larvae, *Plutella xylostella* (Lepidoptera: Plutellidae). They mentioned that a polymer added to the formulation of the nematode improved its persistence and efficacy in the field. Similar results were reported by Somvanshi *et al*., 2006; Nyasani *et al*., 2008). However, Lello *et al.* (1996) reported that high output hydraulic nozzles deposited the greatest number of infective juveniles (IJs) onto foliage and produced up to 98% mortality.

Foliar application of EPNs against other pest species with EPNs has been variable (Georgis *et al*., 2006). For example, research on *S. carpocapsae* and *S. feltiae* demonstrated their potential against some other pests such as the leafminers: *Liriomyza trifolii* (Diptera: Agromyzidae) (Hara *et al.*, 1993; LeBeck *et al*., 1993; Sher*et al*., 2000; Tomalak *et al.*, 2005) *Liriomyza huidobrensis* (Diptera: Agromyzidae) (Williams & Walters, 2000); *S. littoralis* and *A. ipsilon* (Hussein, 2004; Hussein & Abdel-Aty, 2012), *Osterinia nubilalis*, *Sesamia cretica* and *Chilo simplix* (El-Wakeil & Hussein, 2009); *Pieris rapae* (Salem *et al.*, 2007) and *Tuta absoluta* (Lepidoptera: Gelechiidae) (Batalla-Carrera *et al*., 2010).

This work was designed to evaluate the effect of several additives or adjuvants on the performance of two Egyptian isolates of EPNs namely, *Heterorhabditis bacteriophora* BA1 and *Steinernema carpocapsae BA2* in laboratory, semi-field and corn field bioassays against larvae of *S. littorallis* and *A. ipsilon* infesting corn plants (*Zea mays*).

Materials and Methods

2.1. Nematodes

Two Egyptian isolates of EPNs were used in this study, *S. carpocapsae* BA2 and *H. bacteriophora* BA1(Hussein & Abou El-Soud, 2006).The two nematode species were *in vivo* produced using larvae of the greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae) (Woodring & Kaya, 1988).

2.2. Insects

Culture of the *G. mellonella* was maintained on artificial diet according to Woodring & Kaya (1988). Cultures of the black cut worm, *A. ipsilon* and the cotton leaf worm, *S. littoralis* larvae were reared on castor leaves *Ricinus communis* L. according to Hussein (2004).

2.3. Adjuvants

Several formulation adjuvants were used individually or in combinations with both tested nematodes: Tween 80, Paraffin oil, Glycerin, Calcium alginate, Carboxy methyl

cellulose and Arabic gum. They were used at the concentration of 0.1% except Glycerin (3%).

2.4. Effect of adjuvants on nematode survival and infectivity in laboratory

Suspensions of IJs of either *S. carpocapsae* or *H. bacteriophora* were prepared at a concentration of 50 IJs/ml and 50 ml of nematode suspension were put in 9 cm-diameter Petri dish. Adjuvant solutions/or suspensions were added to the dishes at 0.1% concentration and 3% concentration (glycerine). Dishes were kept in 25ºC with a light regime of approximately 16:8 (L:D) for 48h and nematode viability was recorded by counting dead and live juveniles. Each treatment was replicated 3 times. The five-on-one method adapted from Woodring & Kaya (1988) was used for test the effect of formulation adjuvants on nematode infectivity. Either nematode or adjuvant suspension was transferred in 50µL water using a micropipette to a cell-well plate of 24 cells. Cells were padded with two layers of filter paper discs. Controls consisted of water suspensions of nematodes only. A single larva of *G. mellonella* was placed in each cell. Each treatment was replicated 6 times. Plates kept at 28 ± 1°C and larval mortalities were recorded after 48h.

