

Efficiency of Entomopathogenic Nematodes *Steinernema carpocapsae* Against Sunn Pest, *Eurygaster testudneria* Under Laboratory Conditions

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Abstract

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Eurygaster testudneria (Hemiptera: Scutelleridae), is one of the most economic pests that attack and cause serious damage to wheat and barley grains. Filter paper bioassay was conducted under laboratory conditions using different concentrations of *Steinernema carpocapsae* species which is one of the most effective entomopathogenic nematodes (EPNs) applied to control a wide range of insect pests all over the world. Two stages of *E. testudneria* were used in all experiments with three concentrations of EPN. The target pest stages were nymphs and adults and the EPN concentrations used were 50, 100 and 150 IJs/insect with only tap water as the control treatment. The mortality rates were recorded 24, 48, 72, 96 and 120 hours after treatment. Consequently, LC₅₀, LC₉₀ (lethal concentration) and LT₅₀ (lethal time) were estimated for each stage. The results obtained showed that the LC₅₀ values for both stages (adults and nymphs) of *E. testudneria* were 122.9 and 11.3 IJs/insect and the LC₉₀ were 477.6 and 83.9 IJs/insect, respectively. The mortality increased by increasing the exposure time for all concentrations. The LT₅₀ and LT₉₀ for both adults and nymphs were 4.42 and 2.6 days by using 50 IJs/insect, whereas the values of LT₅₀ for both stages were 4.01, 1.9 and 4.02, 1.8 by applying 100 and 150 IJs/insect respectively. The mortality rate of nymphs was 90-100%, whereas that of adults was 65-80%, 7 days after exposure. There was a significant difference among treatments. The current study represents the first report of using EPNs as a biological control agent against *E. testudneria* in Iraq.

Keywords: Biological control, EPNs, *Steinernema carpocapsae*, sunn pest, *Eurygaster testudneria*.

Introduction

Sunn pest, *Eurygaster testudneria* is a widespread insect pest that seriously attack wheat and barley which belongs to the genus *Eurygaster* (Hemiptera Scutelleridae) and prevalent in southeast Turkey, Iran, Iraq, Syria, Lebanon, Jordan, Israel, Kazakhstan, Uzbekistan, Kyrgyzstan, Tajikistan, Afghanistan, and Pakistan and the affected area is around 15 million hectares (Parker *et al.*, 2002; 2011; Trissi *et al.*, 2006). Three species of the genus *Eurygaster* are described in Iraq: *Eurygaster integriceps* (Puton) in the north and *E. Maura* (Linnaeus) and *E. testudinaria* (Geoffroy) in the mid-south (Liqaa, 2019).

In the Near and Middle East countries, classical chemical control is commonly used against sunn pest to keep their population below the economic threshold level. In Iraq, every year a large amount of dangerous chemical pesticides which belong to the pyrethroid group such as sibex (Deltamethrin) and Flash (Alphaspermetrin), and Decstrin are being used against different species of sunn pest. These chemical insecticides require a long time to decompose and thus they are very polluting to the environment. In addition, their toxic effect tends to bio-magnify when moves throughout the food cycle chain affecting animals, humans and beneficial insects.

In the middle of Iraq where flat lands prevail, sunn pest overwinter as adults and spend at least 8-9 months under weeds or close to the soil surface around wheat fields during hibernation. During this period, various natural enemies like

entomopathogenic nematodes are considered as an important agent that could reduce the population density of sunn pest (Canhilal *et al.*, 2007), from end of February until mid-May. When soil surface temperature reaches 20°C, and wheat spikes are formed, the adults migrate to cereal fields to attack spikes directly. After harvest when temperature is still high, sunn pest aestivate under weeds and bushes (Liqaa, 2019).

Entomopathogenic nematodes (EPNs) which mainly belong to the families Steinernematidae (Travassos) and Heterorhabditidae (Poinar) and have been used in classical biological control programs (Kaya & Gaugler, 1993; Lacey & Georgis, 2012; Shapiro-Ilan *et al.*, 2002). The commercial strain of *Steinernema carpocapsae* Weiser (Rhabditida: Steinernematidae) has been employed as a biological control agent against many insect pests that live in the soil for part of their life cycle (Kamali *et al.*, 2013). The entomopathogenic nematodes rapidly kill the host within 24-48 hours, easy to produce commercially in vivo or in vitro, has operative host searching activity for long enough time, easy to use, and has a good ability to mix with a wide range of chemical insecticides and safe for the environment.

