Egyptian Journal of Biological Pest Control, 21(1), 2011, 11-17

Effect of the Entomopathogenic Nematode *Heterorhabditis bacteriophora* (Hp_{88}) and the Garlic Extract *Allium sativum* on the Immune Challenge of the Desert Locust, *Schistocerca gregaria* (Forskal)

Souad A. Shairra and Hanan H. Awad*

Biological Control Dep., Plant Protection Institute, Agriculture Research Center, Giza, Egypt
*Entomology Dep., Faculty of Science, Cairo University, Giza, Egypt E-mail: hananawad19@yahoo.com
(Received: December 10, 2010 and Accepted: January 18, 2011)

ABSTRACT

The 5th nymphal instar of *Schistocerca gregaria* (Forskal) was treated with different doses of the entomopathagenic nematodes *Heterorhabditis bacteriophora* Hp_{88} or *Allium sativum* (garlic) extract, as well as a combination of both of them. Activity of Hp_{88} and garlic extract showed a significant increase in the mortality by increasing the concentration and the post infection time. The study included also defense reaction of the treated nymphs' haemocytes. The role of garlic oil on treated nymphs was studied on the detoxification and the digestive enzymes. Activity of the lactatedehydrogenase (LDH) and the α-esterase increased significantly by increasing the post infection time. On the other hand, the activity of β-esterase increased significantly only after 3 hours post infection, but decreased significantly by increasing the post infection time, whereas the activity of the digestive enzymes and the amylase activity decreased significantly. Obtained results revealed that the studied biocontrol agents can be recommended for controlling *S. gregaria.*

Key words: locust, garlic oil, nematode, detoxification enzymes, digestive enzymes, immunity, haemocytes.

INTRODUCTION

Desert locust, *Schistocerca gregaria* (Forsk.) is a major pest in northern Africa and Middle East. Entomopathagenic nematodes of the family Heterorhabditidae have been used commercially as biocontrol agents of insect pests (Georgis, 1992 & Shairra, 2007).

Insecticidal properties of *Allium sativum* (garlic) extract were attributed to sulfur volatiles produced during degradation of garlic tissues (Auger *et al.*, 2004) and their activities showed greater toxicity on insect pests. Awad (2008) determined the insecticidal activities of garlic extract on *Agrotis ipsilon* larvae. However some investigators have demonstrated the toxic effects of garlic extracts among other medicinal plants extracts (Pavela, 2004). Insect cellular defense mechanisms against invaders include phagocytosis, nodulation, and encapsulation (Miller 1999).

The aim of the present work was to investigate the susceptibility and the haemocytes immune reactions of the 5th nymphal instar of *S. gregaria* to the entomopathogenic nematodes. The activities of the detoxification enzymes (lactate dehydrogenase (LDH), α-esterase and β-esterase) and some digestive enzymes were also studied using garlic extracts. Such studies may throw new highlights for controlling grasshoppers or at least enable to improve strategies of using biocontrol agents in integrated pest management programs (IPM).

MATERIALS AND METHODS

S. gregaria was obtained from the stock culture maintained in Entomology Department, Faculty of Science, Cairo University, Giza, Egypt.

The nematode species, *Heterorhabditis bacteriophora* Hp_{88} used was supplied by Dr.\ M. M. Shamseldean, Zoology and Nematology Department, Faculty of Agriculture, Cairo University, Egypt. The nematode was reared according the method described by Sheble (2002).

Essential oil of *Allium sativum* (Family: Liliaceae) (garlic), was obtained from the AROMISR Company in Giza, Egypt.

Bioassay

To evaluate the susceptibility of 5th nymphal instar of *S. gregaria* towards the nematode species Hp_{88}, individuals were allowed to feed separately on fresh clover leaves sprayed with a suspension of IJs in distilled water at the doses of 400, 200 and 100 IJs/ml for 48h .

The garlic extract concentrations were prepared from the stock solution 1% concentration. The concentrations 1, 0.50, 0.25, 0.125 and 0.063% were prepared by dilution with water and adding 2 drops of Tween 80 as emulsifying agent. The nymphs were allowed to feed separately for 48h on fresh clover leaves sprayed with 5ml of garlic oil emulsion at the above mentioned concentrations.

