J. Plant Prot. and Path., Mansoura Univ., Vol.9 (12): 877 – 881, 2018

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

Samira M. N. Abd-El Wahed[1] and Nabawia M. Elhadidy[2]
[1]Plant Protection Research Institute, Agric. Res. Center, Giza, Egypt
[2]Dept. 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* parasitation on *L. migratoria* immune defense was closely examined, after both 24-48h post treatment with LC_{50} of nematode the activities of phenoloxidase (PO), peroxidase (POD), α and β estrases (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, Perooxidase, α and β esterases, 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 (Magor *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 Capinera 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 peroxidases involve 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 esterases 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-Preczyina, Poland). Next homogenation, supernatants were kept in

freezer at -20ºC 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 2ºC 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 5^0C (Amin 1998).

Estimation of phenoloxidase (PO) activity:

Phenoloxidase activity was specified as (Ishaaya 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% o-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 (ΔOD_{340})/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 substratum, severally. The reaction mixture be composed of 5ml substratum settlement ($3x10^{-4}$M α-or β naphthylacetate, 1% acetone and 0.1M phosphate buffer, pH7) and 20µl of nymphal homogenate. The mixture was incubated for exactly 15min at 27ºC, then 1ml of diazoblue color reagent (prepared by mixing 2 parts of 1% diazoblue B and 5 parts of 5% sodium lauryl sulphate) was added. The developed color was read 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, pH7 (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 pippeted into test tubes and completed to 5ml by phosphate buffer. One milliliter of diazoblue 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-dinitro-phenyl)-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 30ºC for 5min. The increment in absorbance at 340nm was registered against blank including 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 LC_{50} 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, LC_{50} 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 5^{th} 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 humoral 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. carpocapsae 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. carpocapsae. 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 locust. (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.

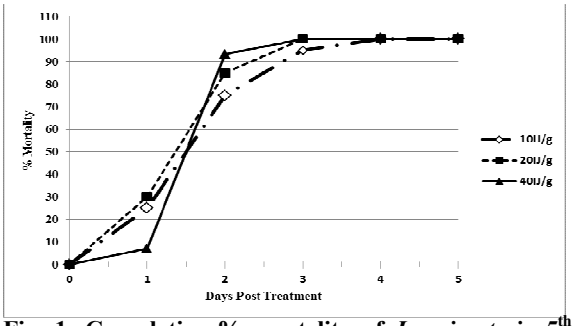

Fig. 1. Cumulative % mortality of *L. migratoria* 5th nymphal instar infected with *S. carpocapsae* with three concentrations of 10, 20 and 40 IJs/g sand. The resultant values are the cumulative of four replicates (n = 60), five nymphs per repeat.

Table 1. Determination of some immunity enzyme activity of 5th nymphal instar of *L. migratoria* treated with LC_{50}=27.426 IJs/g of *S. carpocapsae* at 24-48h.

Enzyme		24h		48h	
		Con.	Trea.	Con.	Trea.
O.D.units x10^3/min/g.b.wt	Phenoloxidase	1678±30.64b	1842±23.07a	1189±23.52b	1728.67±14.01a
	Peroxidase	5848.67±134.23a	4774±114.01b	2910±115.33a	3596.33±100.76a
ΔO.D.x 10^3 /min/g.b.wt	Alpha esterases	309.33±7.37a	598.67±6.028a	319.33±20.03a	504.67±16.5b
	Beta esterases	398.33±12.58b	679.33±17.67a	471.67±11.06a	665.67±12.50a
mmole sub.conjugated /min/g.b.wt	GST	33.4±3.42a	31.17±1.69a	46.97±2.95a	35.87±2.95b

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

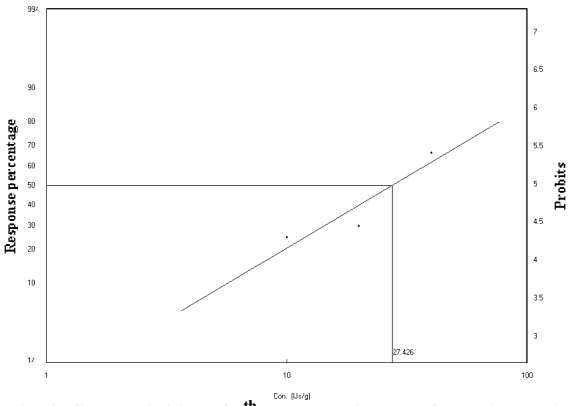

Fig. 2. Susceptibility of 5th nymphal instar of *L. migratoria,* to different concentrations of *S. carpocapsae* (10, 20 and 40 IJs/g.), (LC_{50}=27.426 IJs/g).