In Iraq, under laboratory and field conditions, some research have used native and commercial EPNs to combat many economic insect pests such as *Oryctes elegans* Prell (Coleoptera: Scarabaeidae), *Sesamia cretica Ledereer* (Lepidoptera: Noctuidae), *Anarsia lineatella* Zeller (Lepidoptera: Gelechiidae) and *Earias insulana* Boisduval (Lepidoptera: Nolidae) (Al-Jboory, 2007). Furthermore, EPNs (*S. carpocapsae* and *H. bacteriophora*) were used in

Iraq to control adults and nymphs of German cockroaches *Blattella germanica* L. (Blattodea: Ectobiidae) (Baker *et al.*, 2012). The effectiveness of using EPNs as biological control agents against various termite life stages has been reported (Al-Zaidawi *et al.*, 2019).

The aim of this study was to assess varied concentrations of *S. carpocapsae* for controlling adult and fifth nymph stages of sunn pest *E. testudinaria* (Geoffroy).

Materials and Methods

Insect collection

Overwintering adults of sunn pests, *E. testudinaria* (Geoffroy) as well as fifth nymph stage (immature stage) were collected from sites at AL-Dewinya district in the mid of Iraq "N 44° 46' 23.26" E39.31,32° 3'. Insects were kept in plastic boxes (25 × 35 × 18 cm) covered with a white muslin cloth and kept at a temperature of 27±1°C, 60±10% relative humidity and 16 hours light: 8 hours of darkness photoperiod (Allahyari *et al.*, 2010).

Commercial EPNs used

A commercial strain of *Steinernema carpocapsae* (Capsanem) supplied by Koppert B.V. (Berkel en Rodenr IJs, the Netherlands) was provided by Dr. Jawad Al-Zaidawi, Ministry of Science and Technology, Integrated Pest Management Center, Iraq. EPNs were cultured on late-instar *Galleria mellonella* (Lepidoptera: Pyralidae) larvae using the rearing technique described earlier by Woodring & Kaya (1988). Infective juveniles (IJs) were collected using white trap technique (Canhilal *et al.*, 2005). These IJs were kept in tissue culture flasks at 7- 9°C for 10-15 days (Kung *et al.*, 1990). Nematodes activity (IJs viability) was assessed under the binocular microscope before treatment.

Insect mortality assessment

To examine the sensitivity of sunn pest adults and fifth stage nymphs at concentrations of 50, 100, and 150 IJs/individual, two Whatman No.1 filter paper discs were placed in Petri plates (Woodring & Kaya, 1988) and the IJs were applied and uniformly distributed on the filter paper before the sunn pest adults or nymphs were added (60 min). Three groups of 10 sunn pest insects were introduced into each plate. 1 mL of sterile distilled water with no nematodes was used as a control. All Petri plates used in the experiment were sealed in a double plastic bag and put in a dark incubator at a temperature of 25±1°C and relative humidity of 70±10% (Glazer *et al.*, 1991). All treatments were replicated three times, and the whole experiment was repeated twice. Dead sunn pest insects were dissected using a dissection microscope to confirm the presence of nematode. At the

same time sunn pest cadavers were placed on white trap for monitoring nematodes emergence (White, 1927). The mortality rate (%) was recorded 24, 48, 72, 96 and 120 hours after treatment (Epsky & Capinera, 1994). The corrected mortality rate was calculated using the Abbot formula (Abbott, 1925).

Statistical analysis

Kolmogorov–Smirnov test and Ryan–Joiner exam were used to evaluate the normality and homogeneity of treatments variance (Minitab, 2014). The means of mortality for all treatments were subjected using analysis of ANOVA and means were distinguished by the LSD test. The Petri dishes bioassay for susceptibility of nymphs and adults to different concentrations of EPNs was investigated using Probit analysis to evaluate the lethal concentration (LC₅₀ and LC₉₀) for each stage of sunn pest insect at P=0.05 using POLO plus software (LeOra Software, 2006). Finally, the filter paper bioassay for both stages were examined using analysis of variance (one-way ANOVA) and factorial design by considering EPN concentrations and stages of insect as independent variables (Minitab, 2014).

