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EFFECT OF *HYPTIS BREVIPES* DICHLOROMETHANE EXTRACT ON FEEDING AND HISTOLOGICAL STRUCTURE OF THE MIDGUT AND MALPIGHIAN TUBULES OF *SPODOPTERA LITTORALIS* LARVAE (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT

The growth and development of *Spodoptera littoralis* (Lepidoptera: Noctuidae) larvae strongly inhibited by *Hyptis brevipes* (Lamiaceae) dichloromethane extract. The present study evaluates the antifeedant activity of dichloromethane extract of *H. brevipes* against the 5th larval instar of *S. littoralis* using castor bean leaf disks. The results from the no-choice bioassay showed that this extract caused antifeeding effect by 58.3% ± 6.3and 76.7±5.5 % at concentrations of 2% and 4%, respectively. The histological changes in the midgut and Malpighian tubules of *S. littoralis* larvae elicited by *H. brevipes* dichloromethane extract were also evaluated. Two concentrations (2% and 4%) of this extract were orally applied to the 3rd instar larvae of *S. littoralis* for three consecutive days. The extract induced many histological changes in the tested organs in a time and dose dependent manner. At a concentration of 4% of *H. brevipes* extract, the peritrophic membrane was detached and the mid gut lumen was packed with pycknotic-nuclei-epithelial cells. Extensive destruction of the epithelium with cells lacking nuclei was also observed in the mid gut of larvae treated with 2% after 7 days of treatment. Architecture destruction of the Malpighian tubules of *S. littoralis* larvae treated with 4% of *H. brevipes* extract was observed 4 days of treatment. Histochemically, the total protein content was decreased in the mid gut cells of the treated larvae compared to control. The target organ for this toxic material is the mid gut and Malpighian tubules as indicated by their high sensitivity toward *H. brevipes* extract. This sensitivity could be in part responsible for the antfeeding activity of this plant extract and the deleterious effect on larval mortality and thus enhance the possibility of using this plant extract in the control program of *S. littoralis*.

Keywords: antifeeding activity, Malpighian tubules, midgut, protein, *Spodoptera littoralis, Hyptis brevipes*

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INTRODUCTION

Genus *Spodoptera* (Lepidoptera: Noctuidae) contains a worldwide distribution forms which considered as the most destructive pests (Guerrero et al., 2014). The larval stage of *S. littoralis* caused enormous damage to many crops (Salama et al., 1970). Heavy infestation resulted in defoliation of the attacked plants causing sever loss of crop production (Martinez and VanEmden, 2001). There is renewed interest in using the natural pesticides to control the harmful pests. The secondary metabolites of many medicinal plants are found to be effective alternative to synthetic pesticides. These metabolites are harmless to humans and non-target organisms, host specific, preserve natural enemies and increase the biodiversity of the ecosystem. The secondary metabolites exert their effects against insects through different mode of action such as: antifeedant (Khosravi et al., 2010; Haouas et al., 2010& Pavunraj et al., 2011); larvicidal (Sakr and Abo-El-Mahasen, 2006 & Sakr et al., 2013); repellents (Conti et al., 2011); inhibition of proteas inhibitor (Aguirre et al., 2004), disrupt the normal growth and development (Gatehouse et al., 1990; Weinhold and Shaker, 2011 & Sakr et al., 2013) and others.

Insect midgut and Malpighian tubules play an important role in insect life. The midgut of insect is responsible for the production of enzymes and absorption of the digestive products (Lehane and Billingsly, 1996 & Vatanparast et al., 2012). Histologically, the midgut of *S. littoralis* larva consists of a simple epithelium supported by a basement membrane, striated muscle layers. The epithelium is composed of three main cell types; columnar, goblet and regenerative cells. The food mass is separated from the brush border of the epithelial cells by a thin sheath, the peritrophic membrane (PM). The muscle layers composed of bundles of inner circular and outer longitudinal muscles (Sakr¸ 2007). In most insects, the PM plays an important role in the protection of midgut cells from the invasive pathogen and preventing the damaging of these cells by food particles (Lehan, 1997; Terra, 2001 &Hu et al., 2012). Insect Malpighian tubules are responsible for osmoregulation, elimination of the waste products from the haemolymph and reabsorption of water and useful substances (Prado et al., 1992; Beyenbach et al. 2010& Smitha and Rao, 2012). The Malpighian

tubule consists of a single layer of epithelial cells covered internally by striated border and externally by a basement membrane (Fermino et al., 2010; Smitha and Rao, 2012& Pal and Kumar, 2014).