To detect the combined effect of both the nematode Hp_{88} and the garlic extract, fresh clover leaves were sprayed with 5ml of garlic oil emulsion; the leaves were left to dry and then sprayed by a suspension of Hp_{88} in distilled water at the dose of 100 IJs/ml/. The nymphs were allowed to feed for 48h. All the experiments were conducted under regulated laboratory conditions at 30 ± 2°C, 65 ± 5%

RH. and 14:10 LD. The control was conducted using clover leaves without any treatment. Three replicates were used for each treatment, with a total of 45 nymphs. Fresh clover leaves, 200 mg, were used for each treatment. Data on mortalities were calculated after 24h and compared with that resulted after 48, 72, and 96h post treatment, and then corrected using Abbott's formula (1925).

Studies of haemocytes

Haemocytes of the nymphs treated with *H. bacteriophora* Hp_{88} or *A. sativum* extract, as well as a combination of both of them were examined according to the method of Arnold (1979). The 5th nymphal instar was injected with saline solution (10 µl) containing (5) Hp_{88} IJs / nymph after 48h of feeding the nymphs on treated clover leaves with a concentration of LC_{60} of garlic extract. Another group of nymphs were injected with saline solution (10 µl) after 48h of feeding on clover leaves sprayed with a concentration of LC_{60} of the garlic extract. Moreover, a third group of nymphs were injected with saline solution (10 µl) containing (5) Hp_{88} IJs /nymph. Control insects were injected only with equivalent amount of saline solution.

Biochemical assay

Chemical analysis of the haemolymph of the treated nymphs with garlic extract at the concentration of LC_{60}, after 48 hours of feeding, was performed to detect the activity the detoxification enzymes. The method described for determination of lactate dehydrogenase (LDH), was derived from the formulation recommended by DGKC (1972). Alpha esterase (α- esterase) and beta esterase (β-esterase) were determined according to Van Asperen (1962), using α-naphthyl acetate or β-naphthyl acetate as a substrate, respectively. Digestive enzymes were determined according to the method described by Ishaaya and Swirski (1976) using trehalose, sucrose, and soluble starch as substrates for trehalase, invertase, and α-amylase, respectively. The treated nymphs with garlic extract (LC_{60}) and control were dissected in saline buffer to remove the gut tissue after 48 hours of feeding.

Statistical analysis

Data were analyzed by Student's t test. All analyses were performed using the statistical package for the social sciences (SPSS) for windows (V. 11) Difference between means was significant at $P \leq 0.01$ and $P \leq 0.001$ and the percentage difference was calculated.

RESULTS AND DISCUSSION

Bioassay

Fig. (1) shows that nymphal mortality significantly increased by increasing the dose, (100,200, and 400 IJ/nymph) of Hp_{88} and the post infection time. After 48h, the mortality increased by 30, 53, and 60% as compared to the control. These differences increased by increasing the post infection time to 72h recording 70, 85, and 90%. The mortality percentages increased insignificantly after 96h post infection.

Fig. (2) shows that mortality percentage after the 5th nymphal instar was fed on clover leaves sprayed with the essential oil of garlic for 48h significantly increased as the post infection time increased. The garlic oil had a clear insecticidal effect on *S. gregaria* nymphs. Fig. (3) shows activity of nematode Hp_{88} after 48 and 72h post treatment as the highest mortality percentages at the dose of 100, 200, and 400 IJs/ml/nymph were recorded. The treated nymphs with garlic essential oil at concentration of LC_{60}, (0.063% ml) caused increasing in the initial effect of the nematode and the dose of 100 and 200 IJs/ml caused 90 and 100% mortality after 48h post infection.

Survived nymphs had reduced size and delayed development as compared to the control. Awad (2008) reported a pronounced prolongation in the larval duration and a reduction in the larval weight of *Agrotis ipsilon* when fed on leaves sprayed with garlic oil.

It may be suggested that the nematode produced a synergistic effect to the insect pest treated with garlic extract. This may explain the ability of the nematode to slow down the detoxification of the garlic oil used. Moreover, temporal synergism occurred when both nematode and garlic were used as two bio-insecticides together enhancing the mortality of insects more than either component alone. This synergism may be of economic importance in the control of locusts as well as among numerous harmful insects.

