Journal of Life Sciences Research
ISSN : 2408-9184
Vol. 3, No. 1, 1-7, 2016
http://asianonlinejournals.com/index.php/Lifsc

Immunological and Histopathological Effect of Plant Extract on *Schistocerca Gregaria*

***Badriah M. K. Asiri*[1]**

[1]Department of Biology, Faculty of Science for Girls, King Abdulaziz University, Jeddah, Saudi Arabia

Abstract

The desert Locust, *Schistocerca gregaria* (Forsk) is one of the serious pests attacking vegetables and wide varieties of other field crops in Egypt and other different countries.The present study was also extended to conduct the insecticidal effect of the most potent extracts *Citrullus colocynthis*, and *Guayacum officinalis* extract post formulation on *S. gergaria* larvae. The present study has been conducted to investigate the effect of plant extract on differential and total haemocyte counts and midgut histopathologiocal changes of *S. gregari*a Four types of haemocytes were characterized; prohaemocytes (PRs), plasmatocytes (PLs), granulocytes (GRs) and oenocytoids (OEs). The percentage of PRs decreased in insects fed on leaves treated with *Citrullus colocynthis*, and *Guayacum officinalis* plant The percentage of PLs increased, while the percentage of GRs decreased in all tested insects treated. The percentages of oenocytoids (OEs) increased in insect fed treated compared with control. Results indicated that THCs of insects fed on leaves treated significantly decreased in all insects treated. also, showed highly histopathological disturbances in the midgut and body wall cells of this pest. Among the most recorded observations are vacuolation, destruction of the cells.

Keywords: Grasshopper *schistocerca gregaria*, Immunological, Histopathological studies.

Contents

Citation | Badriah M. K. Asiri (2016). Immunological and Histopathological Effect of Plant Extract on *Schistocerca Gregaria*. Journal of Life Sciences Research, 3(1): 1-7.
DOI: 10.20448/journal.504/2016.3.1/504.1.1.7
ISSN | 2408-9184

1. Introduction

The desert locust, *S. gregaria* Forskal, is the most dangerous herbivore species of locust and attacking vegetables and wide varieties of other field crops in Egypt and other different countries [1]. Specialist and generalist insect herbivore species often differ in how they respond to host plant traits and these responses can include weakened or strengthened immune responses to pathogens [2].

Recently, it has been hypothesized that the immune function of herbivorous insects can be altered by their host plant, thus generating variation in their susceptibility to entomopathogens [3]. When phytophagous insects are reared on host plants with differing nutritional qualities and defence chemicals, it is expected that the baseline defence response would also differ. Invasion by a pathogen can induce the cellular and humoral defence response to higher levels [4]. Poor nutritional quality may result in less investment in immune factors resulting in lower resistance to pathogens or cause physiological changes that may influence the entry of pathogens via the mid-gut or the cuticle [5]; [6]. Showed that diet alters the structure of the peritrophic matrix (PM) in Lepidoptera larvae, a change that can have a profound influence on pathogen infection. Also hemocytes have a key role in the cellular component of immune defense [7]. Accurate methods to measure immune response in the presence and absence of pathogens are necessary to determine whether susceptibility to these natural enemies is reduced or increased by host plant traits [2]

Many studies have reported that plants are one of the richest sources which can be used to pest control agents. They attended to use extract as toxicants, repellents synergists, growth regulators and antifeedants for many insect pests [8, 9]. In Egypt, attempts have been done to monitor insecticidal activity of different plants extracts against many insects [10, 11]. In Egypt, attempts have been done to monitor insecticidal activity of different plant extracts against many insects [10, 11] including *S.gergaria*.

2. Materials and Methods

2.1. Experimental Insects

Desert Locust (*Schistocerca gregaria*, Family: Orthoptera) The susceptible strain of locust. *Schistocerca gregaria* was obtained from Desert Locust Research Department. Ministry of Agriculture. Dokki. Giza being used throughout the present investigation. It was reared on clover leaves. The tests were carried out on the 2th instars nymph.

2.1.1. Tested Plants

The plant used in this study *Citrullus colocynthis* (Cucurbitaceae), and *Guayacum officinalis* (Zygophyllaceae) were collected from fields of Jeddah , Saudi Arabia during flowering stages and identified in Botany department. Faculty of Science, Cairo University. The collected plant leaves were left to dry in air and then in an oven at 50°C [12]. Plants were left to dry in air and then in an oven at and powdered by a mixer.