The secondary metabolites released by many plants play an important role in reducing damage caused by herbivorous insects (Truitt *et al*., 2004). Among these metabolites: alkaloids (Nuringtyas et al., 2014); Phenolic compounds (Santana-Meridas et al., 2014); flavonoids (Sakr et al., 2013); sesquiterpenoids (Portero et al., 2012). *Hyptis brevipes* (Lamiaceae) dichloromethane extract had a desirable impact on growth and development of *S. littoralis* larvae. The treated larvae with this extract had difficulty in shedding their old cuticles and died during ecdysis. Others, showed signs of incomplete moulting and became unable to feed normally (Sakr et al., 2013). The medicinal plant, *H. brevipes* is known to produce a range of terpinoids, flavonoids and pyrons (Deng, 2010 & Sakr et al., 2013). The mode of action of these secondary metabolites toward *S. littoralis* larvae is still unknown. The effect of *H. brevipes* extracts on the histological structure of *S. littoralis* larvae have not been studied so far.

The current study has been carried out as part of our ongoing search of new bioactive natural products (Sakr et al., 2013) and find out their mode of action (Sakr and Abo- El-Mahasen, 2006; Sakr and Hassab El-Nabi, 2007& Sakr, 2007). Therefore, the present study aimed to highlight the effect of *H. brevipes* dichloromethane (CH_2CL_2) extract on the feeding activity and the histological structure of the midgut and Malpighian tubules of *S. littoralis* larvae. The total protein content in the midgut epithelial cells was also evaluated.

MATERIALS AND METHODS

Source of plant extract

The Ecuadorian plant *H. brevipes* CH_2CL_2 extract was kindly provided by Prof. Dr. / Hesham El-Seedi, Prof. of Natural Products, Department of Chemistry, Faculty of Science, Menoufia University, Menoufia, Egypt.

Insect source and maintenance

A laboratory susceptible strain of the cotton leaf worm *S. littoralis* was initially obtained from Agricultural Research Center (Dokki, Giza,

Egypt). The larvae were reared in glass jars covered with muslin tide round the neck by rubber bands at 28 ± 2^{o}C and 65±5%R.H. These larvae were fed exclusively on castor bean leaves (*Ricinus communis*) till pupation. Emerged adults were supplied with 10% (w/v) sucrose solution (Sakr, 2007).

Antifeeding bioassay

To ascertain the antifeeding activity of *H. brevipes* dichloromethane (CH_2Cl_2) extract against the 5th larval instar of *S. littoralis*, the no-choice method was used (Simmonds et al., 1990). *Spodoptera* larvae were individually deprived of food for four hour before being used in this bioassay. *H. brevipes* CH_2Cl_2 extract was dissolved in acetone as a solvent. Ten disks of castor-bean-leave (1cm^2) were treated with 300 µl of CH_2Cl_2 extract at a concentration of 2% and 4% (w/v). Disks treated with acetone alone used as positive control, while that treated with water was considered as negative control. The disks were left to stand (at room temperature) to evaporate the solvent. Each treated leaf disk was introduced to each *S. littoralis* larva in a Petri dish (9cm diam.). Each Petri dish was provided with wet filter paper to avoid drying of the leaf disk. The experiment was terminated when the control larva consumed more than 50% of the disk, 6-9 h). The area of the leaf disks consumed by larvae was then assessed visually by comparing the remaining leaf material with a template of the original disk. This experiment was repeated two times. The antifeeding index was expressed as mean ±S.E and calculated using the following formula:

AFI= [C-T/C+T] X 100

C= is the mean area of food consumed by the total number of larvae in the control group. **T**= area of food consumed by each larva in treated disk.

Statistical analysis

Data were analyzed for significant antifeeding effects using unpaired *t*-test. The level of significant was detected at $P<0.05$ (Minitab release 12.1, Minitab Inc., USA, 1998).

Application of *H. brevipes* extract for histological study

In order to know whether the *H. brevipes* dichloromethane (CH_2CL_2) extract could exert an effect on the histological structure of midgut and Malpighian tubules of *S. littoralis* larvae, two concentrations

were used. Based on our previous published data (Sakr et al., 2013), 2% and 4% (w/v) of *H. brevipes* extract were tested against the 3rd instar *S. littoralis* larvae. Briefly, thirty castor bean leaf discs ($2cm^2$) were treated with 1 ml of each concentration of *H. brevipes* extract. Positive control discs were treated with solvent (acetone) alone. For solvent evaporation, all leaves were left to stand (at room temperature) and then offered to the tested larvae in glass jars for three consecutive days. Thereafter, these larvae were fed on fresh (untreated castor been leaves) leaves till pupation. Randomly selected alive treated and control larvae (5 larvae/concentration/interval), were chosen for the histological study after 3, 4, 6 & 7 days of treatment.