2.5. Semi-field experiments

To evaluate the formulation adjuvants through foliar application on the performance of *S. carpocapsae* BA2 and *H. bacteriophora* BA1 against *A. ipsilon* and *S. littoralis* infesting corn plants in pots,combinations of the tested adjuvants and the tested nematodes were prepared as follows: Tween80 0.1% only; Nematode + Tween80 0.1%; Paraffin oil 0.1% only; Nematode + Paraffin oil 0.1%; Glycerin 3% only; Nematode + Glycerin 3%; Calcium alginate 0.1% only; Nematode + Calcium alginate 0.1%; Arabic gum 0.1% only; Nematode + Arabic gum 0.1%; Arabic gum 0.1% + Tween80 0.1% + Paraffin oil 0.1%; Nematode+ Arabic gum 0.1% + Tween80 0.1% + Paraffin oil 0.1%; Carboxy methyl cellulose (CMC) 0.1% + Tween80 0.1%+ Paraffin oil 0.1%; Nematode+Carboxy methyl cellulose (CMC) 0.1%+Tween80 0.1%+Paraffin oil 0.1%and Nematode+ Calcium alginate 0.1% + Tween80 0.1% + Paraffin oil 0.1%.Controls with water and nematode only were also prepared.

Corn seeds were planted in plastic pots of surface area 314 cm² each filled with sterilized compost soil. Each pot was cultivated with 10 plants. Pots were maintained in doors at 25-28°C, with a light regime of approximately 16:8 (L:D). When plants were three weeks old, nematodes were sprayed at 2 concentrations (2.000 and 5.000 IJs/ pot) with and or without the specific adjuvant according to the experimental treatments. One hour after treatments plants were artificially infested with either 4th instar larvae of *A. ipsilon* or *S. littoralis* at a rate of10 larvae/pot. Each treatment was replicated 4 times. After 3 days of treatment, insect larvae were collected from the pots and inspected for nematode infection.

2.6. Field experiments

To evaluate the effect of formulation adjuvants on the field performance of *S. carpocapsae* BA2 and *H. bacteriophora* BA1 in controlling larvae of *S. littoralis* attacking whorl leaves and *A. ipsilon* attacking stems of corn plants, planted in new reclaimed land in Wadi El-Natron, El Beheira governorate in the north of Egypt. One month-old corn plants were artificially infested with 4th instar larvae of the black cut worm *A. ipsilon* or the cotton leaf worm *S. littoralis* at a rate of 1 larva/ 2 plants and 2 larvae/1plant, respectively. After establishing of the pest larvae on the plants and before acting of

unevaluated mortality factors, nematodes were sprayed at 5x 10^5 IJs/150 plants (about $9x10^4/m^2$ of soil surface) 2 hours before sunset using an 8L-pressure sprayer (Giardino$^{(R)}$ Italiano). A formulation of 0.1% Tween 80, 0.1% Corn oil and 0.1% Carboxy-methyl-cellulose (CMC) was added to the nematode suspension to increase its dispersion and adhesion to corn leaves. Each treatment consisted of 3 replicates. Control plots received only water. Next day after application plants and soil were inspected and larvae were collected and kept individually in plastic cups with pieces of corn plants for 72 hours. Percentages of larval mortality were recorded. The spray was directed at the bases of plants and soil around them. Experimental plots were distributed in completely randomized design. Experiments were conducted during February when air temperature was 18-24 ^oC and relative humidity was approximately 65%.

2.7. Statistical analyses

A one-way ANOVA test was used for comparisons at P>0.05.Larval mortality percents were corrected for the control mortality according to Abbott's formula (Abbott 1925).

Results

3.1. Effect of adjuvants on nematode survival and infectivity in laboratory

No significant adverse effects were noticed on nematode survival when they were exposed to the tested formulation adjuvants for 48h. At the end of exposure time, survival of IJs of either *S. carpocapsae* BA2 or *H. bacteriophora* BA1was between 100 and 93.5%. Most of tested adjuvants had no adverse effect against EPNs infectivity except the glycerin which reduced *G. mellonella* larval mortality for both tested nematodes from 83.3 to 50% for *S. carpocapsae* and from 100 to 50% for *H. bacteriophora* (Table 1).

