Egyptian Journal of Biological Pest Control, 25(2), 2015, 355-358

Evaluation of Entomopathogenic Nematode, *Steirnernema carpocapsae* against the German Cockroach, *Blattella germanica* (L.) under Laboratory Conditions

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(Received: June 24, 2015 and Accepted: August 28, 2015)

ABSTRACT

Virulence of the entomopathogenic nematode, *Steinernema carpocapsae* (All strain) against adults and nymphs of the German cockroach *Blattella germanica* (L.) was studied in small glass chamber cage under laboratory conditions. Three methods of applications were tested with infective juveniles (IJs) of the nematode; Banana bait and cat food, each was mixed with attapulgite clay at ratio 3:7 (w: w) and mixed with the tested infective juveniles concentration, the 3rd treatment was by spraing the infective juveniles suspension in the cage. In every treatment, four concentrations of *S. carpocapsae* (250, 500, 1000 and 1500 IJs/ml) were applied for 20 adults and 20 nymphs. Results showed that the highest mortality percentage (90 and 80%) was recorded at banana bait and cat food bait mixed with IJs at the concentration of 1500 IJs/ml for adult stage. Spray method gave highest mortality (100%) for nymph stage. LC_{50} values for adult, nymph and both stages after 72h of exposure to the nematode infective juveniles were: 345.421, 478.621 and 403.536 IJs/ml, respectively for banana bait. Respective values for spray methods were 300.792, 246.310 and 266.357 IJs/ml. As for cat food bait methods they were 501.036, 727.761 and 599.677 IJs/ml, respectively. Results of toxicity index for cat food bait with spray method at value of LC_{50} against adult, nymph and both stages showed that the highest toxicity index values (toxicity index >1) were recorded in case of cat food by spray method (1.666, 2.955 and 2.251) for adult, nymph and both stages, respectively. On the other hand, the lowest values of toxicity index (toxicity index <1) were recorded in case of spray to cat food bait method (0.600, 0.338 and 0.444) for adult, nymph and both stages, respectively. This proved the relative high toxicity of infective juveniles spray application compared to mixing with cat food bait.

Key words: German cockroach, *Blattella germanica*, Entomopathogenic nematodes, *Steinernema carpocapsae,* Control.

INTRODUCTION

The German cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae) is one of the most predominant urban pests in the world. This species is known to transmit a vast array of pathogenic organisms, also it can contaminate food with bedrail diseases that result in food poisoning (Chanbang, 1997). For the control of cockroaches, boric acid and chemical insecticides have been studied extensively (Appel, 2003 and Wang and Bennett, 2006). However, cockroach resistance has been reported to some insecticides such as; bandiocarb, cypermethrin, permethrin, propoxur, chropyrifos and fipronil gel (Valles and Yu, 1996; Pridgeon *et al.,* 2002 and Srinivasan *et al.,* 2005). Potential microbial biological control agents of cockroaches include fungi that belong to the genera *Metarhizium*, *Paeclomyces*, *Verticillium* and *Aspergillus* (Pathak and Kulshrestha, 1998). Also, *Bacillus thuringiensis* showed cockroach mortality (Payne *et al.,* 1994). Entomopathogenic nematodes (EPNs); Steinernematidae and Heterohabditidae are commonly used as biological control agents of insects in cryptic habitats. Appel *et al.* (1993) and Nguyen and Smart (1996) evaluated the efficacy of steinernematid EPNs for controlling the German cockroach. The infective Juvenile (IJ) stage of nematodes of the genus *Steinernema* harbors symbiotic bacteria of the genus *Xenorhabdus* (Burnell and Stock, 2000 and Forst and Clarke, 2002). When these IJs penetrate the natural openings of the host (mouth, spiracles and anus) or directly cross the cuticle and reach the hemocoel, bacteria are liberated, multiply rapidly and kill the host by septicemia within 24 – 48 h (Adams and Nguyen, 2002). El-Kady *et al.* (2014) studied the pathogenicity of EPN, *Steinernema carpocapsae* against the German cockroach, *B. germanica* under laboratory conditions using two methods; filter paper and nematode bait at different concentrations of IJs.