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. carpocapsae 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 Acetyle-cholinsterase 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 IBC200614 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).

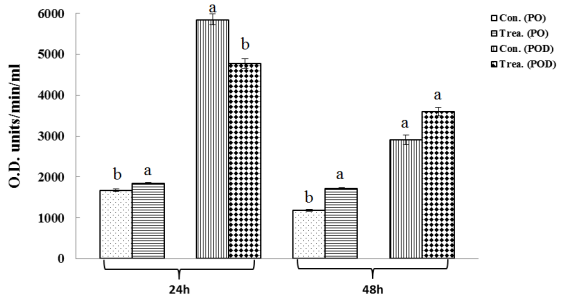

Fig. 3. Phenoloxidase (PO) and peroxidase (POD) activities of *L. migratoria* 5th nymphal instar treated with *S. carpocapsae* post 24-48h in the whole body homogenates (LC_{50}=27.426 IJs/g).

The bars represent mean ± SD of the three repeats. ($p < 0.05$.)

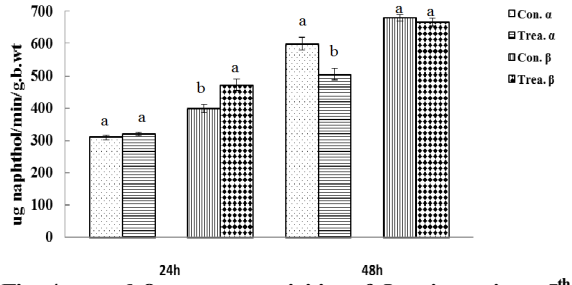

Fig. 4. α and β estrases activities of *L. migratoria* at 5th nymphal instar treated with *S. carpocapsae* 24-48h in the whole body homogenates (LC_{50}=27.426 IJs/g)

The bars represent mean ± SD of the three repeats.

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

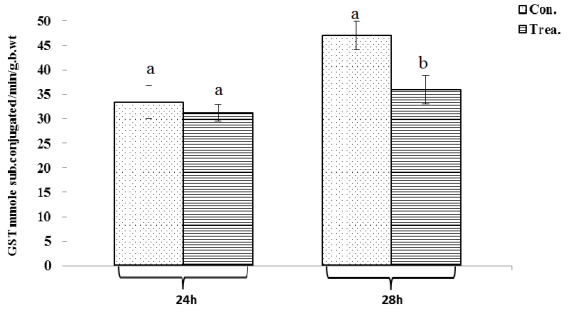

Fig. 5. Activity of glutathione-S-transferase as compared with control after 24 – 48h whole body homogenates of *L. migratoria* at 5th nymphal instar infection with *S. carpocapsae* (LC_{50}=27.426 IJs/g).

The bars represent mean±SD of the three repeats.

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

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.

ACKNOWLEDGMENT

The authors are grateful for the grants, relentless efforts and support provided by Prof. Ahmed Mohamed Azzazy from Plant Protection Research Institute for assistance in the rearing of G. mellonella and isolate S. carpocapsae nematode, also wish to express our sincere and profound gratitude to Dr. Soheer Ellakwah from Plant Protection Research Institute for her effort.

REFERENCES

Amin, T.R. 1998. Biochemical and physiological studies of some insect growth regulators on the cotton leafworm, *Spodoptera littoralis* (Boisd). Ph.D. thesis, Faculty of science, Cairo Univ.

Baker, G.L. and Capinera, J.L. 1997. Nematodes and nematomorphs as control agents of grasshoppers and locusts. *In* ''Microbial Control of Locusts and Grasshoppers'' (M. Goettel, and D. Johnson, Eds.), Memoirs Canadian Entomological Society, Ottawa, Canada, 171: 157-211.

Boemare, N. 2002. Biology, taxonomy, and systematics of photorabdus and xenorhabdus. In: I Gaugler, editor. "Entomopathogenic Nematology", CABI Publishing. New Jersey, USA, pp. 57-78.

Cao, G.; Jia, M.; Zhao, X.; Wang, L.; Tu, X. and Wang, G. (2016). Different Effects of *Metarhizium anisopliae* Strains IMI330189 and IBC200614 on Enzymes Activities and Hemocytes of *Locusta migratoria* L. PLoS ONE 11(5): e0155257. pmid:27227835

Cerenius, L. and Söderhäll, K. 2004. The prophenoloxidase-activating system in invertebrates. Immunol Rev. 1998:116-126. pmid:15199959.

Chapuis, M-P.; Lecoq, M.; Michalakis, Y.; Loiseau, A.; Sword, G. A.; Piry, S. and Estoup, A. 2008. "Do outbreaks affect genetic population structure? A worldwide survey in a pest plagued by microsatellite null alleles". Molecular Ecology. 17 (16): 3640–3653

Costat, 2007. Costat Software Package, CoHort Software Inc., Berkeley, CA, USA, release 5.5. http://www.cohort.com/costat.html.

