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Lactate dehydrogenase activity and calcium content during the control of the African migratory locust Locusta migratoria migratorioides (Orthoptera: Acrididae) oviposition

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Abstract

Biological control is expected to be critical in managing locust aggregations. The effects of two entomopathogenic nematode species, Steinernema sp. (SII) and Heterorhabditis bacteriophora (HP88), on the abdominal muscle fibers of adult female Locusta migratoria migratorioides (Reiche and Fairmaire) (Orthoptera: Acrididae) during oviposition were studied at different concentrations (3000, 6000, 9000, 12000, and 15000 Infected juveniles/cm^2). Lactate dehydrogenase activity and calcium content are important in the metabolism of muscular contraction in adult female locusts. Both entomopathogenic nematodes (EPNs) reduced lactate dehydrogenase activity and calcium content. The LC_{50} of S. sp. was applied to adult female locusts. The abdominal muscular fibers in SII were severely necrotic and atrophy, whereas HP88 LC_{50} treatment resulted in expanded muscle fibers and visible cross-striations.

Introduction

For locust management services, it is required to identify oviposition sites (Latchininsky *et. al*, 2016). Neurohypophysial hormone (AVT) together with ovarian hormones and prostaglandins (PGs) govern oviposition, the physical process of expelling the egg from the oviduct to the external environment, by inducing uterine contractions (Ubuka and Bentley, 2011). The female locust buries her eggs underground in order to protect them and give them the best chance of hatching. It uses two pairs of valves to dig the ground: the dorsal pair excavates the oviposition tunnel while the ventral pair plugs as a wedge (Das *et al.*, 2022).

In locusts and grasshoppers, the highly developed ovipositor is composed of two pairs of shovel-shaped cuticle valves, one ventral and one dorsal that extend over the female's distal end of the abdomen. A noticeable pair of internal apodemes, a ridge-like expansion of the exoskeleton, which the valves are, hinged to at their base, serve the massive supporting muscles (Belanger and Orchard, 1993). These motions are produced by ten muscle groups that the terminal abdominal ganglion innervates. When it comes to insects, only grasshoppers and locusts have ovipositor valves that function

by opening and shutting motions rather than sliding against one another (Vincent, 1976). Multiple natural enemies can be used, and this can benefit biological control programs. In addition to grasslands, forests and coasts, both *Heterorhabditis bacteriophora* (Heterorhabditidae) and *Steinernema* sp (Steinernematidae) can endure a variety of climatic conditions, from hot regions to cold mountains (Bhat *et al.*, 2020 and van der Linden *et al.*, 2022).

The entomopathogenic nematodes (EPNs), *Steinernema* sp. (*S*. sp.) (SII), and *H. bacteriophora* (HP88) have been used against a variety of insect pests, including the sand flies *Phlebotomus papatasi* (El Sadawy *et al*., 2020a) and the red palm weevil *Rhynchophorus ferrugineus* (El Sadawy *et al*., 2020b). The EPNs *Steinernema* sp. and *H. bacteriophora* have been utilized to control a wide range of insect pests, such as the tea mosquito bug *Helopeltis theory* and bunch caterpillar, *Andrea bipunctata* (Amuri and Devi, 2020), locusts, and grasshoppers (Ibrahim *et al*., 2018). The purpose of this study is to determine how the EPNs *Steinernema* sp. SII and *H. bacteriophora* HP88 affect LDH and calcium levels during the oviposition process in the African migratory locust *Locusta migratoria migratorioides* (Reiche and Fairmaire) (Orthoptera: Acrididae).

Materials and methods

1. Locust culture:

Adult African migrating locusts (♂and♀) from the Egyptian town of Abu Rawash in the Giza Governorate, *L. migratoria migratorioides* were collected and reared according to Muhammad *et al.* (2022).

