Physiological Changes of *Schistocerca gregaria* (Forskal.) Nymphal Hemolymph Infected with the Nematode, *Steinernema feltiae*.

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ABSTRACT

Impact of infection with the nematode, *Steinernema feltiae* on the desert locust, *Schistocerca gregaria* (Forskol.) nymphal mortality, physiology and biochemistry, at the nematode concentrations of 10, 50 and 100 IJs/gm soil under the laboratory conditions was studied. All the locust nymphs were susceptible to *S. feltiae* and recorded high mortality rate. The enzyme phenoloxidase activity was elevated at the three concentrations of nematode's suspension. The peroxidase enzyme activity fluctuated between induction and inhibition by the nematode infection. Total insect protein, carbohydrate and lipid contents declined dramatically in all treatments. The results suggest the possibility of safe using the nematode, *S. feltiae* to control *S. gregaria* nymphs.

Key words: *Schistocerca gregaria, Steinernema feltiae*, Biopesticide, Physiological changes.

INTRODUCTION

Locusts are devastating pests of crops in many parts of the world and the desert locust *Schistocerca gregaria* (Forskal.) is a very destructive herbivore causing considerable loss to crops and pastures in the arid and semi-arid areas of Africa and Asia (Bashir *et al.*, 1998). The major control strategy adopted against the desert locust is based on use of chemical insecticides. Most of the insecticides sprayed had potentially negative environmental effects as they kill a broad spectrum of non-target insects and arachnids (Lomer *et al.*, 1997). Thus, alternatives to harmful pesticides have to be found. No evidence of serious side effects of the alternative control agents was recorded on arthropods, when compared with conventional pesticides (Peveling *et al.*, 1994).

The entomopathogenic nematodes (EPN) possess a broad host range and widely used against insect pests but with a limited concern on grasshoppers and locusts as hosts (Baker and Capinera, 1997). Nematodes have the ability to seek out and quickly kill their hosts. They are safe to vertebrate and other non-target organisms as well non-pathogenic to human (Kaya, 1985). *Steinernema feltiae* is an insect pathogenic nematode safely used against insects.

The objective of this study was to evaluate mortality rate and physiological changes in the nymphs of the desert locust, *S. gregaria* when infected with the nematode *S. feltiae* under laboratory conditions.

MATERIALS AND METHODS

S. gregaria nymphs were obtained from the stock culture maintained at the Entomology Department, Faculty of Science, Cairo University. The pest has been reared for several generations under controlled laboratory conditions as described by Vanden *et al.* (1998).

Mass rearing of the greater wax moth, *Galleria mellonella* L.

Galleria mellonella L. larvae were used as a laboratory host for reproduction of the nematode species. The artificial diet, developed by Poinar (1975) was used for its mass rearing in the laboratory in 2007-08.

Mass rearing of the nematode *Steinernema feltiae*

The nematode, *S. feltiae* was obtained from the stock culture maintained for several generations at the Physiology Department of Plant Protection Research Institute, Agricultural Research Centre, Giza, Egypt and reared in the last instar of *G. mellonella* larvae at 25°C according to the method of Dutky *et al.* (1964). Infective Juveniles (IJs) were harvested by White traps method according to White, (1927).

Treatment of *S .gregaria* nymphs with *S. feltiae*

The original method of soil nematode treatment described by Jessics and Andreas (1999), was used with some modifications. Aerated plastic vials were filled to a depth of 1 cm by autoclaved sand. For inoculation, freshly emerged IJs were washed five times by sterile distilled water, viability of IJs was checked and counted under a stereomicroscope, adjusted to the appropriate number, and pipetted in a volume of 200 ml and distributed equally on the moist sand. Sixteen nymphs, 5th instar, were used at each concentration with three replicates per concentration.

Mortality rate

Dead insects were recorded daily and then removed from the plastic vials. Dead nymphs were incubated separately in test tubes with moist cotton under sterile conditions to examine whether they died due to nematode's treatment or not. White traps' technique was used for collecting the infective juveniles.