Studies of haemocytes

Plate (1) shows normal haemocytes of the 5th nymphal instar of *S. gregaria* which were morphologically characterized by Gupta (1979). Several pathological changes were observed due to Hp88 infection. Released bacteria, *Ph. Luminescens*, from the nematode Hp_{88}, started to get out during 12 hours post-infection. The Plasmatocytes (Pls), Granuolocytes (Grs) and Oenocytes (Oes) reacted with these bacteria as shown in Plate (2); the changes showed enlargement in the cell volume with vacuolization in the cytoplasm, compared to the normal cells, there was also a hemolytic response, phagocytes and nodule formation. The Pls and Oes

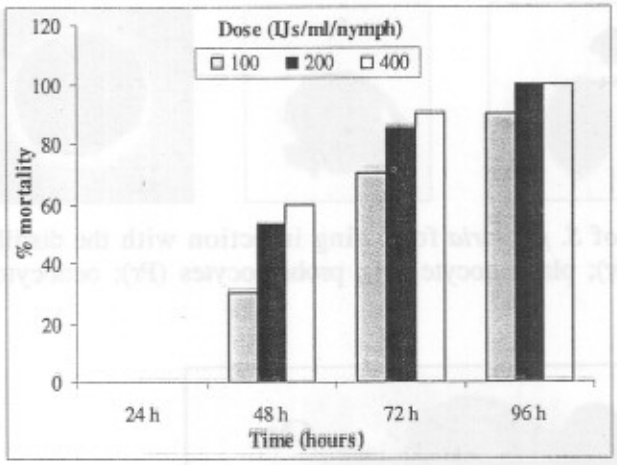

Fig. (1): Mortality of 5th nymphal instar of *S. gregaria* fed on treated clover leaves treated with *H. bacteriophora* (Hp_{88}).

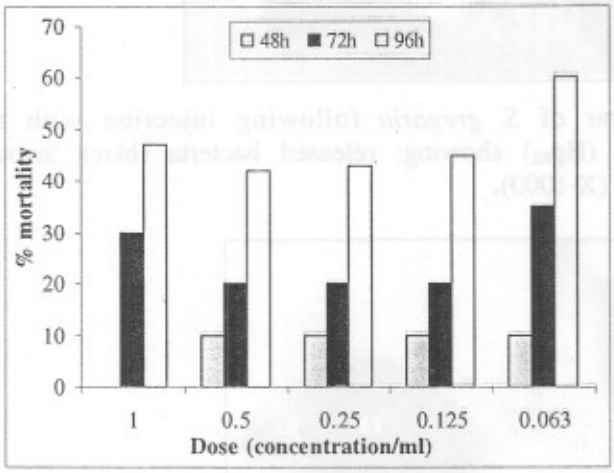

Fig. (2): Mortality of 5th nymphal instar of *S. gregaria* fed on treated clover leaves with garlic essential oils at different concentrations for 48 hours.

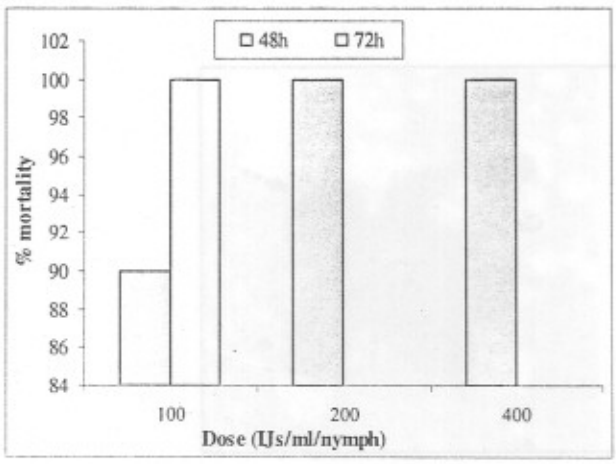

Fig. (3): Mortality of 5th nymphal instar of *S. gregaria* fed on treated clover leaves for 48 hours treated with *H. bacteriophora* (Hp_{88}) at 100 IJs/ml and *Allium sativum* (garlic) essential oils at 0.063% (ml).

were swallowed with ruptured plasma membrane. At the same time, only the Oes showed irregular dark appearance and extrusion of their cytoplasm, whereas, the Grs showed shrinkage with distortion in the cell membrane and undistinguished nucleus. Treated nymphs with garlic oil, showed swollen and enlarged Pls, compared to healthy cells (Plate 3).

