Immunity Changes in Locusta migratoria Linnaeus (Orthoptera: Acrididae) Infected by Entomopathogenic Nematode Steinernema carpocapsae (Rhabditida: Steinernematidae)

Samira M. N. AbdEl Wahed¹ and Nabawia M. Elhadidy²
¹Plant Protection Research Institute, Agric. Res. Center, Giza, Egypt
²Dept. of Zoology, Faculty of Science, El-Arish University, EGYPT

Corresponding author: samiranabel@hotmail.com. Phone number: 01003811283

ABSTRACT

Entomopathogenic nematode (EPNs) Steinernema carpocapsae was tested against the Locusta migratoria L., 5th instar nymphs under laboratory conditions. The mortality of nymphs maintained on three concentrations (10, 20 and 40 IJs/g) nematode-inoculated sand was particularly high. Nematode was effective, causing 100% mortality of fifth nymphal instars after third days of treatment. The impact of S. carpocapsae parasitism on L. migratoria immune defense was closely examined, after both 24-48h post treatment with LC50 of nematode the activities of phenoloxidase (PO), peroxidase (POD), α and β esteras (ESTs) and glutathione-S-transferase (GSTs) enzymes of the whole body were estimated. S. carpocapsae affect locust causing high mortality rate, which significantly increased in concentrations and time dependent manner, this effect might be because of enzyme system distraction. These results demonstrated that, the entomopathogenic nematode broke host immunity, by the changes of enzymes activities; that provide an overview about the efficiency of nematode and its effects on L. migratoria.

Keywords: Locusta migratoria, Steinernema carpocapsae, Immunity, Phenoloxidase, Peroxidase, α and β esteras, Glutathione-S-transferase and enzymes.

INTRODUCTION

Locusts are possibly the most ruinous pest insects in the world. The desert insect Schistocerca gregaria (Foreska) and transitory L. migratoria are the two major types of insects. In the gregarious stage nymphs demonstrate show an aggregating behavior and move in groups to seek food, while adults swarm and migrate over long separations causing significant product and fields harm (Magar et al., 2008). L. migratoria, classified into up to 10 subspecies, swarms in Asia, Africa, New Zealand, and Australia, however has become rare in Europe (Chapuis et al., 2008). The entomopathogenic nematode, have wide host ranges and are broadly utilized for biological control of insect pests, however trialed against hoppers and locusts in just a few cases (Baker and Capineria 1997). Nematodes have the ability to seek out and quickly kill hosts, and they are safe to vertebrate and other non-target organisms as well as non-pathogenic to humans (Kaya 1985). There were plenty of studies didn’t take into consideration that PO phenoloxidase might just turn into stimulated for brief interval timing in particular tissues and furthermore attend in various methods relying on impregnable defy (Nappi and Christensen 2005). The roles of insect POD peroxidas involved detoxification, stability of extracellular matrices, and thinkable included in insect immunity (Zhao et al., 2001). ESTs carry out significant roles within the insect by catabolizing the esters of higher fatty acids, which proceeds vigor in the insect’s flight muscles and allows it to fly, by mobilizing lipids, overall those of the fat body (Roslavtseva et al., 1993), by degrading inert metabolic esters, inclusive different xenobiotics (Terriere et al., 1984). The disintegration inactive poisonous particles with GSTs and ESTs through infectious has an opener function in save insects from pathogens (Dubovskiy et al., 2012). GSTs and ESTs are remarkable groups of enzymes in insects that contribute in the detoxification of different xenobiotics and pesticides, and act significant functions in the resistance of insects to diverse of pesticides (Li, 2007).

The goal of the present work is to study the activity of phenoloxidase, peroxidase, α and β esteras and glutathione-S-transferase in whole body homogenates of the 5th nymphal instars of the migratory locust L. migratoria during the infection with the nematode S. carpocapsae under laboratory conditions.