2. Entomopathogenic nematode species:

Two EPNs, *S*. sp. (SII) and *H. bacteriophora* HP88, were taken from the Department of Parasitology and Animal Diseases at the National Research Centre in Dokki, Egypt, and were raised in the Plant Protection Research, Institute at the Agricultural Research Center for several generations. The larger wax moth *Galleria mellonella*'s larvae in the last instar were housing the infectious juveniles (IJs) of the two EPNs.

3. Lethal activity:

Large plastic boxes packed with 1 kg of moistened, sterilized sand were used for this experiment. Nematode strains were put on the soil surface with (100 ml) water, mixed with the sterile sandy soil, and then placed inside boxes at concentrations of 3000, 6000, 9000, 12000, and 15000 IJs/cm^2. In this study, 30 adult *L. migratoria migratorioides* males and 30 adult females (two weeks post-last molt) were placed in a big plastic box (30 x 15 x 15 cm), covered with plastic lids, and provided with corn leaves (*Z. mays*) as a source of daily sustenance. Three replicates were utilized. The experiment was conducted at the pest physiology department in September at a temperature of 25 ± 2°C open air, with a soil moisture content of 20% and a relative humidity of 55–60 % RH.

4. Oviposition deterrence assays:

As the above experiment, 30 adult *L. migratoria migratorioides* males and 30 adult females (Two weeks post-last molt) were placed in a big plastic box (30 x 15 x 15 cm), with specific cups at the bottom of the plastic box for collecting egg pods. Three replicates were utilized for each EPN at the LC_{50}. Longevity and Fecundity (The number of eggs or offspring produced by the female) were determined after 72 hrs. of treatment. Oviposition deterrent indices (ODI), was calculated as follows (Huang *et al*., 1994) , ODI=100 /(C−T) C+T, where C and T are the mean number of eggs laid on control and treated locust, respectively. Reduction in hatching percentage (% H) was calculated using the following equation of Kumar *et al*., 2012 and Banerjee *et al*., 2014: % H= ((HC−HA) / HC) x100. Where HC is the

hatching rate in the control group, and HA is the hatching rate in the EPNs-treated groups.

5. Biochemical studies:

Preparation of the samples: The adult mature female is murdered and the abdomen is taken out in order to prepare the sample. To prepare the tissues for sampling, an average sample of 9 mm wide by 3 cm long is homogenized in distilled water and spun on a Beckman GS-6R centrifuge at 6000 rpm for 10 min at 5 °C. After centrifugation, tiny aliquots (0.5 ml) of the supernatant fluid were obtained and stored at 20 °C until the primary components were examined. For each sample, three replications were completed. Samples were evaluated for each biochemical parameter 24, 48, 72, 96 and 120 hrs. after being treated with the LC_{50} of both EPNs. The method described by Vanderlinde (1985) was used to measure lactate dehydrogenase (LDH). Determination of Calcium Content: Calcium content was determined for control and treated adult females by the 0-cresolphthalein complexone method (Sarkar and Chauhan, 1967).

6. Histopathological effects:

Ten adult female locusts were used as controls, and the same number of them was given the LC_{50} concentrations of the EPNs from both species. Insects were dissected in Ringer solution (pH 6.8), and the abdomen was separated, **fixed in Bonn's fluid, and embedded in paraffin, according to Nasiruddin and Mordue (1993).**

7. Statistical analysis:

Using the SPSS version 27 software **(SPSS, 2020),** the LC_{50}, LC_{90}, lower bound, and upper bound (95% confidence limits) of the lethal activity of EPNs species were calculated. When examining the rate of reproduction of the tested EPNs species and the effect of EPNs on biochemical tests, $P<0.05$ showed a significant difference between groups.

Results and discussion

1. Lethal activity:

When SII was administered, the LC_{50} values for adult male and female locusts were (11221, 8643.1 IJs/cm^2), respectively (Table 1). For the male and female adults, the LC_{50} values for the treatment with HP88 were (14063 and 12080 IJs/cm^2. soil), respectively (Table 1).