2.1.2. Extraction

Solvent (methylene chloride) was used for extraction. A stock extract was prepared soaking 200 g of the plant powder in 800 ml of methylene chloride for 4 days at room temperature (25±1±C),. The combined extract was filtered over anhydrous sodium sulphate. The solvent was then evaporated under reduced pressure using a rotator evaporator at 30^{o}. Afterwards, the remaining powder was soaked again in 800 ml of methylene chloride by the same procedure. The extracted solutions were left away for complete dryness to obtain the crude extracts. The extracts of *Citrullus colocynthis, Guayacum officinalis* were carried out by Freedman, et al. [13] method with some modification the crude extract was transferred quantitively to a clean and weighted flask and kept in the refrigerator until used for Biological and toxicological investigation.

2.2. Preparation of the Tested Concentrations

Consider the crude extract as 100% a known weight of the crude was added to a similar volume of the solvent (acetone) to obtain stock solution. Stock solution of each extract was made prior to use. Successive dilutions were carried out to obtain the tested solutions.

2.3. Bioassay

The different concentrations of each plant extract were tested on the 2^{nd} instar nymph of *S. gregaria*. Leaf dipping technique was used. The same sizes of clover leaves for *S. gregaria* were dipped in each tested concentration of plant extracts and in the control for 20 seconds and left to dry. The dried leaves were put singly in plastic cups. Tens insects were transferred to each cup and allowed to feed on the treated leaves for one day. Three replicates for each concentration were done. After 24h surviving insects were transferred to clean cups and supplied daily with untreated leaves until the end of experiment. Group of insects was left unexposed under the same laboratory conditions as control. Mortality was recorded daily until the end of experiment and corrected according to Abbott [14]. Mortality values 24 days after exposure were analyses by profit analysis (LDP line) to obtain LC_{25}, LC_{50} LC_{90} and slope for each extract according to method adopted by Finney [15]. The most effective plant extracts were selected for father experiments.

2.4. Immunological Studies

Afterfeeding on treated leaves with sublethal concentration (LC_{25}) of the extracts for 48 hours, alive insects were collected and allowed to feed on normal leaves after 48 hours for *S. gregaria*. A specific number of pests was taken and subjected directly for Immunological studies.

2.4.1. Differential Haemocyte Counts (DHCs)

After 48 hrs of feeding on treated leaves, the haemolymph samples were withdrawn from the coxal corium according to Hoffmann [16]. Haemocytes (blood films) were stained by Giemsa stain and examined by light microscopy. The haemocytes are classified according to the classification scheme.

Various haemocytes were differentially counted by examining approximately 100 cells per slide. 10 slides prepared from 10 locusts / count. The percentages of haemocyte types were calculated by the formula:

$$\% = \frac{\text{Number of each haemocyte type X100}}{\text{Total number of haemocytes examined}}$$

2.4.2. Total Haemocyte Counts (THCs)

The oozed haemolymph was collected directly into Thoma- white blood cell diluting pipette to the mark 0.5. Diluting solution (NaCl- 4.65 g, KCl- 0.15 g, CaCl2- 0.11 g, crystal violet- 0.05 g and acetic acid- 1.25 ml/liter distilled water) was taken up to the mark 11 on the pipette (dilution is 20). The mixture was dispensed to both chambers of the counting slide (the chamber depth is 1.0 mm) and the total number of cells were counted according to the formula of Jones [17]**:**

$$\frac{\text{Haemocytes in 1 mm squares X dilution X depth of chamber}}{\text{Number of 1mm squares counted}}$$

2.5. Histopathological Examination

Group of *S. gregaria* insect feed on leaves treated with LC_{25} of *Citrullus colocynthis* for 48 and second group feed on leaves treated with LC_{25} of *Guayacum Officinalis* and third groups feed on leaves untreated(Control).

Samples of tested groups and control from the S. gregaria insect were dissected after 48 hours from exposure. Body wall and mid gut of *S. gregaria* were fixed separately in alcoholic Bouin s solution for 24 hours, washed in ethanol (70%) then dehydrated in an according series (70-100%) of ethyl alcohol. Infiltration embedding of sample was carried out in Paraffin wax. Sections were stained in Ehrlischs haematoxylin and cosin.

2.6. Statisitical Analyses

Date obtained were analysed by student (t) test according to the equation. Significant difference were established at $P<0.05$ and $P<0.01$ levels.

3. Results

3.1. Toxicity Testing

The result recorded in Table (1) showed that, the *C. colocynthis* and *G.Offici* plant was affected on ^{2} instar nymph of *S. gregaria*. The recorded LC_{50} and LC_{90} values of *C. colocynthis* plant extract were 8.2 and 14 ppm and 11ppm (mg/ml), respectively. On the other hand, the recorded LC_{50} and LC_{90} values of *G.Offici were* 9.1 and 15,6 ppm, respectively.