MATERIALS AND METHODS

Insect culture:

The mass rearing of L. migratoria was obtained from Locust And Grasshopper Department, Plant Protection Research Institute, Agriculture Research Center (A.R.C.), Dokki, Giza, EGYPT, nymphs was breed constantly for many offspring under exact lab conditions as mentioned by (Vanden Broeck et al., 1998).

Examined nematode:

Nematode of S. carpocapsae was gained from a supplies colony bred constantly many progeny in Physiology Department, Plant Protection Research Institute, Agriculture Research Center (A.R.C.), Dokki, Giza, EGYPT.

Mass rearing of Galleria mellonella (Lepidoptera: Pyralidae):

Mass rearing of the greater wax moth G. mellonella larvae used in the present study as a suitable host for reproduction of entopathogenic nematode was breed in the Physiology Department, Plant Protection Research Institute according to (Poinar 1975).

Mass rearing of S. carpocapsae nematode:

The nematode species S. carpocapsae applied in this examination was breed in the last stages of G. mellonella larvae at 25°C in accord with procedures by (Dutky et al., 1964). Obtained IJs were preserved at 16 C for minimal seven days after prior the experiment.

Exposing the locust nymphs to the nematode:

The original method of nematode-inoculated sand described by (Jessica and Andreas 1999).

Mortality estimation:

Departed insects were listed each day and constantly taken away from plastic container. Dead nymphs were incubated separately in test tubes with moist cotton under sterile conditions to examine whether they died because of nematode or not. White traps are used for collecting entomopathogenic nematodes (IJs).

Determination some biochemical changes of L. migratoria treated with S. carpocapsae

Enzyme preparation:

The whole bodies of 5th nymphal instar for biochemical tests were homogenized in a cooled glass Teflon tissue homogenizer (ST–2 Mechanic-Preczyjna, Poland). Next homogenation, supernatants were kept in
freezer at -20°C till usage for biochemical analyses. Double beam ultraviolet-visible spectrophotometer (spectronic 1201, Milton Roy Co., USA) was used to measurement absorbance of dyed materials or metabolic component.

**Preparation of insects for analysis:**

The insects were homogenized in purified water (50mg/1ml). Homogenates were centrifuged with 8000r.p.m. for 15min at 2°C within a frozen centrifuge. The residues were neglected and the supernatants, which is indicated as enzyme extractor, can be stocked as a minimum seven days without appreciable loss of efficiency when stockpiled at 5°C (Amin 1998).

**Estimation of phenoloxidase (PO) activity:**

Phenoloxidase activity was specified as (Ishaya 1971) using Catechol as the substrate. Absorbance was registered after 1 min from the initiation of the reaction, the optical density was determined. Zero modification was against sample blank at 405 nm.

**Estimate of peroxidase (POD) activity:**

Peroxidase activity was estimated as (Vetter et al., 1958) was followed with some changes. To the sample (200µl), in which the color is to be formed, the following reagents are added: 1ml of 1% α-phenylenediamine and 1ml of 0.3% hydrogen peroxide (in distilled water). The reaction is allowed to proceed for 5 minutes at which time it is stopped by adding 2ml of saturated sodium bisulfite. The enzyme activity was expressed as the change in absorbency at 340 m (ΔOD405)/min/gm.

**Estimation of Nonspecific esterases (ESTs):**

Esterases (α and β) were specified in accord with (Van Asperen I962) using α-naphthyl acetate or β-naphthyl acetate as substrate, severally. The reaction mixture be composed of 5ml substratum settlement (3x10^4M α- or β naphthylacetate, 1% acetone and 0.1M phosphate buffer, pH17) and 20µl of nympha homogenate. The mixture was incubated for exactly 15min at 27°C, then 1ml of diazobule color reagent (prepared by mixing 2 parts of 1% diazobule B and 5 parts of 5% sodium lauryl sulphate) was added. The developed color was red at 600 or 555nm for α- and β-naphthol produced from hydrolysis of the substrate, relatively. A-and β-naphthol standard curves were prepared by dissolving 20 mg α- or β-naphthol in 100ml phosphate buffer, pH17 (stock solution). Ten milliliters of stock solution were diluted up to 100ml by the buffer. Aliquots of 0.1, 0.2, 0.4, 0.8 and 1.6ml of watered solution (equal to 2, 4, 8, 16 and 32µg naphthol) were pipetted into test tubes and completed to 5ml by phosphate buffer. One milliliters of diazobule reagent was added and the developed color was determined as mentioned before.