Assessment of physiological changes in *S. gregaria* treated with *S. feltiae*

Haemolymph assays

Blood samples were taken after 24 h post treatment of *S. gregaria* nymphs. Prior to bleeding, the nymphs were chilled for 30 min. at 4 °C. A leg was surface sterilized by 70% ethanol and severed between the coxa and the femur. Haemolymph samples were obtained according to the method of Abdel El-Kawy (1981).

Phenoloxidase (PO) activity

Phenoloxidase activity was determined as described by Ishaaya (1971), using catechol as a substrate. Absorbance was recorded 1 min after the initiation of the reaction. Optical density was determined. Zero adjustment was against sample blank at 405 nm.

Peroxidase (POD) activity

Peroxidase activity was determined according to Vetter *et al.* (1958), with some changes. Sample size was (200µl) and the following reagents were added: 1ml of 1% o-phenylenediamine and 1ml of 0.3% hydrogen peroxide (in distilled water). The reaction was allowed to proceed for 5 minutes and stopped by adding 2 ml of saturated sodium bisulfite. Enzyme activity was expressed as a change in absorbancy at 430 nm (ΔOD_{430})/min/ ml.

Total protein content

Total proteins were determined by the method of Bradford (1976). Protein reagent was prepared by dissolving 100 mg of the stain Coomassie Brilliant blue G-250 in 50ml 95% ethanol. 100 ml 85% (W/V) phosphoric acid was added to the solution. Sample solution (50 µl) for the preparation of the standard curve 50µl of serial concentrations, containing 10 to 100 µg bovine serum albumin was pipetted into test tubes. The volume was adjusted to 1ml with phosphate buffer (0.1 M, PH 6.6). Absorbance, at 595nm, was measured after 2 min. and before 1 hr against blank prepared from 1ml of phosphate buffer and 5 ml protein reagent.

Total carbohydrates content

Total carbohydrates were estimated in heamolymph by the phenol-sulfuric acid reaction of Dubios *et al.* (1956). Total carbohydrates were extracted from the heamolymph and prepared for assay according to Crompton and Birt (1967). Samples of haemolymph were homogenized in 5 ml of 0.3 M $HCLO_4$ at 0°C for 1 min. Insoluble matter was removed by centrifugation for 3 min. at 2000 RPM and washed twice in ice-cold HCLO4 (5 ml) by re-dispersion and centrifugation. 100 µL of the haemolymph were added to 0.5 ml of phenol (20 percent w/v). Total carbohydrate was expressed as: µg glucose/ml haemolymph.

Total lipids content

Total lipids were estimated by the method of Knight *et al.* (1972), using phosphovanillin reagent prepared by dissolving of o.6 gm pure vanillin in 10 ml ethanol and completed to 100 ml with distilled water. 250 ml of sample were added to conc. sulphuric acid (5 ml) in a test tube and heated in a boiling water bath for 10 min. After cooling to room temperature, the digest was added to phosphovanillin reagent (6 ml). After 45 min, developed color was measured at 525 nm against reagent blank. Optical density was compared to that of a reference standard and results expressed as mg lipids/ ml haemolymph.

Data analysis

Data were recorded, tabulated and subjected to statistical analysis using a software (SPSS 11.0, SPSS Inc., Chicago, IL) test program. Significance of the main effects was determined by ONE-way analysis of variance (ANOVA) and significance of various treatments was evaluated by Range test ($P \leq 0.01, 0.05$).

RESULTS AND DISCUSSION

Obtained results showed that the nematode *S. feltiae* affected all the treated locust nymphs significantly in a concentration dependent manner (Table 1). Significant difference for the cumulative percent mortality of treated nymphs ($F_{(3, 24)} = 16.23$, $P \leq 0.01$) was found. The locusts treated with the highest concentration began to die as early as the first day post nematode treatment (Fig. 1). Results indicated that the nematode was highly effective against 5th instar locust nymphs at the three concentrations, which is in agreement with Georgis *et al.* (1992) who reported that exposure of the locust *Schistocerca nitens* to the nematodes *S. glaseri* and *S. scapterisci* caused 39 and 57 % mortality, respectively within the first four days.