3.2. Immunological Studies

3.2.1. Differential Haemocyte Counts (DHCs)

The present results in Table 1 showed that the percentages of different haemocyte types of adults (2-4 days) fed on leaves treated with plant extracts. The percentage of Prohaemocytes (PRs) decreased in insects fed on leaves treated with *C. colocynthis* (3.20 %) compared to the control (14.50 %). PRs disappeared In insects fed on leaves with *G.Offici*. The results also recorded that the highest number of these Plasmatocytes (PLs) was in insects fed on *G.Offici*. (75.10%) than control insects(53.8). Granulocytes (GRs) percent decreased when insects fed on *C. colocynthis* and *G.Offici*.(21&18.2respectively) compared with control insects. The percentages of Oenocytoids (OEs) increased in insect fed on on *C. colocynthis* and *G.Offici*.(7.9&4.1respectively) compared with control.

3.2.2. Total Haemocyte Counts

The present results in Figure (1) showed the effects of LC_{25} of *plant extracts* on *S. gregaria* adults. The THC values showed a significant decrease ($P < 0.05$) with all adults fed on leaves treated with *C. colocynthis* and *G.Offici* (8120±42.12, 7940±21.2 cells/mm^3, respectively) compared with controls (920±42.12, 9324±21.2 cells/mm).

3.3. Histopathological Chanes

3.3.1. Histopathological Chanes in the Body Wall of Treated *Schistocerca Gregaria*

The present results showed that the epithelial cells (EC) were detached or displaced from their basement membrane and some dissolution of cytoplasmic material was observed beneath the epithelial layer after 48h of treatment with LC_{25} of *C. colocynthis* with compared to the control (Fig.1).

Examinattion of body wall after 48h of treatment with LC_{25} of *G.Offici* showed complete dissolution of the epithelial cell walls(Ec), dissolution, granulation of their cytoplasm and destrruction of some nuclei and fragementation of muscle fibers were observed. (Fig.3).

3.3.2. Histopathological Changes in the Midgut of Treated *S.Gregaria*

The present data showed that enlargement of the columnar epithelial cells(EC) and rupture of some cells after the treatment with LC_{25} of *C. colocynthis* for 48 hour (Fig 5). Also, vacuolation of the striated border and dissolution of the epithelial cells boundaries was observed as compared to control (Fig. 4).

The present results in Figure 5. Showed that severs destruction of the brush bordes, the columnar epithelial cells seemed to be swollen with dissolution of cytoplasm and showed some vacuolations between the striated borders the columnar epithelial cells seemed to be swollen with dissolution of cytoplasm and showed some vacuolations between the striated borders after 48h of treatment with LC_{25} of *G.Offic* with compared to the control (Fig.4).

4. Discussion

Circulating haemocytes have important functions on the immune system, metabolism, and detoxification, and play a crucial role in the defense of xenobiotics or microbial infection [18]. This work proceeded to the impact of *C. colocynthis* and *G.Offici* extracts on differential and total haemocyte count of adult locust Four types of haemocytes were found in locust fed on each experimental plant (PRs, PLs, GRs and OEs).

The present results investigated that normal locusts fed on leaves treated with *C. colocynthis* and *G.Offici* extracts (high protein diet) had the highest percentage of PLs (75.10%) and the highest percentage of GRs (21%) was found in normal locusts fed on leaves treated with *C. colocynthis* sorghum (high carbohydrate diet). Szymaś and Jędruszuk [19] stated that GRs cells may act as storage cells in insect. Idowu and Sonde [20] also found that Pls numbers are the most in cassava (high protein diet) fed grasshopper and are the lowest in acalypha (low protein diet) fed grasshopper while GRs numbers are the highest in grasshopper fed highest carbohydrate diet. Further,