3.2. Semi-field experiments

3.2.1. Effect of formulation adjuvants on nematode performance against S. littoralis

Through the data obtained, it is possible to verify that tested adjuvants induced significant improvement in performance of *S. carpocapsae* BA2 against 4th instar larvae of *S. littoralis* infesting whorl leaves of corn plants in pots (Table 2). The nematode suspension alone (Cn) caused 59.5% mortality in pest larvae within 72 h. Several single adjuvants (T1, T2 and T4) did not significantly add to the nematode effect. Other additives (T3 and T5) caused an adverse effect on the nematode performance and decreased the larval mortality to 41.67 and 33.61%, respectively. The positive significant effect on the nematode performance resulted from T6, T7 and T8 (combinations of surfactants, oils and polymers) which enhanced the mortality of *S. littoralis* larvae to 85.83, 72.02 and 66.67%, respectively. The treatment T7 (CMC 0.1%+ Tween 80 0.1% + paraffin oil 0.1%) was selected for field application experiments because it composed of available and relatively cheaper constituents. *Heterorhabditis bacteriophora* BA1 that sprayed against larvae of *S. littoralis* on corn plants in pots without any additives (Cn) caused 20.03% larval mortality. The treatments T1, T2, T7 and T8 significantly improved the nematode performance. The highest effect was induced by T8 (calcium alginate 0.1%+ tween 80 0.1% + paraffin oil 0.1%) which increased the nematode effect from 20.03 to 45.49% mortality in the pest larvae. T1 and T2 treatments increased the nematode effect to

Table 1. *Effect of some adjuvants on survival of infective juveniles of Steinernema carpocapsae BA2 and Heterorhabditis bacteriophora BA1and their infectivity to larvae of Galleria mellonella.*

Adjuvant	Code	% Nematode survival		% Infectivity to larvae of *Galleria mellonella*	
		Steinernema carpocapsae BA2±SE*	*Heterorhabditis bacteriophora* BA1±SE*	*Steinernema carpocapsae* BA2	*Heterorhabditis bacteriophora* BA1
Tween	**T1**	100	97.43±2.57	100	83.3
Paraffin	**T2**	97.23±1.38	99.33±0.87	83.3	83.3
Glycerin	**T3**	96.00±1.15	93.90±0.20	50	50
Alginate	**T4**	97.27±1.36	96.17±2.17	100	83.3
Gum	**T5**	97.27±1.37	95.37±0.68	100	66.6
Tween+ Paraffin+ Alginate	**T6**	95.33±0.67	96.80±1.68	83.3	100
Tween+ Paraffin+ CMC	**T7**	96.67±3.33	96.87±1.62	100	83.3
Tween +Paraffin+Gum	**T8**	93.50±3.25	96.17±2.17	83.3	100
Control	**C**	100	97.87±3.13	100	83.3

*SE: standard error

38.19 and 38.33%, respectively. The treatments T3, T4, T5 and T6 however, did not add significantly to the nematode performance through these semi-field experiments (Table 2). It was remarked that combinations of adjuvants (surfactant + polymer + oil) improved nematode performance more than did the individual adjuvants. *S. carpocapsae* was always higher in its efficacy against *S. littoralis* larvae than *H. bacteriophora.* Treatments T6 (for *S. carpocapsae* only), T7 and T8 added significantly to the efficacy of both nematodes against the pest larvae. T7 was chosen for field experiments for both nematodes.

3.2.2. Effect of formulation adjuvants on nematode performance against A. ipsilon

The nematode suspension alone (Cn) caused a very high mortality to the 4th instar *A. ipsilon* larvae (93.33%). None of adjuvant formulations used had significant additional effect on the nematode performance against this particular pest. Because *A. ipsilon* starting from the 4th larval instar feeds of plant stems at the soil surface, it received the maximum effect of nematodes without need to additional adjuvants. Similarly, *H. bacteriophora* BA1 applied at a rate of 2000 IJs/pot without adjuvants formulations induced 75% mortality in *A. ipsilon* larvae (Table 3). None of the tested adjuvants, either alone or

Table 2. *Mortality in larvae of Spodoptera littoralis infesting corn plants in pots after spraying Steinernema carpocapsae BA2 or Heterorhabditis bacteriophora BA1with different formulation adjuvants.*