Depending on the previous results, goal of the preset work was to study the efficacy of an application method of *S. carpocapseae* on its pathogenicity as a biocontrol agent against German cockroach using different application methods in small glass chambers as field model under laboratory conditions.

MATERIALS AND METHODS

The entomopathogenic nematode *S. carpocapseae* was maintained in laboratory on wax moth larvae (*Galleria mellonella* L.). The German cockroach *B. germanica* was reared in Plant Protection Department, Suez Canal University, Ismailia as described by El Kady *et al.* (2014).

Evaluation of the nematode

A black glass cage (60×30×40 cm) closed by muslin cloth was used as a kitchen model for this investigation.

Application methods

Three application methods were tested: 1) A bait of dry cat food; 2) a bait of dry banana (each of them was mixed with attapulgite clay at ratio 3:7 (w: w) a total of 10 gm/bait in small cups (Maketon *et al.*, 2010) and 3) spray of the nematode IJs in the cage before introducing the tested cockroach stages.

Bioassay

Virulence of *S. carpocapseae* against different stages of the cockroach (20 adults and 20 nymphs) was tested by applying several concentrations of IJs (250, 500, 1000 and 1500 IJs/ml) in the kitchen model. Each Petri-dish concentration was mixed with the bait in a Petri dish (Petri dish containing water served as control). The glass cage was kept in the laboratory at 25±2°C and 60–65% RH. Mortality rates were recorded daily for 72h. Each concentration was repeated three times.

Statistical analysis

Standard probit analysis (SAS Institute 2002) was used to calculate different LC values of *S. carpocapseae*. Toxicity index values were also calculated as LC_{50} value of row/LC_{50} of Coolum.

RESULTS AND DISCUSSION

Percentages of mortality of German cockroach after 72h of exposure to different concentrations of (IJs) of *S. carpocapseae* were shown in table (1). Highest mortality percentages (90 and 80%) were recorded in adult stage when allowed to feed on both banana and cat food baits mixed with the nematode at the concentration of 1500 IJs/ml. Similarly highest mortality percentages for nymphs were 100% when sprayed with nematodes at the same concentration of IJs.

The study of Manwieler *et al.* (1993) showed that applying *S. carpocapceae* at a concentration of 50 IJs /cm^2 for killing all the tested adult cockroaches after 3 days of exposure at 25°C. Also, Maketon *et al.* (2010) found that *S. carpocapceae* at a concentration of 1×10^6 IJs/ml caused 86.7±4.7% mortality of the German cockroach when applied mixed with cat food and attapulgate clay at ratio of 3:7 (w:w).

The LC_{50} values of *S. carpocapceae* and toxicity parameters are presented in tables (2 & 3). LC_{50} values of the tested *S. carpocapceae* for adult, nymphs and both of the two stages of the cockroach were calculated 72h post exposure. The values were 345.421, 478.621 and 403.536 IJs/ml when applied with banana bait, 501.036, 727.761 and 599.677 IJs/ml with cat food bait and 300.792, 246.310 and 266.357 IJs/ml when applied as spray (Table 2). Moderate values of slope and X^2 were recorded. Lowest LC_{50} values of IJs recorded when applied as spray indicated that both cockroach stages were more susceptible to the spray method than the bait.

Toxicity index was in table (3) showed that the highest toxicity index values (Toxicity index > 1) were recorded between cat food and spray methods; 1.666, 2.955 and 2.251 for adult, nymph and both stages, respectively . On the other hand, the lowest values of toxicity index (Toxicity index < 1) were recorded between spray method and cat food bait method; 0.600, 0.338 and 0.444, respectively. These results clearly indicated that spray method was more effective than other methods used against both stages of the cockroach (Toxicity index > 1). Morover, banana bait method was more effective than cat food bait method (Toxicity index < 1).