Following the locusts fed on leaves with plant extracts, the PLs increased and the GRs decreased at post-injection in all treatments than control. The increase in PLs was attributed to the release of sessile haemocytes after infection, and the decrease in GRs may be attributed to their involvement in phagocytosis and nodule formation. This concept is supported by the observations of Anandakumar and Michael [21] on silk worm larvae infected with *Bti*. Hillyer, et al. [22] shed light on GRs which play an important role to phagocytose bacteria and showed that a reduced GRs population in insects may affect their capacity to clear high infection levels. In addition, insects fed with protein might invest more resources in certain types of haemocytes (e.g. Grs or PLs) at the expense of others types [23]. Also, Mori [24] suggested that the decrease of PRs after injection may be explained with the transformation of PRs (stem cells) into PLs and GRs which are needed in phagocytosis and nodule formation. From our observation to haemocyte pathological conditions we suggested that toxins secreted by injected bacteria cause lysis of haemocytes or induce programmed cell death. The low values presented by OEs in desert locust fed on leaves treated with plant extracts in this study was confirmed by Silva, et al. [25] who reported the absence of quantitative alterations of OE in response to pathogens in the haemocoel of *Anastrepha oblique* larvae. It is known that the OE cells are involved in the production of prophenoloxidase, an enzyme that actively participates in the mechanisms of defense in insects [26, 27]. These results suggest that the abundance and diversity of environmental resources can have a direct impact on insect immune system. Higher haemocyte concentrations are expected to be associated with high protein diet and higher resistance to disease. Natural proteinaceous food is an important factor in the function of the cellular system of insect haemolymph where a lack of protein in the diet causes considerable changes in the function and structure of the cellular system of the haemolymph [19]. One possibility is that the high food quality stimulates increased proliferation of one or more haemocytes types [28]. Generally, the THC is positively correlated with the rate of phagocytosis and nodule formation. The total number of haemocytes in haemolymph is likely to reflect the capability of immune system to deal with pathogens or chemical molecules [29]. The present results indicated that THCs decreased significantly after treated with plant extracts than controls. Similar observations were also reported by Anandakumar and Michael [21] who noticed that about 15.3% of the total haemocytes were decreased after bacterial infection in silk worm larvae when compared to normal worms. They explained the decrease in total THCs infected worm with *Bacillus* was due to the depletion of prohaemocytes, which accounts for most of the lowered total counts compared to normal. Banville, et al. [30] stated that immune response requires a high degree of resources to be maintained at an optimum level to fight infection. Also authors observed that the higher level of haemocytes and antimicrobial peptide expression in unstarved *Galleria mellonella* larvae leads to significantly greater ability to curtail or eliminate the pathogen than starved insects. In the present study, vacuolation, necrosis and destruction of epithelial cells and their boundaries are highly recognized in both epidermal and mid gut cells of insect. In addition, the disturbances in the function of the internal organs as a consequence of structural damage may lead to inhibition of lipid synthesis.

The histopathological changes occurred in *S.gergaria* treated with *C. colocynthis* and *G.Offici* extracts were vacuolation and necrosis of the epithelial cells and destruction of epithelial cells and their boundaries. Vacuoles may occur as a result of cell elongation or a result of excessive fat droplets which dissolve during fixation and dehydration process. Similar observation were also obtained by many authors for neem extract and other plant extracts against *S. gregaria* and various insect species belonging to different order.

The histopathological changes occurred in the larval midgut of *S. littoralis* treated with *C. colocynthis* and *G.Offici* extracts were observed necrotic and swollen. Similar observation were also obtained by many authors for other plant extracts against *S. gregaria* and various insect species belonging to different order. Against *S.littoralis*, Salam and Ahmed [31] found that, the *Melia azedarach* extract caused destruction of epithelial cells. Also, Younes, et al. [32],observed the degeneration of the epithelial cells and decay of its boundaries when *S. littoralis* larvae with the extracts of both Clerodendro inerme and Conyza dioscoridis caused slight and severe disintegration of the epithelium, fading of the boundaries of epithelial cells and detachment of epithelial cells [33]. In contrast to these observations, Szymaś and Przybył [34] found no effect of azadirachtin on the midgut epithelium of *Epilachna varivestis* larvae. This might reflect the variable susceptibility of different insect species.

5. Conclusion

Finally, we can concluded that, *S. littoralis* adults feed on leaves treated with *C. colocynthis* and ***G.****Offici* extracts had considerable variation in the differential and total haemocyt counts. Also, The present histopathological destruction caused by the investigated plant insecticides may suggest that any of these extracts are capable of

causing death of an insect when entering into tissues in adequate amounts. In conclusion , the results of the present study show that the emulsifiable concentrate of *C. colocynthis* and ***G.Offici*** extracts has high toxicity on *S. gergaria*

Table-1. Toxicological evaluation of plant extract against the 2nd instar nymph of *S. gregaria.s*

	Toxicity of match Ppm			**Slop Function**
	LC_{25}	**LC_{50}**	**LC_{90}**	
C. colocynthis	5.1	8.2	14	2.3
G.Offici	6.4	9.1	15,6	1,8