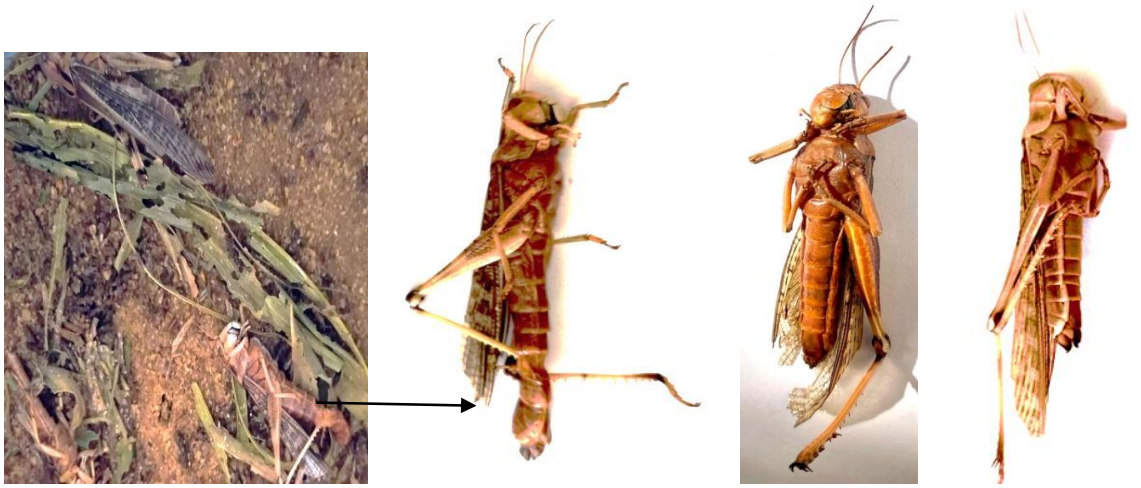
Table (1): Lethal activity of two entomopathogenic nematodes (EPN), *Steinernema* sp. (*S.* sp.) (SII) and *Heterorhabditis bacteriophora* HP88 against male and female adult *Locusta migratoria migratorioides* at 25 ± 2 °C and 60% RH.

EPNs	Adult Stage	LC_{50} (Ijs/cm^2)	95% Confidence limit (Ijs/cm^2)		LC_{90} (Ijs/cm^2)	95% Confidence limit (Ijs/cm^2)		Slope± S.E	Chi square (χ^2)
			LB	UB		LB	UB		
Steinernema sp. (SII)	Male	11221	9677	13352	20659	17441	26737	1.1 ±o.2	3.6
	Female	8643.1	7300	10086	16950	14646	20852	1.2 ±0.2	5.21
Heterorhabditis bacteriophora (HP88)	Male	14063	12217	17240	23264	19391	31265	1.2 ± 0.3	1.3
	Female	12080	10582	14229	20677	17626	26363	1.1±0.3	1.9

$L.C_{50}$: lethal concentration brings out 50% mortality and $L.C_{90}$: lethal concentration brings out 90% mortality. (LB, UB): Lower Bound and Upper Bound.

2. Oviposition deterrence assays:

The damaged to adult female abdomen caused by the EPN, SII is depicted in (Figure 1a). Also, the treatment of adult female locusts with *H. bacteriophora* HP88 is depicted in (Figure 1b). These results revealed statistically significant reduction in longevity ($d f = 2$, $f = 14.6$, $P > 0.05$), fecundity ($d f = 2$, $f = 132.7$, $P < 0.001$), ODI ($d f = 2$, $f = 2772.4$, $P < 0.001$), and reduction in hatching % ($d f = 2$, $f = 883.1$, $P< 0.001$) (Table 2).



a **b** **c**

Figure (1): a. Dead adult female *Locusta migratoria migratorioides* treated with *Steinernema* sp. (SII) at LC $_{50}$ showed elongated and damaged abdomen.
b. Adult female *Locusta migratoria migratorioides* treated with *Heterorhabditis bacteriophora* HP88 at LC $_{50}$.
c. Control adult female *Locusta migratoria migratorioides.*

Table (2): Effect of LC_{50} of the two entomopathogenic nematodes (EPN), *Steinernema* sp. (*S.* sp.) (SII) and *Heterorhabditis bacteriophora* HP88 on the longevity, fecundity, ODI, (%) reduction in hatching percentage of adult female *Locusta migratoria migratorioides* at 25 ± 2 °C and 60% RH.