Histological study

The effect of *H. brevipes* CH_2CL_2 extract on the histological structure of both midgut and Malpighian tubules of *S. littoralis* larvae was determined. In addition, the total protein content of midgut epithelium was also evaluated. Selected treated and control larvae (as mentioned above) were dissected and the midguts and Malpighian tubules of each group were removed. Parts of these organs were fixed for 24h in Boun's solution. Following fixation, all specimens were dehydrated by ethanol, cleared in xylol, embedded in parablast and section at 5µ thick. Sections were stained with hematoxyline and eosin (Sakr, 2007). The method of Mercury bromophenol blue (Bonhag, 1955) was used for protein determination in the larval midgut cells. The stained sections were mounted on glass slides in DePeX-mounting medium under cover slips. Microscopic examination and photographs were carried out by Olympus microscope attached with Olympus digital camera.

RESULTS

Antifeedant activity

Dichloromethane extract of *H. brevipes* at concentrations of 2% and 4% (w/v) elicited antifeeding activity against the 5th larval instar of *S. littoralis* using castor bean leaf disks. The results from the no-choice bioassay showed that this extract caused significant antifeedant activity, in a dose-dependent manner ($P<0.05$). The feeding index (AFI) values were 58.3% and 76.6% at concentration of 2% and 4%, respectively (Table 1).

Table 1: Antifeedant activity of dichloromethane extract of *H. brevipes* against the 5th larval instar of *S. littoralis* (no-choice assay)

Concentration %	*Antifeeding index (%Mean ± S.E.)
2	58.3 ±6.3
4	76.7 ±5.5

*Antifeeding index **(AFI) = [C-T/C+T] X 100** (Simmonds et al., 1990); where **C**= is the mean area of food consumed by the total number of larvae in the control group. **T**= area of food consumed by each larva in treated disks.

Histological structure of mid gut and Malpighian tubule

Normal structure of the midgut of the 5th larval instar of *S. littoralis* is illustrated in Figure (1a-b). The gut consists of a simple columnar epithelium (composed of three main cell types: columnar, goblet and regenerative cells) supported by a basement membrane and striated muscle layers. The muscle layers are composed of bundles of inner circular and outer longitudinal muscles. The food mass is separated from the brush border of the epithelial cells by a thin sheath, the peritrophic membrane (Fig. 1a-b). The normal structure of Malpighian tubule of control *S. littoralis* larva consists of a single layer of epithelial cells which covered internally by striated border and externally by a basement membrane (Fig. 2).

Histological alteration of midgut and Malpighian tubule

Feeding *S. littoralis* larvae on castor bean *H. brevipes* CH_2CL_2 extract elicited many histological changes in the larval midgut in a dose and time dependent pattern (Figs. 3-4). Four days of treatment with 4%, the peritrophic membrane was detached (Fig. 3a) and the gut lumen was filled with many apoptotic epithelial cells that have pycknotic nuclei (Fig.3b). Extensive destruction of the midgut epithelium with cells lacking nuclei was also observed after 4 days (Fig. 3c-d) of treatment with 4% of *H. brevipes* extract. The same effect (Fig. 4) was observed after 7 days of treatment with 2% of *H. brevipes* extract. The effects of the extract extend to the Malpighian tubules of *S. littoralis* larvae (Fig. 5a-b). Architecture destruction of the tubules (Fig. 5a) and flattened epithelial cells (Fig. 5b)

was observed in the larvae fed castor bean leaves treated with 4% four days of treatment.

Effects of *H. brevipes* extract on protein content of the midgut cells

In control midgut of *S. littoralis* larvae, the protein contents appeared as intensely bluish colouration in the midgut cells (Fig. 6). All structures of the cells exhibited positive stain ability with varying degrees reaching its maximum in the nucleus (Fig. 6). Oral application of 4% and 2% of *H. brevipes* CH_2CL_2 extract to *S. littoralis* larvae exhibited an obvious decrease in the protein content in the cytoplasm of the midgut cells in a dose and time dependent manner (Figs. 7-8).