Table-1. Differential haemocyte counts (DHCs) of *S. gregaria* adults (2-4 days old) fed on leav at 48 hr post-injection

Experimental plants	**Haemocyte type %●**							
	Prohaemocytes		**Plasmatocytes**		**Granulocytes**		**Oenocytoids**	
C. colocynthis	14.5	3.2	53.80	65.9	31.90	21.00	5.80	7.90
G.Offici	14.4	0.0	56.4	75.1	30.80	18.2	4.50	4.10

●n = 10 replicates

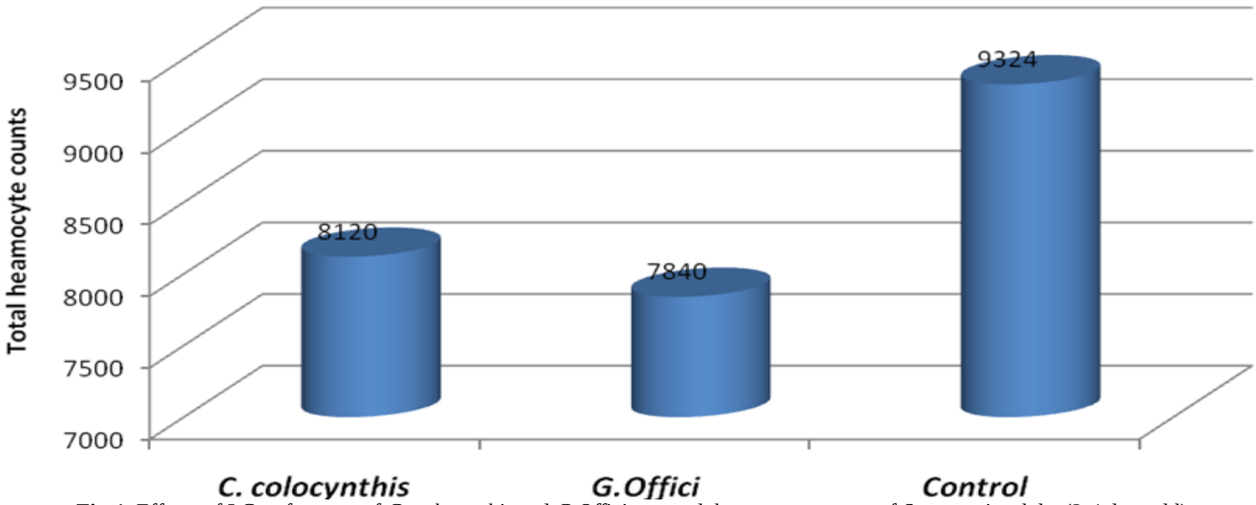

Fig-1. Effects of LC_{25} of extract of *C. colocynthis* and *G.Offici* on total haemocyte counts of *S. gregaria* adults (2-4 days old).

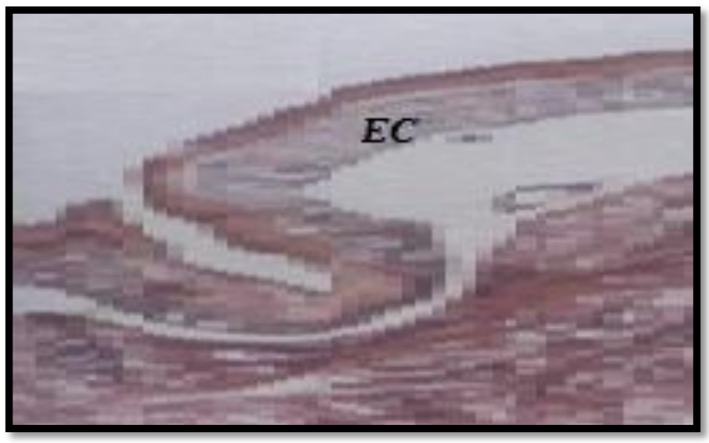

Fig-1. Cross section in the Body wall of untreated *Schistocerca gregaria* larvae with showing epithelial cell (ec).