**Estimation of glutathione-S-transferase (GST) activity:**

GST catalyzes the combination of reduced glutathione with 1-chloro 2, 4-dinitrobenzene (CDNB) via the SH group of glutathione. The conjugate, S-(2, 4-dinitrophenyl)-L-glutathione was revealed as (Habig et al., 1974).

The reaction mixture comprised of 1ml of potassium phosphate buffer (pH 6.5), 200µl of GSH and 200µl of haemolymph. The reaction begun by the addition of 25µl of the substrate CDNB solution. Enzymes and reagents were placing in incubator at 30°C for 5min. The increment in absorbance at 340nm was registered against sample blank inclusing the enzyme to mark the nanomole substrate conjugated/ min/ nymph using a molar extinction of coefficient of 9.6/mM/cm.

**Statistical analysis:**

The data were recorded, tabulated and subjected to statistical analysis using Ldp-line” log-Probit analysis software developed by Dr. Ehab Bakr, (Plant Protection Research Institute http://www. Ehabsoft.com according to (Finney 1971)), to obtain LC50 and slope. Also significantly different means were identified by analysis of variance (Tukey’s honestly significant difference) at P<0.05 using CoStat®software (Costat 2007).

**RESULTS AND DISCUSSION**

It is notable that; S. carpocapsae is most effective against treated fifth nymphal instars of the L. migratoria at three different concentrations (10, 20 and 40 IJs/g). The accumulative 100% of death was reached within 72h post treated; all nymphs were affected significantly by the nematode S. carpocapsae. The significant increase in commutative mortality of treated nymphs compared as control (Fig. 1). These results are in agreement with (Shairra 2009) who revealed increased mortality percent of S. gregaria nymphs with time increased at 48, 72 and 96h post infection with Steinernema glaseri nematode. And according to (Shereen et al., 2013) they test the effect of nematode Steinernema feltiae at concentrations 10, 50 and 100IJs/g were applied on the S. gregaria, whole fifth nymphal instars were susceptible to S. feltiae, with rise mortality rate registered. The data appeared that, the mortality of L. migratoria because of treated by the highest dose (40 IJs/g) of nematode that shows up post 24h after treatment. The data agree with (Boemare 2002) who noticed that; while the nematode (IJs) reaches the haemolymph of a host, the bacteria spreads and increases quickly in the haemolymph. Generally the mortality occurs during 24-72h despite the bacterium is basically in charge of the death of many insect hosts; the nematode additionally delivers a toxin that is deadly to an insect. Steinernema infectious juveniles (IJs) contain on cells of symbiotic bacteria in its gut and spread it into insect-haemolymph. The insect death caused by bacteria, giving perfect state for improvement and duplication of the nematodes. Almost three Steinernema reproductions can improve inside one insect dead body driving to the duplication of thousands of IJs (Zenner 2011). Parallel to evaluations of mortality, a few enzymes activity was estimated. All enzymes activity was elevated after 24-48h post treatment, LC50 value was 27.426 IJs/g sand (Fig. 2) with sample size was 75 insects. Parallel to evaluations of L. migratoria mortality, a few enzymes activity was evaluated. Phenoloxidase (PO) is a remarkable tool against different pathogens (Cerenius and Söderhäll, 2004). Information represented in (Table 1, Fig. 3) uncovered that, the nematode causes increasing in phenoloxidase activity, there was significant increase between treated and untreated nymphs after both 24-48h in the 5th nymphal instar, post treatment with S. carpocapsae. Results in agreement with Söderhäll and Cerenius (1998), who reported that; the major function of PO in melanogenesis is to transform phenols to quinones that consequently polymerize to shape melanin. Liu et al., (2009) indicated that PO may act a vital function in the rising resistance of pests to pesticides. Cao et al., (2016) The virulence of Metarhizium anisopliae IMI330189 was importantly higher than that of M. anisopliae IBC200614 to L. migratoria, IMI330189 might control the hemolymph immunity of locust by inhibiting the activities of phenoloxidase (PO) in locust. However M. anisopliae raise the activities of POD peroxidase in locust. The alterations of enzymes activities in time of the infection demonstrated that the time period between the second and the fifth day post treatment is necessary in the pathogenic development. Also,