EPNs LC_{50} ($IJs/cm^{2)}$	Female longevity (days) (Mean± S.E)	Number of egg pods (Mean± S.E)	Number of eggs/ pod (Mean± S.E)	ODI (Mean± S.E)	% H (Mean± S.E)
Control	36 ± 2.9 a	3.33± 2.9 a	111.7 ± 5.8 a	100 a	-
***Steinernema* sp. SII**	14.3 ± 2.6 c	1.33 ± 0.31 c	45.3 ± 2.9 c	42.3 ± 0.6 b	77.8 ± 0.5 a
***Heterorhabditis bacteriophora* HP88**	20.2 ± 0.9 b	2.3 ± 0.29 b	86.7 ± 8.7 b	12.6 ± 0.8 c	19.2 ± 0.4 b

The letters (a, b, c) indicate significant differences between the control and treatment groups, at P<0.05.

3. Biochemical studies:

The results of the lactate dehydrogenase activity in adult females of *L. migratoria migratorioides* treated with LC_{50} of the EPNs, *S.* sp. (SII), and *H. bacteriophora* (HP88) are shown in (Figure 2). These results revealed a statistically significant decrease in lactate dehydrogenase activity in adult females (d f = 2, f = 7.03, P < 0.001) in comparison to control adult females. Whereas, adult females exposed to LC_{50} of the EPNs, (SII), (HP88) demonstrated a statistically none significant decrease in the calcium content compared to untreated adult females (d f = 2, f = 2.66, P > 0.05) (Figure 3).

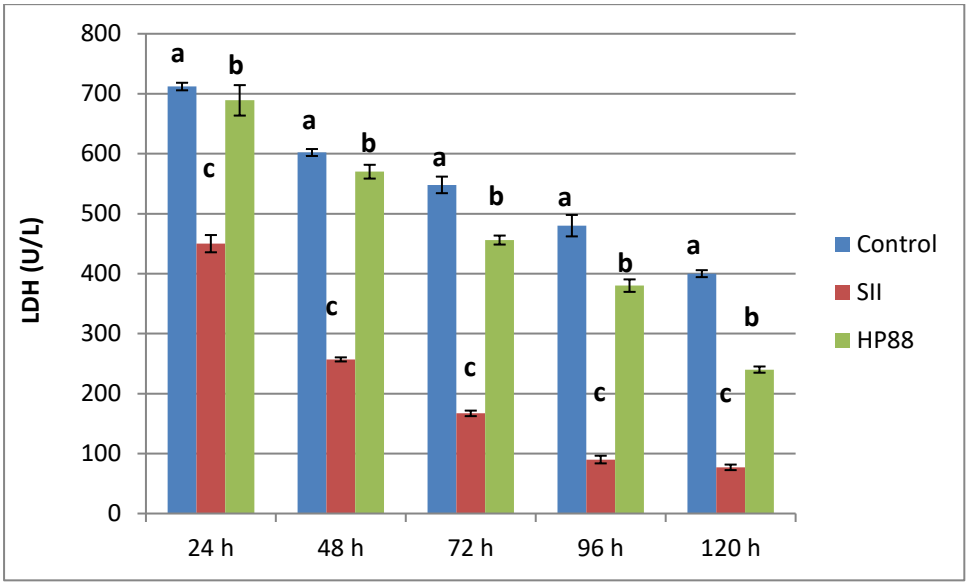


Figure (2) : Changes of lactate dehydrogenase activity (U/L) in adult females due to using LC_{50} of entomopathogenic nematodes (EPNs) in various time intervals (h). Bars (Mean ±SE) in the same and between time intervals with the same letter(s) are significantly different (P<0.001).