Concerning the combined effect of the entomopathogenic nematodes Hp_{88} and garlic oil, the Grs appeared damaged as indicated by vacuolization, lysis of cell membrane with irregular appearance, extrusion of their cytoplasm and nodules were observed, there was hemolytic response, phagocytes and nodule formation, from the engulfed bacteria by the Grs, which had an irregular outline and the bacteria, that directed toward the cells were bending and adhering at the cell boundary (Plate 4).

From the present data, it is clear that the haemocytic responses of the insects were variable and the nodules appeared to be combined with melanin deposition on the periphery. The damaged cells died due to the phagocytosis of the bacteria or due to the toxic particles of garlic and melanization of the foreign materials. Ribeiroand and Brehélin (2006) stated that the size, maturation and the durability of nodules depend upon pathogenicity of the invaders and the immune response of the insects' species studied. Similar results were obtained by Brakat *et al.* (2002) on *S. gregaria* injected with bacteria. Dunphy and Webster (1988) stated that the nodule formation in response to the injection of *S. gregaria* nymphs with the nematode Hp_{88} together with its symbiotic bacteria *Ph. luminescens* may indicate the important role this bacterial species plays in inducing host insect immune reactions. On the other hand, damage and death of haemocytes by the haemocytotoxin "lipopolysaccharide" released from the outer membrane of bacteria into the haemolymph; these toxins contain fatty acid that damage the haemocyte, or it may follow the suggestion that the initial haemocyte reaction could be analogues to a margination effect, where the haemocytes would tend to adhere to the wall of the haemocoel and to each other, after which some cells return into circulation (Ayaad *et al.*, 2008).

Gupta (1979) concluded that the cellular immune reactions may involve the GRs, PLs and Oes. He added that the oenocytoids contained large amount of phenoloxidase playing a role in recognition of foreign invaders. Abd El-Aziz and Awad (2010, a&b), reported that degenerative changes lead to lyses of haemocytes on *A. ipsilon* larvae following bacterial infection and showed significant decrease in the oenocytoids count and significant increase in the activation of phenoloxidase.

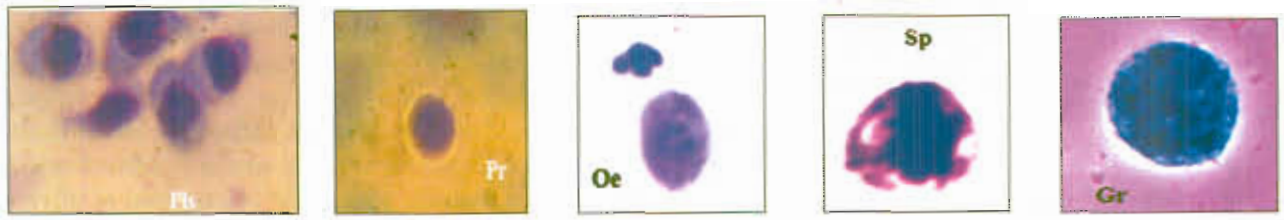

Plate (1): Haemocytes of the 5th nymphal instar of *S. gregaria* following injection with the distilled water served as control showing: granulocyte (Gr); plasmatocyte (Pl); prohemocytes (Pr); oenocytoid (Oe) and spherulocytes (Sp). (X 1000).