Treatment	Code	% Larval mortality (Mean±SE) in *Spodoptera littoralis*	
		Steinernema carpocapsae BA2	*Heterorhabditis bacteriophora* BA1
Nematodeonly	**Cn**	59.5 c ±5.09	20.03d±4.71
Nematode + tween80 0.1%	**T1**	65.18c±4.70	38.19b ±5.60
Nematode + paraffinoil 0.1%	**T2**	60.95 c ±2.02	38.33b ±4.41
Nematode + Glycerine 3%	**T3**	41.67 d±6.31	20.63 d±4.51
Nematode +Calciumalginate 0.1%	**T4**	62.95 c±7.19	20.00 d±4.03
Nematode + Arabicgum 0.1%	**T5**	33.61 e±5.45	16.46 d±5.66
Nematode+ Arabic gum 0.1% + Tween80 0.1% + paraffin oil 0.1%	**T6**	85.83 a±4.79	23.61cd±2.19
Nematode+ Carboxy methyl cellulose (CMC) 0.1% + Tween80 0.1%+ paraffin oil 0.1%	**T7**	72.02 b ±3.93	27.22c±3.56
Nematode+ Calcium alginate 0.1% + Tween80 0.1% + paraffin oil 0.1%	**T8**	66.67 b±3.37	45.49 a±5.88
F		9.66818	4.9466
P		$3.2E^{-01}$	0.000766
dF		8	8
LSD		7.1	6.6

in combinations significantly added to the nematode effect.

3.3. Field experiments

For *S. littoralis* infesting corn whorl leaves, the effect of adding formulation adjuvants to nematode suspensions resulted in significant improvement in performance of both nematode species against the pest larvae. Formulation adjuvants increased the effect of *S. carpocapsae* BA2 from 89.13 to 100% larval mortality and increased the effect of *H. bacteriophora* BA1 from 23.94 to 33.57% larval mortality (Table 4). For *A. ipsilon* larvae feeding at the soil surface and confined mostly under the soil, the efficacy of *S. carpocapsae* BA2 against the pest larvae was 96.66% therefore, the formulation adjuvant did not add significantly to the nematode effect. Nevertheless the formulation adjuvant increased the field performance of *H. bacteriophora* BA1 from 68.69% to 83.81% (Table 4). The field performance of the Egyptian hetrorhabditid nematode looked weaker during February cold weather than the Steinernematid did. Air temperature was 18-24 ºC during the experimental work. In Elsaadawy and Saleh (1999), native steinernematids were more suitable for lower temperatures than heterorhabditids.

Table 3. *Mortality in 4th instar larvae of Agrotis ipsilon infesting corn plants in pots after spraying Steinernema carpocapsae BA2 or Heterorhabditis bacteriophora BA1 with different formulation adjuvants.*

Treatments	Code	% Larval mortality	
		Steinernema carpocapsae BA2	*Heterorhabditis bacteriophora* BA1
Nematodeonly	**Cn**	93.33 ±6.6	75 ±6.84
Nematode + Tween80 0.1%	**T1**	100	71.42 ±8.25
Nematode + Paraffinoil 0.1%	**T2**	93.33±6.6	77.97±3.81
Nematode + Glycerin 3%	**T3**	87.5± 7.2	67.85±3.57
Nematode + Calciumalginate 0.1%	**T4**	88.88±11.11	85.71±5.83
Nematode+ Calcium alginate 0.1% + Tween80 0.1% + Paraffin oil 0.1%	**T5**	93.33±6.6	82.14 ±6.84
Nematode+ Carboxy methyl cellulose (CMC) 0.1% + Tween80 0.1%+ Paraffin oil 0.1%	**T6**	100	85.71±5.83
F		0.6428	1.322
P		0.69515	0.2907
dF		6	6

Table 4: *Mortality in larvae of Spodoptera littoralis and Agrotis ipsilon infesting corn plants in the field after treatment with Steinernema carpocapsae BA2 and Heterorhabditis bacteriophora BA1 with or without formulation adjuvant.*

Treatment	% Larval mortality (Mean± SE)	
	Spodoptera littoralis	*Agrotis ipsilon*
Steinernema carpocapsae+adjuvant*	100 [a]	100 [a]
Steinernema carpocapsae	89.13 [b] ± 2.11	96.66 a ±3.33
Heterorhabditis bacteriophora + adjuvant*	33.57 [c] ± 5.75	83.81 b ±8.46
Heterorhabditis bacteriophora	23.94 [d] ± 2.68	68.69 c ±4.28
F	132.18	8.006
P	$13.7E^{-07}$	0.00857
dF	3	3
LSD	5.88	7.9