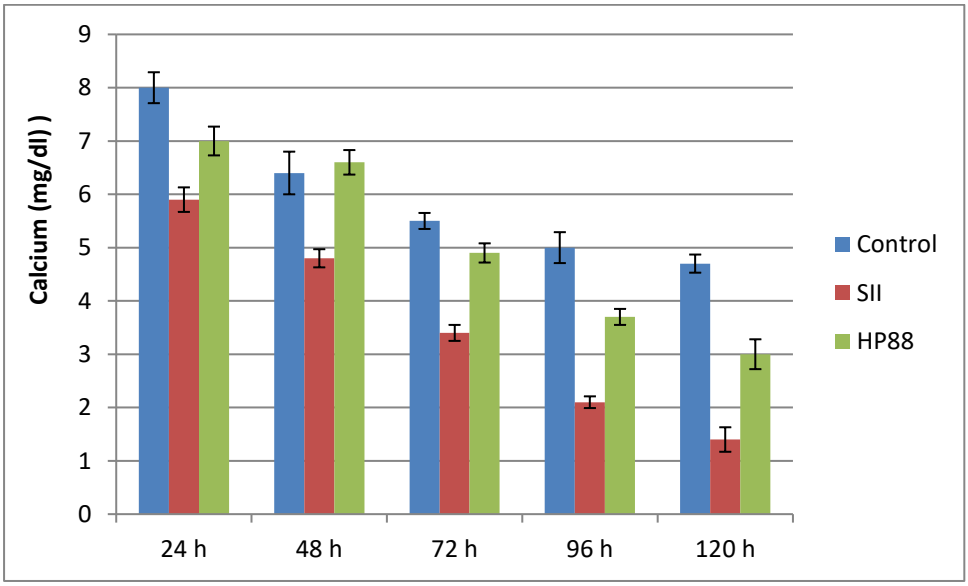
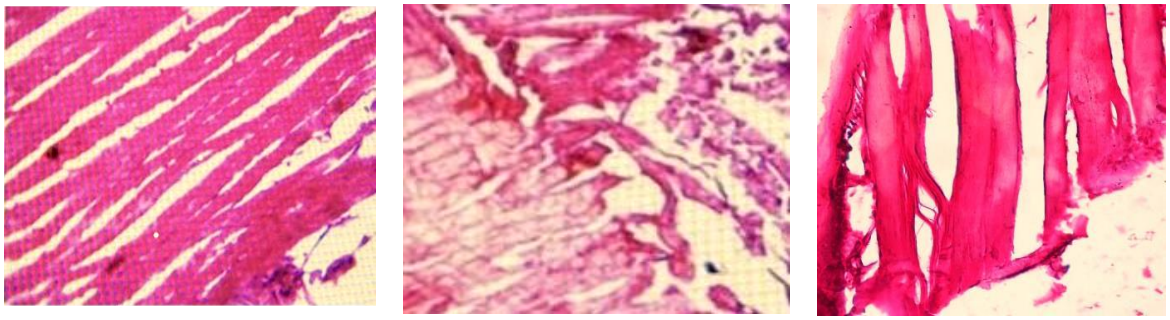


Figure (3): Changes of calcium (mg/dl) due to using LC_{50} of entomopathogenic nematodes (EPNs) in various time intervals (h). Bars (Mean ±SE) in the same and between time intervals are none significantly different (P > 0.05).