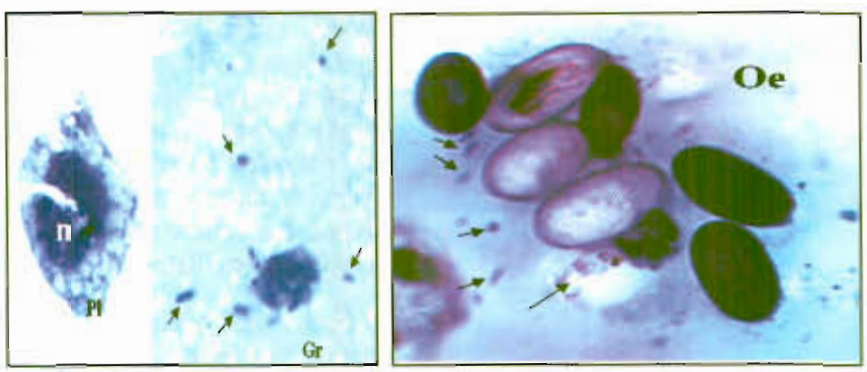

Plate (2): Haemocytes of the 5th nymphal instar of *S. gregaria* following injection with the entomopathogenic nematodes, *H. bacteriophora* (Hp_{88}) showing: released bacteria (black arrow), granuolocytes (Gr), nucleus (n), plasmatocyte (Pl). (X 1000).

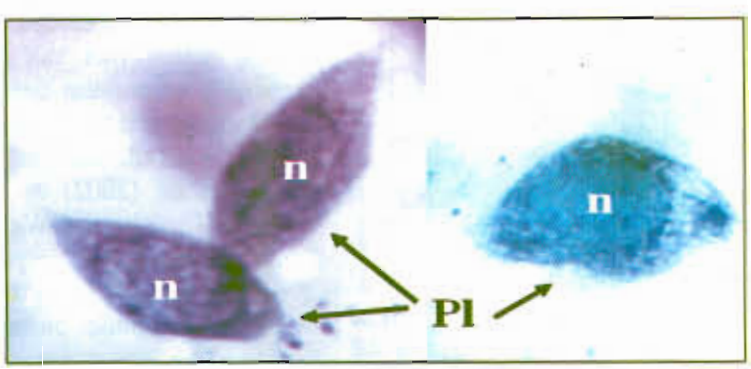

Plate (3): Haemocytes of the 5th nymphal instar of *S. gregaria* fed on clover leaves treated with garlic extract and injected with saline showing: swollen and enlarged plasmatocyte (Pl), nucleus (n) (X 1000).

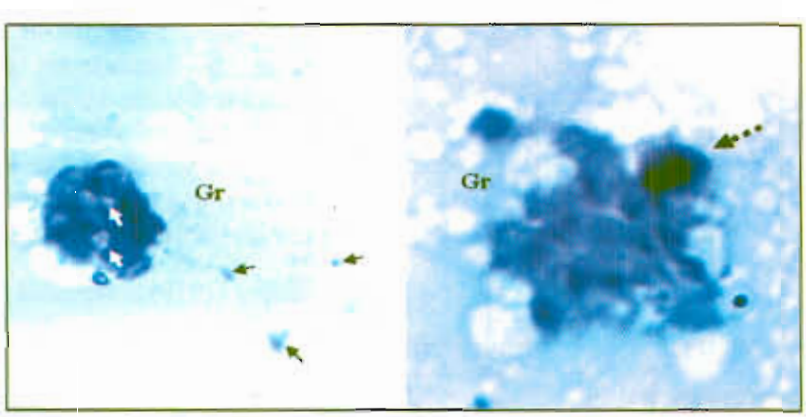

Plate (4): Haemocytes of the 5th nymphal instar of *S. gregaria* following injection with the entomopathogenic nematodes, *H.s bacteriophora* (Hb_{88}) and fed on clover leaves treated with garlic extract showing: granuolocyte (Gr), nodule (dashed arrow),engulfed bacteria (white arrow), bacteria (black arrow). (X 1000).

Biochemical analysis using garlic extract

Due to the high mortality of the treated nymphs with the nematode Hp_{88} after 48h, the percentage of mortality was 90 and 100% with the lowest and highest doses used, respectively. So it was not possible to carry out the biochemical studies on Hp_{88} infected *S. gregaria* nymphs. Also, preliminary test was carried out under light microscopy to ensure that garlic oil had a negative effect on the symbiotic bacteria *Ph. luminescens*.