Dahi, H.F.; El-Sayed, Y.A.; El-Barkey, N.M. and Abd-ElAziz, M.F. 2009. Toxicological and biochemical studies of methylamine avermactin, a new type of bioinsecticide against the cotton leaf worm, *Spodoptera littoralis* (Boisd). Egypt. Acad. J. Biol. Sci., 2(1): 103-116.

Diamantino, T.C.; Amadeu, E.; Soaresa, M.V. and Guilherminoc, L. 2001. Lactate dehydrogenase activity as an effect criterion in toxicity tests with *Daphnia magna* Straus. Chemosphere, 45: 553-560.

Dubovskiy, I.M.; Martemyanov, B.B.; Vorontsova, Y.L.; Rantala, M.J.; Gryzanova, E.V. and Glupov, V.V. 2008. Effect of the bacterial infection on the antioxidant activity and lipid peroxidation in the midgut of larvae *Galleria mellonella* L. (Lepidoptera: Pyralidae) Comp. Biochem. Physiol., 148: 1-5.

Dubovskiy, I.M.; Slyamova, N.D.; Kryukov, V.Y.; Yaroslavtseva, O.N.; Levchenko, M.V. and Belgibaeva, A.B. 2012. The activity of nonspecific esterases and glutathione-S-transferase in *Locusta migratoria* larvae infected with the fungus *Metarhizium anisopliae* (Ascomycota, Hypocreales).Entomol. Rev.92 (1):27-31

Dutky, S.R.; Thompson J.V. and Cantwell, G.E. 1964. A technique for the mass propagation of the DD-136 nematode. J. Insect Pathol. 6, 417-422.

Finney, D.J. 1971. Probit analysis, A statistical treatment of the sigmoid response curve, 7th edition, Cambridge University Press.

Gillespie, J.P.; Burnett, C. and Charnley, A.K. 2000. "The Immune Response of the Desert Locust *Schistocerca gregaria* during Mycosis of the Entomopathogenic Fungus *Metarhizium anisopliae* var acridum," J. Insect Physiol. 46, 429–437.

Habig, W.H.; Pabst, M.J. and Jakoby W.B. I974. Glutathione-S transferase the first enzymatic step in mercapturic acid formation. J. Biol. chem., 249:7130-7139.

Ishaaya, I. I971. Observations on the phenoloxidase system in the armored scales Aonidiella aurantii and chrysomphalus aonidum. Comp. Biochem. physiol., V39B:935-943.

Jessica, S. and Andreas, W. 1999. Successful parasitological of locusts by entomopathogenic nematodes is correlated with inhibition of insect phagocytes. Journal of Invertebrate Pathology 73, 154-161.

Kaya, H.K. 1985. Entomogenous nematodes for insect control in IPM systems. 283-302 pp. In: Biological control in Agricultural IPM Systems. (Eds.). Hoy, M.A. & Heezog, D.C. Academic Press, New York.

Li. X. 2007. Schuler MA, Berenbaum MR. Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics. Annu Rev Entomol. 52:231–253. pmid:16925478.

Liu, S.; Niu, H.; Xiao, T.; Xue, C.; Liu, Z. and Luo, W. 2009. Does Phenoloxidase Contributed to the Resistance? Selection with ButaneFipronil Enhanced its Activities from Diamondback Moths. The Open Biochemistry Journal, 2009, 3, 9-13.

Magor, J.I.; Lecoq, M. and Hunter, D.M. 2008. Preventive control and desert locust plagues. Crop Prot. 27:1527-1533.

Müller, P.; Donnelly, M.J. and Ranson, H. 2007. Transcription profiling of a recently colonised pyrethroid resistant *Anopheles gambiae* strain from Ghana. BMC Genomics. 8:36–47. pmid:17261191

Nappi, A.J.; and Christensen, B.M. 2005. Melanogenesis and associated cytotoxic reactions: applications to insect innate immunity. Insect Biochem Mol Biol. 35:443–459. PMID: 15804578.

Nath, B.S. 2000. Changes in carbohydrate metabolism in hemolymph and fat body of the silkworm, *Bombyx mori* L., exposed to organophosphorus insecticides. Pestic. Biochem. Physiol., 68: 127-137.

Nathan, S.S.; Kalaivan, K. and Chung, P.G. 2005. The effect of azadirachtin and nucleopolyhedrovirus on midgut enzymatic profile of *Spodoptera litura* Fab. (Lepidoptera: Noctuidae). Pestic. Biochem. Phys., 83 (1): 46-57.