4. Histopathological effects:

A histopathological evaluation of the effects of LC_{50} of SII and HP88 on the adult female abdomen of *L. migratoria migratorioides* was conducted. These outcomes were demonstrated, and they are discussed in (Figure 4). The control female's abdominal muscle fibers were well-organized and had normal nuclei (Figure 4 a). After receiving LC_{50} of SII, adult female locusts displayed severe necrosis and atrophy of the abdominal muscular fibers (Figure 4 b). With LC_{50} of HP88 treatment, adult female locusts displayed more expanded muscle fibers and noticeable cross-striations (Figure 4 c).



a **b** **C**

Figure (4): a. Control of normally organized muscle fibers and entire nuclei in the abdominal muscles (H&EX200).
b. Muscle fibers in the abdominal muscles are degrading, dying, and atrophying while losing their transverse striations and nuclei (H&EX200).
c. The muscle fibers are further apart cross-striations are evident (H&EX200).

L. migratoria migratorioides is one of Africa's most serious agricultural pests. The locust has a high fecundity and consumes a large amount of food, causing severe damage to a variety of crops including, corn, sorghum, and rice (Hu *et al*., 2022). The adult female locust digs a hole in the sand about 8-9 cm deep to lay her eggs. The ovipositing part of the abdomen is normally about 2.5 cm long. Part of the length increase is accomplished by unfolding the telescoped intersegmental membranes, resulting in a two-fold extension.

The remaining increase is obtained by stretching a thickened section of the intersegmental membrane) by up to ten times its unstretched length (Malek, 1958). There are two main theories for the mechanism of abdominal extension: (a) internal pressure (Agarwala, 1951), and (b) pulling the ovipositor valves as they dig the hole (Uvarov, 1966). While digging its hole, the locust frequently releases its grip on the hole's walls, both to rest and to probe deeper. If the membranes were elastic, as the membranes contracted, the abdomen would be drawn back up the hole (Vincent and Wood, 1972). When different concentrations of EPNs, SII and HP88, were applied to adult females, there was a decrease in longevity, fecundity, oviposition deterrent indices, and hatching percentage when compared to untreated adult females.

This was agreed with (Sharaby *et al*., 2013), who found that the LC_{50} of the alcoholic 80% extract of *Euphorbia pulcharrima* (Ephorbiaceae) (0.714%), essential oil of garlic plant *Allium sativum* (Liliaceae) (0.067%), and entomopathogenic nematodes (EPNs) of *Steinernima carpocapsae* (Stienernematidae) and *Heterorhabditis bacteriophora* (Heterorahbditidae) were tested for their single and/or combined toxic effects on female fecundity and egg fertility of the treated grasshopper, *Heteracris littoralis* (Orthoptera: acrididae), in comparison to control females. LDH (lactate dehydrogenase) is a key enzyme in the anaerobic metabolic pathway.

The enzyme's function is to catalyze the reversible conversion of lactate to pyruvate (The main glycolytic product) by reducing NAD+ to NADH and vice versa (Schumann *et al*., 2002). In some insects, glycolysis is the primary source of energy for muscular contraction; the concentration of lactate dehydrogenase in an insect muscle provides information about the contractile process's metabolism (Kitto and Briggs,

1962). It is also involved in carbohydrate metabolism and has been used to indicate chemical stress exposure (Wu and Lam, 1997). LDH is a widely used parameter in toxicology and clinical chemistry to diagnose cell, tissue, and organ damage. However, the potential of this enzyme as a predictive criterion in invertebrate toxicity tests has received little attention (Ribeiro *et al*., 1999).

In addition, calcium is important in the physiological function and intermediary metabolism of insects (Clark, 1958). The use of EPNs, SII, and HP88 resulted in a significant decrease in LDH but not in calcium levels. This is consistent with the findings of Soliman *et al*. (2022), who found that LDH activity and calcium content were significantly reduced in infected adult green bugs, *Nezara viridula* (L.) (Hemiptera: Pentatomidae) treated with the entomopathogenic fungus *Beauveria bassiana*. Histopathological changes in treated female locusts revealed a degrading, dying, atrophying, and loss of transverse striations and nuclei in the abdominal muscles as a result of SII treatment; additionally, the abdominal muscles of HP88-treated female locusts revealed further apart cross-striations of muscle fibers. This was agreed upon (Nouh and Abo Abdalla, 2016 and Kokhia *et al*., 2010).

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