Table (1) shows that the LDH activity in *S. gregaria* nymphs had significant increase by 1.12, 2.89, and 2.10% as compared to the control after 6, 12 and 24 h, respectively. This implies the release of LDH that may be used as an indicator of cell lyses (Wu and Lam, 1997). Nathan *et al.* (2005) mentioned that feeding of *Spodoptera litura* on leaves treated with azadirachtin and nucleopolyhedrovirus decreased the amount of this enzyme in midgut that demonstrated low nutritional efficiency of the larvae. Therefore, it may be concluded that in the infected and dead cells in the gut tissue, their LDH was released and found its way into the haemolymph. It was also postulated that the haemocyte damage in the tested nymphs treated with garlic oil and the clear increase in the LDH activity might offer a marker for monitoring the biotoxicity of the insecticidal action of the garlic oil. Wu *et al.*, (1992) clarified that LDH is an important glycolytic enzyme being present in virtually all tissues; it is also involved in carbohydrate metabolism and has been used as an indicative criterion of exposure to chemical stress. LDH catalyzes the conversion of lactate pyruvate, this is an important step in the energy production in cells (Zibaee *et al.*, 2008).

α-esterase showed a significant increase by 1.47, 1.83, 1.55, and 1.05% as compared to the control, after 3, 6, 12 and 24 h, respectively. β-esterase showed a significant increase by 1.04% as compared to the control, after 3 hours and a significant decrease by 0.90, 0.94, and 0.32% as compared to the control after 6, 12, and 24 h, respectively (Table 1).

This indicates that the enzyme was consumed during the metabolism of the treated ingested food with garlic extract. The defensive mechanisms and biochemical reactions are involved in the detoxification processes against any chemical insecticides. These mechanisms predominantly involve either metabolic detoxification of the insecticide before it reaches its target site, or the sensitivity changes of the target site so that it is no longer susceptible to insecticide inhibition The most common metabolic resistance mechanisms involve esterase. However, the general decrease in the activity of β - esterase may indicate that esterase was not involved in the detoxification process of garlic oil. The enzyme inhibition was significantly higher than control. In addition to the antifeedant activity of garlic oil, added a stress on the enzyme expression system to synthesize new and higher amounts of detoxification enzymes which could be the possible reasons for the arrested growth and increase mortality percentage recorded. Survived nymphs had reduced size and delayed development as compared to the control ones. A growth regulator such as juvenile hormone could be assumed to be the cause of the long nymphal periods due the high non-specific esterase induction. On the other hand, numerous studies have demonstrated that esterase plays an important role in conferring or contributing to insecticide detoxifications in insect (Fahmy and Dahi, 2009).

Table (1): Determination of lactate dehydrogenase (LDH) and α-esterase, *β*-esterase in the haemolymph of 5th nymphal instar of *Schistocerca gregaria* treated with LC_{60} garlic extract

Enzyme		Control	Treated	% difference	Significance
Lactate Dehydrogenase (LDH) (U x 10^3 /ml)	3h	66±0.87	65±1.15	- 1.52	n.s.
	6h	74±1.73	83±2.13	12.16	*
	12h	80±1.15	231±2.31	188.75	**
	24h	177±2.31	373±4.62	110.73	**
α – esterase μg α – naphthol released/min./ml	3h	4478±31.76	6589±93.53	47.14	**
	6h	1521±5.77	2778±52.24	82.64	**
	12h	1081±13.28	1675±8.08	54.95	**
	24h	1172±3.46	1233±6.35	5.20	*
B – esterase μg β – naphthol released/min./ml	3h	896±8.66	926±12.7	3.35	*
	6h	600±4.04	579±1.73	- 3.5	*
	12h	480 ± 5.77	452±3.46	- 5.8	*
	24h	545±6.93	177±1.73	-67.52	**

Values represent the means ± SEM and (n= 45) *p≤0.01, ** p≤0.001 significant, (n.s.) non significant

Table (2): Determination of the digestive enzymes in the gut of 5th nymphal instar of *Schistocerca gregaria* treated with LC_{60} garlic extract

Enzymes µg glucose/min/gm gut	Control	Treated	% difference	Significance
Trehalase	194±2.89	189±1.15	-2.58	n.s.
Invertase	1407±24.25	1346±9.24	- 4.34	n.s.
Amylase	62±1.73	29±1.1	-53.23	**

Values represent the means ± SEM and (n=45) ** $p \leq 0.001$ significant, (n.s.) non significant

Table (2) shows the decrease in the gut digestive enzymes in treated nymphs. The amylase activity decreased significantly to nearly half that of the control. However, trehalase and invertase activities showed insignificant decrease as compared to the control.