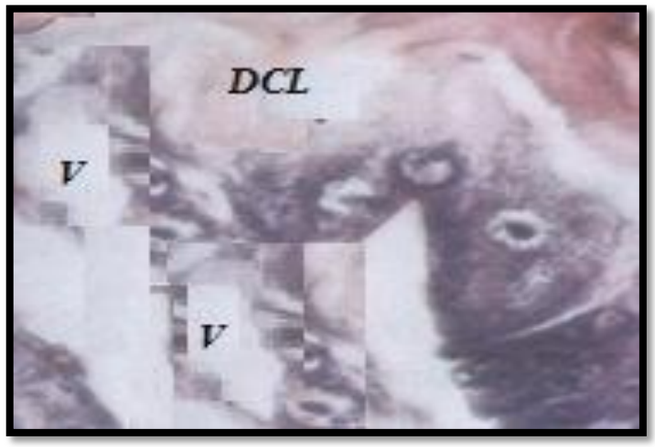

Fig-2. Body wall sections of *Schistocerca gregaria* larvae treated with LC_{25} formulated extract of *C. colocynthis* showing detachement cuticular Lamellae (del). vacuolation(v) after 48 h of treatment DCL : detached epithelial cell (x400.1000 H and E).

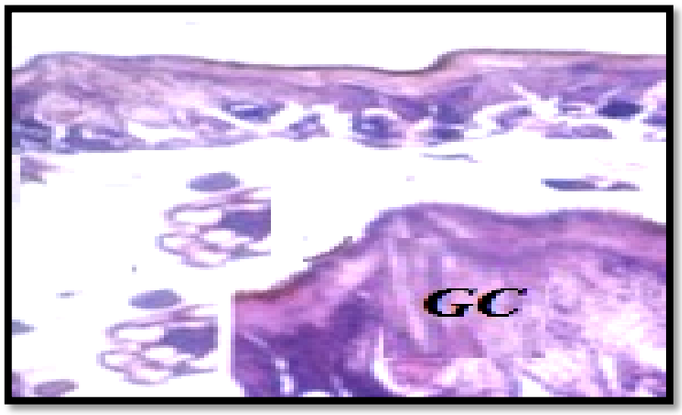

Fig-3. Body wall section of *Schistocerca gregaria* larvae with LC_{10} formulated extract of *G.Offici colocvnthis*showing granulated cytoplasm (gc) after 48 h of treatment) (x 400.1000H and E)

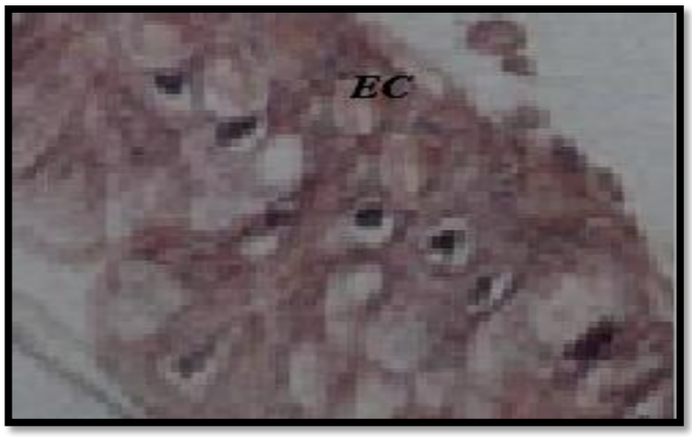

Fig-4. Cross sections in the midgut of untreated ***Schistocerca gregaria*** larva showing epithelial cells(ee) (a) after 48 h from the beginning of the experiment and brush

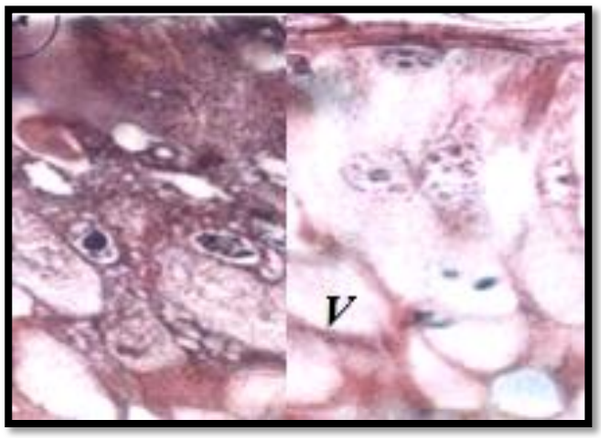

Fig-5. Cross sections in the midgut of *Schistocerca gregaria* larva treated with LC_{25} extract of *C. colocynthis* after 48 h of treatment (x400 H and E).