Poinar, G.O. 1975. Entomogenous Nematodes-A Manual and Host List of Insect-NematodeAssociations. Brill, Leiden.

Roslavtseva, S.A.; Bakanova, E.I. and Eremina, O.Y.u. 1993. Esterases in arthropods and their role in the mechanisms of insect acaricide detoxication. Izv. RAN Ser. Biol. 3:368–375.

Shairra, S. A. (2009). Parasitizm of Locust by Entomopathogenic Nematode in Relation to Insect Microaggregation Inhibitor. J. biolog. Sci., 2 (2): 221-230

Shereen M. Elbanna; Nabawia M. Elhadidy and Ahmed, M. Azazy 2013. Physiological Changes of *Schistocerca gregaria* (Forskal) Nymphal Haemolymph Infected with the Nematode *Steinernema feltiae*. Egypt. J. Bio. Pest control. 23(1), 65-69.

Shereen M. Elbanna; Nabawia M. Elhadidy; Fayez M. Semida and Tayseer Abdel-Rasool. 2012. Physiological and biochemical effect of entomopathogenic fungus *Metarhizium anisopliae* on the 5th instar of *Schistcereca gregaria* (Orthoptera: Acrididae). J. Res. Environ. Sci. Toxicol. Vol. 1(1) pp. 007-018, February.

Söderhäll, K. and Cerenius, L. 1998. Role of the prophenoloxidase-activating system in invertebrate immunity. Curr Opin Immunol. 10(1):23–28. pmid:9523106.

Terriere, LC. 1984. Induction of detoxication enzymes in insects. Ann Rev Entomol. 29:71–88.

Van Asperen, K. I962. Astudy of house fly esterase by means of sensitive colourimetric method.J.Insect physiol., 8:401-41.

Vanden Broeck, J.; Chiou, S.J.; Schoofs, L.; Hamdaoui, A.; Vandenbussche, F.; Simonet, G.; Wataleb, S. and De Loof, A. 1998. Cloning of two cDNAs encoding three small serine protease inhibiting peptides from the desert locust *Schistocerca gregaria* and analysis of tissue-dependent and stage-dependent expression. Eur J Biochem 254: 90-5.

Vetter, J.L.; Steinberg, M.B. and Nelson, A.I. 1958. Quantitative determination of Peroxidaese in sweet corn. Agric. and food chem., vol. 6, No. 1:39-41.

Wu, Q.J.; Zhang, Y.J.; Xu, B.Y. and Zhang, W.J. 2011. The defending enzymes in abamectin resistant*Plutella xylostella*. Chinese Journal of Applied Entomology. 48(2):291–295.

Zenner, Annemie N. R. L. 2011. Factors influencing the outcome of male-male encounters in the entomopathogenic nematode *Steinernema* spp. Ph.D. thesis, Faculty of science, National University of Ireland Maynooth.

Zhao, X.; Smart, C.T.; Li, J. and Christensen, B.M. 2001. Aedes aegypti peroxidase gene characterization and developmental expression. Insect Biochem. Mol. Biol.,volume 31, page 481-90.

التأثيرات المناعية فى الجراد الإفريقى المهاجر *Locusta migratoria L.* بفعل النيماتودا الممرضة *Steinernema carpocapsae*

سميرة محمد نبيل عبد الواحد[1] و نبوية محمد الحديدى[2]

[1] معهد بحوث وقاية النباتات- مركز البحوث الزراعية- الدقي- جيزة - مصر

[2] قسم الحيوان – كلية العلوم – جامعة العريش – مصر

تم اختبار فعالية النيماتودا الممرضة *Steinernema carpocapsae* على حوريات العمر الخامس للجراد الافريقي المهاجر *Locusta migratoria L.* تحت الظروف المعملية. تمت معاملة الرمل باستخدام ثلاثة تراكيزات من النيماتودا (10، 20، 40 فرد نيماتودا/جرام رمل). وكانت النيماتودا فعالة وتسببت في موت حوريات العمر الخامس بنسبة 100٪ بعد اليوم الثالث من المعاملة. تم قياس نشاط انزيمات فينولوأكسيداز - البيروكسيديز - ألفا وبيتا استريزس - الجلوتاثيون اس ترانسفيرز المتحصل عليها من طحن الجسم بالكامل بعد 24- 48 ساعة بعد المعاملة بـ LC_{50} من النيماتودا. تسببت النيماتودا في موت حاد في الحشرات المعاملة مع الوقت، ويعود هذا للخلل في نظام نشاط الانزيمات ومناعة الحشرة. وهذا يعطي لمحة عامة عن كفاءة النيماتودا المستخدمة ضد حوريات الجراد الافريقي المهاجر.