It was clear that the digestive enzyme synthesis in the treated nymphs were highly decreased compared to the control. This depends mostly on the secretion rate of the enzymes. Trehalase, invertase and amylase. The results agree with those of Terra and Ferreira (1994). This is particularly true on the treatment with the garlic extract. The amylase activity significantly decreased compared to trehalase and invertase. On the other hand, the gut amylase decreased and was more sensitive to garlic oil than trehalase, and invertase. In Orthoptera, *S. gregaria* nymphs, the salivary enzymes contribute to the early stages of digestion within the gut, an amylase is commonly present. Thus, the mastication process is important in digestion. The studied enzymes, decreased steadily during the treatment with garlic oil and this clears that the enzyme production decreased due to the treatment of the ingested food with garlic extract and leads to the control of the enzyme synthesis. On the other hand, trehalase is an important enzyme in which insects degrade trehalose to glucose for internal energy supply, thus the activity of trehalose might serve as an indicator of energy reserves resulting from availability of carbohydrate nutrients. Trehalase activity is closely linked to alteration in physiological conditions or development, indicating that this enzyme plays an important role in such biological functions as homeostasis and developmental events (Silva, *et al.*, 2004).

It may be concluded that the high nymphal mortality of *S. gregaria* treated with entomopathogenic nematodes, (Hp_{88}), and garlic extract, alone or combined, may be due to the toxic effects of these treatments. Since each treatment alone resulted in high mortality rate, the single application of each is recommended and may be investigated both bioinsecticides could be used on a wide scale together in integrated pest management programs on different pests in the field, since they are safe and do not cause the environmental pollution, which is the most dangerous drawback of chemical insecticides. Alternative strategies have included the search for new types of pesticides which are often effective against a limited number of specific target species, are biodegradable into nontoxic products and are suitable for use in integrated pest management programs. The use of botanical pesticides is now emerging as one. The infection of *S. gregaria* with the biopesticides, nematode and garlic oil is characterized by specific increase of the activity of the detoxification enzymes and cytopathological changes in haemocyte composition. These changes may play a role in the cellular immune response of insects to foreign substances. The present work is a contribution to assist in understanding the biochemical and pathological response of the insects to the used bioinsecticide as well as the resistance mechanisms that may arise in the future.

REFERENCES

Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18, 265–267.

Abd El-Aziz, N. IM. and H. H. Awad 2010a. Changes in the haemocytes of *Agrotis ipsilon* larvae (Lepidoptera: Noctuidae) in relation to dimilin and *Bacillus thuringiensis* infections. Micron 41 (2010) 203–209.

Abd El-Aziz, N. IM. and H. H. Awad 2010b. Immune response in *Agrotis ipsilon* larvae (Lepidoptera: Noctuidae) induced by *Bacillus thuringiensis* and Dimiline. Egypt. J. Boil. Pest Cont., 20 (1):7-13.

Arnold, J.W., 1979. Controversies about haemocyte types in insects. In: Gupta, A.P. (Ed.), Insect Haemocytes. Cambridge University Press, Cambridge, pp. 231–508.

Auger, J., I. Arnault, S. Diwo-Allain, M. Ravier, F. molia, M. Pettiti, C. Innophyt and O. Cedex 2004. Insecticidal and fungicidal potential of *Allium* substances as biofumigants. Agroindustria. (3): pp 5-8.

Awad, H. H. 2008. Physiological and Histopathological effects of essential oils of some local medicinal plants on the control of the black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae). Egy. J. Zool, 88:50-67.

Ayaad T.H., N. M. Zohdi, S. A. Shairra and A. A. Ibrahim 2008. Effects of entomopathogenic nematodes and some pharmaceutical inhibitors of eicosanoid biosynthesis on the desert locust *Shistocerca gregaria* (Forskal).J. Biol. Pet Control. 18(1): 105-118.