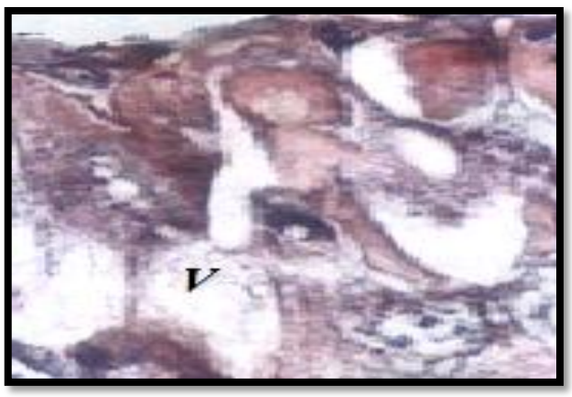

Fig-6. Cross sections in the mid gut of *Schistocerca gregaria* larva treated with LC_{25} formulated extract of *C. colocynthis* after 48 h of treatment (x 400 H and E).

References

[1] S. Singh, P. J. Ran, and S. Singh, "Effect of ageratum conyzoide on feeding development and reproduction of schistocerca gregaria (Forck)," *J. Pesticide Research,* vol. 12, pp. 204-209, 2000.

[2] E. Lampert, "Influences of plant traits on immune responses of specialist and generalist herbivores," *Insects,* vol. 3, pp. 573-592. doi:10.3390/insects3020573, 2012.

[3] F. Vogelweith, D. Thiery, B. Quagliett, Y. Moret, and J. Moreau, "Host plant variation plastically impacts different traits of the immune system of a phytophagous insect," *Functional Ecology,* vol. 25, pp. 1241–1247, 2011.

[4] J. P. Gillespie, M. R. Kanost, and T. Trenczek, "Biological mediators of insect immunity," *Annual Review of Entomology,* vol. 42, pp. 611-643, 1997.

[5] I. Shikano, J. D. Ericsson, J. S. Cory, and J. H. Myers, "Indirect plant-mediated effects on insect immunity and disease resistance in a tritrophic system," *Basic and Applied Ecology,* vol. 11, pp. 15–22, 2010.

[6] R. Plymale, M. J. D. C.-F. Grove, N. Ostiguy, and K. Hoover, "Plant-mediated alteration of the peritrophic matrix and baculovirus infection in lepidopteran larvae," *Journal of Insect Physiology,* vol. 54, pp. 737-749, 2008.

[7] M. R. Strand, "The insect cellular immune response," *Insect Science,* vol. 15, pp. 1–14, 2008.

[8] M. Nakatani, S. A. M. Abd El-Galeil, J. Kurawaki, H. Okamura, T. Iwagawa, and M. Doe, "Antifeedant ring B and D opened limonoids from Khaya senegalensis," *J. of Natural Products,* vol. 64, pp. 1263-1265, 2001.

[9] G. H. Schmidt and S. Assembe-Tsoungui, "Effect of oral application of Melia fruit extract on growth, development and gregarization of the desert locust schistocerca gregaria (Forsk) (Caelifera, Acridiae)," *Zeitschrift fur Pflanzenkrankheiten und Pflanzenachutz,* vol. 109, pp. 38-56, 2002.

[10] N. A. Farag, "Effect of haphyllum tuberculatum extracts on an insect on an insect pest spodoptera littoralis and the predator, coccinella und ecimpunctata," *Ann. of Agric. Sci. Cairo.,* vol. 47, pp. 1097-1105, 2002.

[11] M. M. Sadek, "Antifeedant and toxic activity of adhatoda vesica leaf extract against spodoptera littoralis (Lep. Noctuidae)," *J. Applied Entomology,* vol. 127, pp. 396-404, 2003a.

[12] P. Trinder, "Determination of glucose in blood using glucose oxidase with an alternative oxygen accepter," *Ann. Clin. Biochem.,* vol. 6, p. 24-27, 1969.

[13] B. Freedman, L. J. Nowak, W. F. Ewalek, E. C. Berry, and W. D. Guthrie, "A bioassay for plant derived pest control agents using European corn borer," *J. Econ. Ent.,* vol. 72, pp. 541-545, 1979.

[14] W. S. Abbott, "A method of computing the effectiveness of an insecticide," *J. Econ. Ent.,* vol. 18, pp. 265-267, 1925.

[15] D. J. Finney, *Propit analysis*, 3rd ed. London: Cambridge Univ. Press, 1971.

[16] D. Hoffmann, "Induction of antibacterial activity in the blood of the migratory locust, locusta migratoria L," *Journal of Insect Physiology,* vol. 26, pp. 539-549, 1980.

[17] J. C. Jones, "Current concepts concerning insect haemocytes," *American Zoologist,* vol. 2, pp. 209- 246, 1962.