Results

The commercial nematode product of *S. carpocapsae* caused mortality of the last nymph stage and adults of *E. testudneria* (Figure 1). Seven and 10 days after treatment, the mean mortality values were 90% and 100% for nymphs and 65 and 75% for adults when 100 and 150 IJs/insect concentrations were used, respectively (Figure 2). There were significant differences between concentrations in each stage (nymph and adult) (df= 6, F_(2,7)=3.83, P= 0.02). In addition, the differences were significant between the two treatments (nymph and adult) by using the same concentration (df= 3, F_(5,13)=10.50, P=0.01). The correlation between concentration and mortality rates was strong and positive for both stages (nymph and adult) which were 0.79 and 0.83, respectively. The lethal concentrations (LC₅₀ and LC₉₀) of the commercial entomopathogenic nematodes *S. carpocapsae* 10 days after treatment are shown in Table 1. It was evident that mortality rate increased by increasing the exposure time for all concentrations and on both insect stages (nymph and adult). therefore *S. carpocapsae* may be useful for control of Overwintering adults of *E. testudneria* at the soil surface (Figure 3). The lethal time of *S. carpocapsae* on nymphs was 1.8-2.6 days, whereas the lethal time on adults was between 4.01-4.42 days. There was a significant difference between the two treatments (nymphs and adults) (F_(5,13)=6.00, df=2, P<0.01) (Table 2).

Table 1. Mean lethal concentrations (LC_{50,90}) of *S. carpocapsae* against adults and fifth stage nymphs of *E. testudneria* 10 days after treatment.

Treatment	Lethal concentration (LC)	95% confidence interval	Chi-square value	Regression equation	Correlation coefficient	
Adults	LC ₅₀	122.9 IJs/insect	63.11-177.05	6.2	X=0.5Y+2	0.83
	LC ₉₀	477.6 IJs/insect	356.25-822.65	7.1		
5 th stage nymphs	LC ₅₀	11.3 IJs/insect	26.99-29.65	31.8	X=0.16Y+7	0.79
	LC ₉₀	83.9 IJs/insect	63.26-134.14	26.4		

Table 2. Mean lethal time (LT₅₀) of *S. carpocapsae* against adults and fifth stage nymphs of EPN *E. testudneria*.

Treatment	EPN concentration IJs/insect	Lethal time (LT ₅₀)	95% confidence interval	Chi-square value
Nymphs	50	2.6	0.96 - 3.17	11.4
	100	1.9	0.58 - 2.23	19.1
	150	1.8	0.87 - 2.98	22.3
Adults	50	4.42	3.66 - 5.24	28.9
	100	4.01	3.75 - 5.02	9.1
	150	4.02	3.49 - 5.01	19.8



Figure 1. *Steinernema carpocapsae* invading the adult of *E. testudneria* and multiplying within the hosts' body (black arrows refer to EPNs *S. carpocapsae*).

Discussion

No previous research on the effectiveness of EPNs against the sunn pest *E. testudneria*. (Hemiptera: Scutelleridae) under laboratory conditions has been conducted in Iraq. Sunn pest *E. testudneria* that has led to huge loss in quantity and

quality of Iraq's wheat harvest. Sunn pest was reported earlier in wheat fields in northern Iraq cold region (Ali, 2011) at the beginning of the twentieth century, but now due to climate change, this insect has become an invasive pest in wheat fields in central and southern Iraq hot and dry regions.

Laboratory studies indicated high infectiveness of sunn pest by entomopathogenic nematodes (Canhilal *et al.*, 2007; Gülcan *et al.*, 2017). Under laboratory conditions, Gözel *et al.* (2020) investigated EPN *S. carpocapsae* (Sakarya isolate) from Turkey on sunn pest (*E. integriceps*) adults. Trials showed that 100% mortality of sunn pest was reached when using 1000 IJs/insect 7 days after application. In another laboratory study (Peçen & Kepenekci, 2022) using native EPNs *S. carpocapsae*, *S. feltiae* and *Heterorhabditis bacteriophora* against the wheat stink bug *Aelia rostrata* Boheman (Hemiptera: Pentatomidae) adults, EPN *S. carpocapsae* showed the highest mortality rate (75%) on wheat stink bug when 200 IJs/cm² concentration was used.