Table (1): Mortality percentages of *Blattella germanica* population exposed to different concentrations of entomopathogenic nematode *S. carpoapceae* using different application methods at 25°C

Cockroach stage	• Mortality % of German cockroach at different concentrations of IJs			
	250	500	1000	1500
Banana bait				
Adult	7 (35%)	14 (70%)	16 (80%)	18 (90%)
Nymph	6 (30%)	11 (55%)	13 (65%)	17 (85%)
Both	13 (32.5%)	25 (12.5%)	29 (72.5%)	35 (87.5%)
Spray methods				
Adult	8 (40%)	15 (75%)	17 (85%)	19 (95%)
Nymph	10 (50%)	17 (85%)	19 (95%)	20 (100%)
Both	18 (45%)	32 (80%)	36 (90%)	39 (97.5%)
Cat food				
Adult	4 (20%)	12 (60%)	15 (75%)	16 (80%)
Nymph	3 (15%)	9 (45%)	12 (60%)	14 (70%)
Both	7 (17.5%)	21 (52.5%)	27 (67.5%)	30 (75%)

• Number cockroach tested; 20 nymph – 20 adult (M&F)

Table (2): Toxicity parameters of *S. carpocapceae* against different stages of German cockroach at different application methods

Toxicity parameter Methods/stage	LC_{20}	LC_{50}	LC_{90}	Slope	Alpha $_{0.05}$ CHI^2
1-Banana bait					
Adult	133.458 (57.78- 308.24)	345.421 (223.37-534.31)	1464.83 (801.46-2677.28)	3.068	0.66<6.00
Nymph	126.329 (69.64-378.37)	478.621 (319.73-716.48)	2474.017 (1048.64-5836.87)	3.577	0.73<6.00
Both	144.540 (74.82-265.08)	403.536 (299.2-544.27)	1919.928 (1146,71-3214.53)	3.354	1.16<6.00
2-Spray method					
Adult	128.397 (58.62-281.23)	300.792 (195.1-463.75)	1096.0 (669.0-1796.5)	2.728	0.59<6.00
Nymph	131.322 (68.36-252.29)	246.310 (167.17-362.9)	640.393 (443.33-925.06)	2.099	0.49<6.00
Both	123.03 (72.05-210.09)	266.357 (195.89-362.17)	861.165 (634.94-1167.99)	2.486	0.95<6.00
3-Cat food bait					
Adult	200.146 (104.45-383.50)	501.036 (355.43-706.29)	2019.788 (1049.5-3887.3)	2.950	1.50<6.00
Nymph	261.280 (138.81-491.80)	727.761 (510.73-1037.17)	3450.293 (1348.2-8829.8)	3.345	0.70<6.00
Both	224.909 (141.65-357.1)	599.677 (470.27-764.7)	2660.493 (1516.65-4667.02)	3.177	2.12<6.00

Table (3): Toxicity index values of *S. carpocapceae* against the German cockroaches at ratio of LC_{50} using different application methods

Methods- T.I.	Banana bait	Spray	Cat food bait
A) Adult stage			
Banana bait	1.0	1.148	1.451
Spray methods	0.871	1.0	1.666
Cat food bait	0.689	0.600	1,0
B) Nymph stage			
Banana bait	1.0	0.515	1.521
Spray methods	1.943	1.0	2.955
Cat food bait	0.658	0.338	1.0
C) Both stage			
Banana bait	1.0	0.660	1.486
Spray methods	1.515	1.0	2.251
Cat food bait	0.673	0.444	1.0

Manweiler *et al.* (1993) reported that during a 4 week long test in (32×25×10cm) cage in which 24 adult cockroaches (12 females and 12 males) were replaced each week, prototype bait containing 2 million nematodes of *S. carpocapceae* caused a weekly mortality of about 80%. Elkady *et al.* (2014) recorded the recommend dose of EPNs against the German cockroach using filter paper and nematode bait assay under laboratory conditions. El Sharabasy *et al.* (2013) tested eight food items, four carbohydrate-rich foods and four protein-rich foods and found that banana was the preferred food item. Kochler *et al.* (1992) stated that among alive cockroach species, the American cockroach *Periplaneta americana* was the least susceptible to infection with *S. carpocapsae* whether the nematode was applied directly or in baits; in particular no mortality occurred with bait stations.

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