[18] Q. Zhu, Y. He, J. Yao, Y. Liu, L. Tao, and Q. Huang, "Effects of sublethal concentrations of the chitin synthesis inhibitor, hexaflumuron, on the development and hemolymph physiology of the cutworm, spodoptera litura," *Journal of Insect Science,* vol. 12, pp. 1-13, 2012.

[19] B. Szymaś and A. Jędruszuk, "The influence of different diets on haemocytes of adult worker honey bees, apis mellifera," *Apidologie,* vol. 34, pp. 97–102, 2003.

[20] A. B. Idowu and O. A. Sonde, "The contribution of food plants to the growth, development and fecundity of zonocerus variegates," *African Journal of Biotechnology,* vol. 2, pp. 350-355, 2003.

[21] M. D. Anandakumar and A. S. Michael, "Haematology and haemochemistry of silkworm, Bombyx Mori L. infected with bacillus thuringiensis," *International Journal of Environmental Sciences,* vol. 2, pp. 451- 457, 2011.

[22] J. F. Hillyer, S. L. Schmidt, J. F. Fuchs, J. P. Boyle, and B. M. Christensen, "Age-associated mortality in immune challenged mosquitoes (Aedes Aegypti) correlates with a decrease in haemocyte numbers," *Cellular Microbiology,* vol. 7, pp. 39–51, 2005.

[23] C. Alaux, F. Ducloz, D. Crauser, and Y. Le Conte, "Diet effects on honeybee immunocompetence," *Biology Letters,* vol. 6, pp. 562-565, 2010.

[24] H. Mori, *Embryonic haemocytes. In: Origin and development in insects haemocytes. (Eds. Gupta, A.P.)*. Cambridge: Camridge University Press, 1979.

[25] J. E. B. Silva, I. C. Boleli, and Z. L. P. Simões, "Hemocyte types ,total and differential counts in unparasitized and parasitized anastrepha oblique (Diptera, Tephridae) larvae," *Brazilian Journal of Biology,* vol. 62, pp. 689-699, 2002.

[26] M. D. Lavine and M. R. Strand, "Insect hemocytes and their role in immunity," *Insect Biochemistry and Molecular Biology,* vol. 32, pp. 1295-1309, 2002.

[27] F. G. Andrade, M. C. C. Negreiro, S. M. Levy, I. C. B. Fonseca, F. Moscardi, and A. M. F. Falleiros, "Hemocyte quantitative changes in anticarsia gemmatalis (Lepidoptera: Noctuidae) larvae infected by AgMNPV," *Brazilian Archives of Biology and Technology,* vol. 53, pp. 279-284, 2010.

[28] J. C. Castillo, A. E. Robertson, and M. R. Strand, "Characterization of hemocytes from the mosquitoes anopheles gambiae and aedes aegypti," *Insect Biochemistry and Moleculer Biology,* vol. 36, pp. 891–903, 2006.

[29] S. A. A. Mo'men, "The role of prophenoloxidase activation system in cellular defense mechanisms in the haemolymph of the desert locust, schistocerca gregaria," Msc Thesis in Ain Shams University, 2010.

[30] N. Banville, N. Browne, and K. Kavanagh, "Effect of nutrient deprivation on the susceptibility of Galleria mellonella larvae to infection," *Virulence,* vol. 3, pp. 497–503, 2012.

[31] A. Salam and A. Ahmed, "Evaluation of using the extract of chinaberry fruits Melia azedarach L. in the control of the cotton leafworm, spodoptera littoralis in Egypt," presented at the International Conf. on Pests in Agriculture,(6-8 Jan.1997), Le corum, Montpellier, France, 1997.

[32] M. F. Younes, F. Abd-Dahab, and A. Assar, "Histopathology studied on the effect of some botanical extracts on the cotton leafworm, spodoptwea littoralis (Boisd) (Lepidoptera: Noctuidae) II – effect on rhe integument, the midgut and fat bogy," presented at the 2th Sci. Conf. on the Role of Science in the Development of Egyptain Society and Enviroment,Zagazig Unif/. Fac. of Sci. Benha, 1999.

[33] A. G. Emara and A. Sser, "Biological and histopathological, biological and hisopathology activity of some plant extracts against the cotton leafworn, spodoptera littoralis (Boised)," *Lepidoptera: Noctuidae J. Egypt, Ger. Soc, Zool.,* vol. 35, pp. 1-25, 2001.

[34] B. Szymaś and A. Przybył, "Midgut histological picture of the honey bee (Apis Mellifera L.) following consumption of substitute feeds supplemented with feed additives," *Nauka Przyroda Technologie,* vol. 1, pp. 39-48, 2007.