Barakat, E. M. S., W. S. Meshrif, and M. G. Shehta, 2002. Changes in the haemolymph of the desert locust *Shistocerca gregaria* (Forskal) after injection with *Bacillus thuringiensis* J. Egypt. Acad. Soc. Environ. Develop., 2(1): 95-115.

Deutsche Gessellschaft für Klinische Chemie 1972. Empfehlungender Deutschen Gessellschaft für Klinische Chemie (DGCK). J. clin. Chem. Biochem.10:182-193.

Dunphy, G.B. and Webster, J.M. 1988. Virulence mechanisms of *Heterorabditis heliothidis* and its bacteria associated with *Xenorhabdus luminescens* in non-immune larvae of the greater wax moth, *Galleria mellonella.* J. Pathol., 18:779- 737.

Fahmy, N. M. and H. F. Dahi 2009. Changes in detoxifying enzymes and carbohydrate metabolism associated with spinetoram in two field-collected strains of *Spodoptera littoralis* (Biosd.). Egypt. Acad. J. biolog. Sci., 1(1): 15-26.

Georgis, R.1992. Present and future prospects for entomopathogenic nematode products . Biocontrol Sci .Tech., 2: 83-89.

Gupta, A.P. 1979. Identification of insect blood cells. In: Gupta, A.P. (Ed.), Insect

Hemocytes. Cambridge University Press, London, pp. 527–529.

Ishaaya, I. and E. Swirski 1976. Trehalase, invertase and amylase activities in the black scale *Saissetia oleae*, and their relation to host adaptability. J. Insect. Physiol., 16: 1025-1029.

Miller, J. S., R. W. Howard, R. L. Rana, H. Tunaz, and D. W. Stanley, 1999. Eicosanoids mediate nodulation reactions to bacterial infections in adults of the cricket, *Gryllus assimilis*. J. Insect Physiol., 45: 75-83.

Nathan, S. S., K. Kalaivani and P. G. Chung 2005. The effects of azadirachtin and nucleopolyhedrovirus on midgut enzymatic profile of *Spodoptera litura* Fab. (Lepidoptera: Noctuidae). Pestic. Biochem. Physiol. 83:46–57.

Pavela, R. 2004. Insecticidal activity of certain medicinal plants. Available on line (17 November 2004).

Ribeiroand, C. and M. Brehélin 2006. Insect haemocytes: What type of cell is that? J. insect Physiol. 52: 417-429.

Shairra, S. A. 2007. Effects of entomopathogenic nematodes and some pharmaceutical inhibitorsof eicosanoid biosynthesis on the desert locust *Schistocerca gregaria* (Forscal)(.Ph D. Thesis, Fac. Sci., Cairo Univ., Egypt, 65.

Sheble, D E. A. 2002. Reproduction potential of five entomopathogenic nematodes of the Genus *Heterorhabditis* .Egypt .J .Biol .Pest Control., 12 (1): 5-9.

Silva, M.C.P., W.R. Terra, and C. Ferreira 2004. The role of carboxyl, guanidine and imidazole groups in catalysis by a midgut trehalase purified from an insect larvae, Insect Biochem. Mol. 34:1089-1099.

Terra, R.W. and C. Ferreira 1994. Insect digestive enzymes: properties, compartmentalization and function. Comp. Biochem. physiol. 109 (b). 1-62.

Van Asperen, K. 1962. A study of housefly esterase by means of sensitive colourimetric method .J. Insect Physiol. 8: 410-416.

Wu, R.S.S. and P.K.S. Lam 1997. Glucose- 6-phosphate dehydrogenase and lactate dehydrogenase in the greenlipped mussel (Perna viridis). Possible biomarker for hypoxia in the marine environment. Water Res. 31:138-142.

Wu, J., A. J. Daugulis, P. Faulkner and M. F. A. Goosen 1992. Correlation of LDH activity with loss of insect cell viability: An assessment of the L.D.H. Assay. BioTech. Techniques, (6): 4, 335-340.

Zibaee A., J.J. Sendi, F. Alinia and K. Etebari 2008. A study on biochemical differences among five different groups of rice striped stem borer *Chilo suppressalis* Walker (Lepidoptera: Pyralidae). I.S.J. 5: 20-29.