Discussion

Previous studies of Hara & Kaya (1982) and Rovesti & Deseo (1990) reported the tolerance of the entomopathogenic nematodes to agricultural agjuvants or even insecticides. Our results also showed no adverse effect against survival of nematode IJs and only one substance affected negatively the nematode infectivity to larvae of *G. mellonella* in the laboratory. This insures the tolerance of IJs to agricultural adjuvants or even insecticides. Laboratory testing of substances that intended to be mixed with nematode formulations was necessary before field application.The double layered integument as well as the non-feeding nature of the nematode IJs may protect them against probable harmful effects of these substances (Hara & Kaya (1982) and Rovesti & Deseo (1990). The semi-field experiments showed that most of tested adjuvants significantly improved the performance of both nematodes against the cotton leaf worm, *S. littoralis* while their effect was insignificant against the black cut worm, *A. ipsilon* (Table 2). In the case of *S. littoralis* infesting corn whorl leaves, the effect of nematodes alone was relatively weak in such air environment so that the additives were essential for improvement nematode performance against the pest larvae. However, in case of *A. ipsilon* attacking corn stems at the soil surface, the effect of nematodes alone was relatively higher and the formulation adjuvants did not add significantly to the nematode performance. This could be interpreted as the EPNs activity is usually the best in confined habitats, like the soil, due to the protection against rapid desiccation and death caused by air environment and sun rays (Beggley, 1990; Zervos et al., 1991; Grewal et al., 1994; Arthurs et al., 2004).

Semi-field results also showed that combination of thickener, surfactant and oil, T7 for example, (Carboxy methyl cellulose + tween 80 + paraffin oil) improved performance of both nematode isolates against both foliar pests more than did any of single additive (Table 2). In agreement with this result, Mason *et al.*(1998) recommended the use of combinations of surfactants and polymers for improving the nematode effect on plant foliage. They mentioned that surfactants improve nematode deposition on leaves. Schroer & Ehlers (2005) reported also that surfactant alone did not enhance the efficacy of the Egyptian nematode isolate *S. carpocapsae* S2 against larvae of diamond back moth *P. xylostella* infesting cabbage leaves and the addition of polymers seemed to be the key factor for improvement of EPNs efficacy.

Field results again insured semi-field results that formulation adjuvants were more useful against *S. littoralis* larvae infesting whorl corn leaves rather than *A. ipsilon* larvae attacking corn stems at the soil surface. The results agreed with the findings of several authors (Mason *et al.,* 1998; Schroer & Ehlers, 2005; Shapiro-Ilan *et al.,* 2012) who stated that formulation adjuvants were useful against foliar pests than against soil pests. The noted lower effect of the heterorhabditid Egyptian nematode isolate against *S. littoralis* larvae in whorl corn leaves was due to the low temperature during February, when the experiment was carried out. Elsaadawy and Saleh (1999) mentioned that Egyptian steinernematids were more suitable for field application at low temperatures than heterorhabditids.

In the present study, *S. carpocapsae* BA2 was more efficacies (with or without adjuvants) against 4th instar larvae of both *S. littoralis* and *A. ipsilon* in semi-field (Table 3) and field (Table 4) trials than *H. bacteriophora* BA1. Reviewing many literature citations on the effects

of steinernematid and/or heterorhabditid nematodes against noctuid larvae it was found that the nematode efficacy depended on many variables including: the nematode species, strain and dose, the insect species and stage, and other environmental variables (El-Kifl & Amin 1991; El-Magraby *et al.*, 1991; Hussein, 2004).

This work adds evidence that several formulation adjuvants are useful in improving field performance of EPNs in controlling the larvae of the cotton leaf worm *S. littoralis* infesting leaves of corn seedlings while there is no need for adjuvants in case of controlling larvae of the black cut worm *A. ipsilon* attacking corn stems at the soil surface.

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