At the same time, in order to specify the humoral immune reaction of *S. gregaria* 5th instar nymph to the nematode; *S. feltiae*, phenoloxidase and peroxidase activities were determined after one day post treatment (Table 2). When EPN infects the

Table (1): Effect of different concentrations of the nematode, *Steinernema feltiae* on cumulative percent mortality of *Schistocerca gregaria* 5th instar nymphs

Locust	Control	Nematode concentration (IJs/gm sand)		
nymphs	Mean ±SE	10	50	100
Mortality	0.00± 0.00	76.19 ±13.17	80.95±14.29	90.48±7.14

Values within rows represent mean of three separated group's ± SE ($P \leq 0.05$).

Table (2): Phenoloxidase and peroxidase activity changes on *Schistocerca gregaria* treated with different concentrations of the nematode, *Steinernema. Feltiae*

Parameters	Control	Nematode concentration (IJs/gm soil)		
(O.D. units/min/ml haemolymph)	Mean ± SE	10	50	100
Phenoloxidase	7.42±0.77	9.95±0.08	10.74±0.25	8.53±0.12
Peroxidase	8.70±0.26	8.58±0.08	9.03±0.04	7.77±0.18

Values within rows represent mean of three separated group's ± SE ($P \leq 0.05$). O.D. = Optical Density

Table (3): Total protein, carbohydrate and lipid contents changes in *Schistocerca gregaria* treated with different concentrations of the nematode, *Steinernema. Feltiae*

Parameters	Control	Nematode concentration (IJs/gm soil)		
(mg/ml haemolymph)	Mean ± SE	10	50	100
Total proteins	84.93±1.26	83.83±1.11	83.20±1.40	67.47±1.60
Total carbohydrates	28.70±1.72	23.60±0.56	16.97±0.26	17.17±0.61
Total lipids	4.36±0.08	3.68±0.09	4.32±0.06	2.21±0.03

Values within rows represent mean of three separated group's ± SE ($P \leq 0.05$).

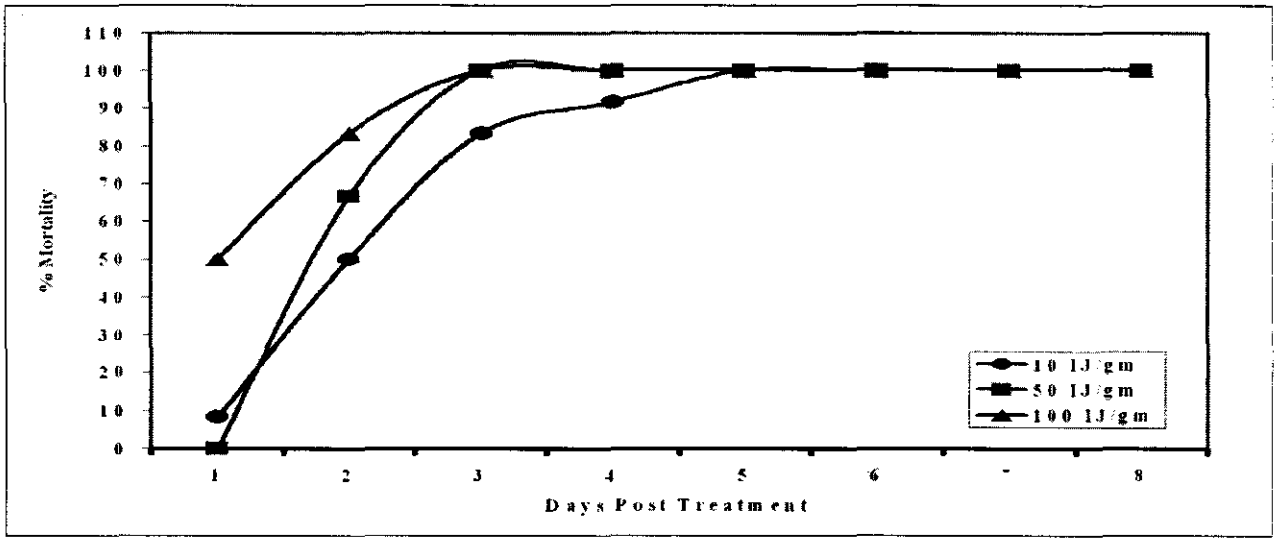

Fig. (1: Cumulative percentage mortality of *Schistocerca gregaria* nymphs treated with different concentrations of the nematode *Steinernema feltiae.*