(Nappi and Christensen 2005) the positive connection among PO phenoloxidase activity and succeeded pathogen defense hasn’t achieved wide support due to several studies were unsuccessful to determine the seriousness of the pathogen challenge used, for example failing to calculate the medium lethal dosage, or didn’t think that PO phenoloxidase might use for different functions moreover immunity, like pigment synthesis, egg production, molting or sclerotization. The fluctuating of Peroxidase (POD) activity was recorded at 24-48h post treatment. After 24h, there was significant decrease between treated and untreated nymphs, but after 48h values insignificantly different between treated and control group (Fig. 3). Our results in accordance with (Müller et al., 2007 and Wu et al., 2011) they obtained that, the increase in the activity of POD was in connection with the resistance of insects to pesticides (Table 1, Fig. 3). Also with (Shereen et al., 2012) who noted that; S. gregaria enzymes peroxidase, phenoloxidase activities were affected, altered between rising and declining by entomopathogenic fungus M. anisopliae, infectious. Total proteins, carbohydrates and lipids contents were drastically decreased in each treatment. Treatment with S. carpopocpsae induced significant alterations in α and β esterases activity when compared with control values. Nematode lead to alterations increasing and decreasing in esterases (ESTs) at 24-48h post treatment. In α esterase activity, there was no significant differences between treated and control after 24h. However, after 48h the activity declined significantly between treated and untreated nymphs in 5th nymphal instar, post treatment with S. carpopocpsae. In β esterase activity, after 24h, there was significant increase between treated and untreated nymphs. However, after 48h, there was insignificant between them (Table 1, Fig. 4). Our results in accordance with (Cao et al., 2016) who illustrated that the L. migratoria treated with M. anisopliae IMI330189, could control the humoral immunity of locust by inhibiting esterases activities in locusts. (Dubovskiy et al., 2012) mentioned that, the action of M. anisopliae was found to be accompanied by activation of detoxicating enzymes on L. migratoria after the 3rd day of the infection. Analysis of nonspecific esterase activity in the whole body homogenates of young instar of L. migratoria revealed a significant two-fold increase in esterase activity on the 3rd day after treatment by M. anisopliae.

![Cumulative % mortality of L. migratoria 5th nymphal instar infected with S. carpopocpsae with three concentrations of 10, 20 and 40 LIs/g sand. The resultant values are the cumulative of four replicates (n = 60), five nymphs per repeat.](image1)

**Table 1. Determination of some immunity enzyme activity of 5th nymphal instar of L. migratoria treated with LC₅₀=27.426 LIs/g of S. carpopocpsae at 24-48h.**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>24h Con.</th>
<th>Trea.</th>
<th>48h Con.</th>
<th>Trea.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenoloxidase</td>
<td>16.7±6.4</td>
<td>18.2±3.7</td>
<td>11.9±2.3</td>
<td>17.2±6.7</td>
</tr>
<tr>
<td>Peroxidase</td>
<td>58.4±6.7</td>
<td>20.1±1.4</td>
<td>73.9±7.6</td>
<td>123.5±8.6</td>
</tr>
<tr>
<td>Alpha esterases</td>
<td>30.9±7.3</td>
<td>59.6±6.2</td>
<td>31.9±2.0</td>
<td>50.4±6.7</td>
</tr>
<tr>
<td>Beta esterases</td>
<td>39.8±12.5</td>
<td>67.9±17.7</td>
<td>47.1±11.0</td>
<td>66.5±12.0</td>
</tr>
<tr>
<td>GST</td>
<td>33.4±3.4</td>
<td>31.6±1.7</td>
<td>46.9±2.9</td>
<td>35.8±2.9</td>
</tr>
</tbody>
</table>

Con. = control (untreated nymphs) and Trea. = Treated nymphs.