The results obtained in this study showed that *S. carpocapsae* caused high mortality on the last nymph stage and adults of *E. testudneria*. Ten days after treatment, the mean mortality values were 90 and 100% for nymphs and 65 and 75% for adults when concentrations of 100 and 150 IJs/insect were used, respectively. In Iraq, sunn pest adults hibernate (overwinter) before migration to infest the wheat crop, 2-3 cm under soil surface at 15% soil humidity, which is very appropriate for *S. carpocapsae* pathogenesis (Koppenhöfer *et al.*, 1995; Yadav, 2012) which makes it a potential biological control agent against sunn pest *E. testudneria*. It can be concluded from this study that *S. carpocapsae* is a likely candidate to be utilized as a biological control agent against the nymphs and adults of the sunn pest *E. testudneria*. For practical purposes, future research should focus on field effectiveness, field application, and persistence of this entomopathogenic nematode as a pesticide in the Iraqi environment.



Figure 3. Overwintering adults of *E. testudneria* at the soil surface in the mid-lands of Iraq.

الملخص

جبار، أحمد شمخي، أحمد سعيد محمد وأحمد محمد حسين. 2024. فعالية النيماطودا المتطفلة على الحشرات *Steinernema carpocapsae* ضد حشرة السونة (*Eurygaster testudneria*) تحت الظروف المختبرية. مجلة وقاية النبات العربية، 42(1): 108-112.

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تعدّ *Eurygaster testudneria* (Hemiptera: Scutelleridae) من أكثر الآفات الاقتصادية التي تهاجم حبوب القمح والشعير وتسبب أضراراً جسيمة لها. تم إجراء الاختبار الحيوي بواسطة أطباق ورق الترشيح تحت الظروف المختبرية باستخدام تراكيز مختلفة من النيماطودا *Steinernema carpocapsae*، والتي تعدّ واحدة من أكثر الديدان الخيطية الممرضة للحشرات (EPNs) والمستخدم في مكافحة مجموعة واسعة من الآفات الحشرية في جميع أنحاء العالم. تم استخدام طورين (الحوريات والبالغات) من حشرة *E. testudneria* في جميع التجارب، مع ثلاثة تراكيز من EPN (50، 100 و 150 يرقة فعالة/حشرة). وتم تسجيل نسب القتل بعد 24، 48، 72، 96 و 120 ساعة من المعاملة. وبناء على ذلك، تم تقدير LC₅₀، LC₉₀ و LT₅₀. أظهرت النتائج أن قيم LC₅₀ لكل طور (البالغات والحوريات) الحشرة *E. testudneria* كانت 122.9 و 11.3 يرقة فعالة/حشرة، وأما قيمة LC₉₀ فكانت 477.6 و 83.9 يرقة فعالة/حشرة، على التوالي. زادت نسبة القتل بزيادة زمن التعرض لجميع التراكيز. كانت قيم LT₅₀ و LT₉₀ لكل من البالغات والحوريات 4.42 و 2.6 يوماً باستخدام 50 يرقة فعالة/حشرة، في حين كانت قيم LT₅₀ لكل طور للحشرة 4.01، 1.9، 4.02 و 1.8 بتطبيق 100 و 150 يرقة فعالة/حشرة على التوالي. كان نسبة قتل الحوريات 90-100%، في حين كان نسبة القتل في الحشرات البالغة 65-80%، بعد 7 أيام من المعاملة. وكان هناك اختلاف معنوي كبير بين المعاملات. تعدّ الدراسة الحالية هي الأولى لاستخدام EPNs كعامل مكافحة حيوية ضد *E. testudneria* في العراق.

كلمات مفتاحية: مكافحة حيوية، الديدان الخيطية الممرضة للحشرات (EPNs)، *Steinernema carpocapsae*، حشرة السونة، *Eurygaster testudneria*.

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