nymphs, release the bacteria in the hemoceol and the immune reactions, including activation of some enzymes, are evident. Phenoloxidase activity was significantly increased than in the control, when the concentrations of EPN used to infect the nymphs were 10, 50 and 100 IJ/gm soil. There was a significant difference for the PO activity of *S. gregaria* ($F_{(3, 8)}$ =55.94,P≤ 0.01). Peroxidase activity also showed significant alteration of infested locust nymphs ($F_{(3, 8)}$ = 10.79, $P \leq 0.003$). Dimarcq *et al.* (1990) found that the injection of foreign molecules into the insect haemolymph induce the synthesis of immune protein. *S. gregaria* responded to the nematode *S. feltiae* infection, with an increase in PO. Hung and Boucias, (1996) noted that the fungus *Beauveria* infection increase active PO levels in the grasshopper, *Melanoplus sanguinipes* and the army cutworm, *Spodoptera exigua.*

The immune reaction of insects responds to an invasive organism by mounting both cellular and humoral reactions. Cellular response involves different classes of haemocytes that remove foreign objects from the haemolymph by phagocytosis, nodulation or encapsulation. The humoral response consists of the activation of enzyme system like phenoxidase system (Gillespie *et al.*, 2000), peroxidase (Rocktein, 1978). In the present experiment, when the locust nymphs were infected by the nematodes, phenoloxidase titre significantly increased, indicating that the activity was a reaction for the infection with EPN. Shelby and Popham (2006) stated that phenoloxidase was primarily responsible for antiviral activity present in *Heliothis virscens* larval plasma after infection by nucleohydrovirus. It was observed that the nematode *S. feltiae* had antifeedant activity against *S. gregaria* nymphs (not measured), where most of the introduced food, not consumed by the locust nymphs, especially at highest nematode concentration 100 IJs/ gm soil. Reduced food consumption was the most likely cause. *S. gregaria* ate less when infected with the fungus *Metarhizium* spp. (Seyoum *et al.*, 1994), while it stopped feeding in case of *Manduca sexta* (Dean, *et al.*, 2002).

In the same manner, the results represented in (Table 3) showed that the total protein contents were dramatically declined, especially at highest concentration. There was a significant difference for the total protein contents of treated insects ($F_{(3, 8)} = 37.37$, $P \leq 0.01$). Gillespie *et al.* (2000) reported that the nematodes; *H. bacteriophora* and *S. riobrave* severely depleted proteins in the haemolymph of larval *Pseudomonas aegyptiaca*.

Total carbohydrate and lipid contents of infected nymphs was significantly different ($F_{(3, 8)} = 34.28$, $P \leq 0.01$) ($F_{(3,8)} = 199.95$, $P \leq 0.01$), respectively (Table 3). Total carbohydrate and lipid contents decreased whenever the concentration of the nematode increased. Results recorded in the present study indicated a decrease in total carbohydrates and total lipids contents of treated nymphs after 24 hr post infection with *S. feltiae* dependently on concentration. Results agree with Rutherford and Webster (1978) who reported changes in the individual carbohydrates in the haemolymph of infected locusts as well as the lipids contents. It has been noticed that, there was an actual decrease in the total lipid contents of infected hosts after 10, 20, 30, and 40 hr from infection with the nematode.

In conclusion, the nematode *S. feltiae* caused activation of locust's enzyme system in addition to reduction in haemolymph protein, carbohydrate and lipid contents. This activation and metabolic depletion led finally to increase accumulative mortality in *S. gregaria* nymphs. Further field experiments are needed to confirm the potential of EPN on the desert locust nymphs.

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