![Susceptibility of 5th nymphal instar of L. migratoria to different concentrations of S. carpopocpsae (10, 20 and 40 LIs/g), LC₅₀=27.426 LIs/g.](image2)

The present results revealed that; GST showed suppression in activity at treated nymphs comparing to control group after 24h, there was insignificant between treated and untreated nymphs. GST enzyme activity was significantly diminished because of application of S. carpopocpsae post 48h (Table 1, Fig. 5). Such fluctuation in GST activity might be because of chemical stress of biopesticides (Diamantino et al., 2001). (Nathan et al., 2005) revealed that numerous natural plant compounds applied in the control of insect pests are known to influence the enzymatic profiles. GST fluctuations could be the outcome of abnormalities in the functioning of the insect detoxificative system as consequences of the biopesticide treating. These fluctuations and the abnormal functions of GST can increase the sensitivity of the treated insects as noted by (Dubovskiy et al., 2008 and Dahi et al., 2009) mentioned that the application of biopesticides guides to decline in the Acetycholinsterase activity because of the sensitivity of insects to biopesticides, or guides to its rise because of the novel mode of action of recently biopesticides. Reduction in GST activity during the infection at 24-48h of nematodes may be associated with effective inhibition of the host defense systems by entomopathogenic nematodes. This suggestion is supported by studies of defense reactions of the desert locusts during the progress of M. anisopliae mycosis. In particular, an abrupt reduction in activity of phenoloxidases, antibacterial activity, and the total number of hemocytes was recorded in infected insects (Gillespie et al., 2000). The harmfulness of M. anisopliae IMI330189 was fundamentally higher than M. anisopliae IBC200614 to L. migratoria,
IMI330189 might control the humoral immunity of locust by increasing the activities of glutathione-S-transferases (GSTs). However, M. anisopliae IMI200614 inhibit the activities of GSTs in locust, the time span of infection among the second and the fifth day post treatment is basic in the pathogenic development (Cao et al., 2016).

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**REFERENCES**


It is obvious from these data that; the normal immunity functioning of L. migratoria is greatly disturbed after treated with nematode S. carpocapsae. The disturbance in detoxification enzymes activities showed that the toxic compounds contained in the present biopesticides may directly or indirectly influence the synthesis of these enzymes by modifying the cytomorphology of the cells (Nath 2000). Based on the obtained data, it can be suggested that the detoxicating system of locusts participates in the defense reactions against entomopathogenic nematode. It should be noted that the modern biotechnological methods is search for ways of alterations activity of insect defense systems, which would increase their susceptibility to entomopathogens, in particular, to nematode.

**Fig. 3.** Phenoloxidase (PO) and peroxidase (POD) activities of L. migratoria 5th nymphal instar treated with S. carpocapsae post 24-48h in the whole body homogenates (LC₅₀=27.426 IJs/g). The bars represent mean ± SD of the three repeats. (p < 0.05)

**Fig. 4.** α and β estrases activities of L. migratoria at 5th nymphal instar treated with S. carpocapsae 24-48h in the whole body homogenates (LC₅₀=27.426 IJs/g). The bars represent mean ± SD of the three repeats. (p<0.05)

**Fig. 5.** Activity of glutathione-S-transferase as compared with control after 24 – 48h whole body homogenates of L. migratoria at 5th nymphal instar infection with S. carpocapsae (LC₅₀=27.426 IJs/g). The bars represent mean±SD of the three repeats.

Con. = control (untreated nymphs) and Trea. = Treated nymphs. (p < 0.05)
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Steinernema carpocapsae