DISCUSSION

The present data clearly demonstrate the antifeeding activity and the potent insecticidal action of *H. brevipes* CH_2CL_2 extract toward the histological structure of two important organs of *S. littoralis* larvae. The no-choice experiment is actually a choice between eating and starvation of insect larvae. The rejection of castor bean leaf disks treated with *H. brevipes* extract by the 5^{th} larval instar of *S. littoralis* can be explained by the presence of antifeedant compounds in this extract, the most of which is alkaloids and flavonoids. This plant is known to produce a range of terpinoids, flavonoids and pyrons (Deng, 2010 & Sakr et al., 2013). The antifeeding activity observed in the present study could be in part due to the direct damage of the epithelial tissue of the midgut (this will be discussed below) by the active compounds persist in *H. brevipes* CH_2CL_2 extract.

The present result is consistent with data reported by Raja et al. (2005); Haouas et al. (2010); Pavunraj et al. (2011), Dowd et al. (2011) & Pavunraj et al. (2014 a and b). The ethyl acetate extract of *H. suaveolens* at 1000 ppm elicited antifeeding activity against *S. litura* and *Helicoverpa armigera* by 65.3 % and 71.0%, respectively (Raja et al., 2005). Phytochemical analysis of the ethyl acetate extract of *H. suaveolens* showed the presence of terpenoids and alkaloids (Pavunraj et al., 2014 a). *Chrysanthemum segetum* methanol extract at 10000ppm, gave the highest significant antifeedant activity by 78.5%± 24.3 against the 3^{rd} instar larvae of *S. littoralis* (Haouas et al., 2010). Maximum antiffedant activity was recorded in ethyl acetate leaf extract of milkweed *Pergularia daemia* (Pavunraj et al., 2011). They

reported that this extract caused antiffedant activity against *H. armigera* and *S. litura* by 70.3% and 71.8%, respectively. This activity is attributed to the presence of 6-(4'7-hydroxy-heptyl) quinine isolated from ethyl acetate extract of *P. daemia*. This isolated compound showed 80.2% activity when tested at 2000 ppm against *H. armigera* (Pavunraj et al., 2011). The activity of saponins from different plant species was evaluated against the 1st larval instar of *H. zea* and *S. frugiperda* (Dowd et al., 2011). They stated that sapogenol B (isolated from soybean) appeared to have antifeedant properties to *S. frugiperda*. Pavunraj et al. (2014 b) evaluated the antifeeding activity of *Spilanthes acmella* dichliormethane extract against three different insect species. They reported that 5% of this extract exhibited antifeeding activity by 53.2%, 65.4% and 56.7% against *H. armigera, S. litura* and *Earias vitella*, respectively (Pavunraj et al., 2014b).

The results from the current study showed that *H. brevipes* extract acts through more than one mode of action toward *S. littoralis*. One major route of toxicity is the direct damage of midgut and Malpighian tubules in addition to the dramatic deceased of the protein content of the mid gut cells. The high sensitivity of the midgut toward *H. brevipes* extract (as indicated by the histological changes observed in the epithelial cell) considered this organ as a primary target organ for toxic material. These effects are similar to those induced by other plant extract and natural products on the midgut of lepidopteron insects (Sakr and Abo-Elmahasen¸ 2006; Sakr, 2007; Adel *et al*., 2010; Rawi *et al.* ¸ 2011; Adel and Sammour, 2012& Ghribi *et al.* ¸ 2012). For instance, Sakr and Abo-Elmahasen (2006) reported that the CH_2CL_2 extract of *Artemisia monosperma* at concentration of 9% caused dramatic histological changes in the midgut cells of treated larvae. The cell boundaries were dissolved, regenerated cells were absent and some cells came to lie between the epithelial cells and the peritrophic membrane (Sakr and Abo-Elmahasen, 2006). Deleterious effects such as histolysis and cytoplasmic vacuolation with signs of pycknosis were observed in the midgut of 3rd larval instar of *S. littoralis* exposed to a thin film of *Streptomyces lavendulae* (Streptomycetaceae) culture filtrate at concentration of 226 CFU/cm^2 (Sakr¸ 2007). In (2010) Adel et al. reported that after treatment of *S. littoralis* with *A. monosperma* hexane extract for

two days, the epithelial membrane was completely destroyed, the epithelial cells were strongly vacuolated and the cell boundaries were disappeared. *Bacillus subtilis* SPB1 biosurfactant caused histopathological changes in the midgut of the treated *S. littoralis* which were: vesicle formation of the apical region, cellular vacuolation and destruction of the epithelial cells and their boundaries as well (Ghribi et al., 2012). The SPB1 biosurfactant bind to a protein of 45 kDa (corresponding to its putative receptor) which is differing from those recognized by *Bacillus thuringiensis* toxins (Ghribi et al., 2012).

The architecture destruction observed herein in the Malpighian tubules of the treated *S. littoralis* larvae is in agreement with that of Cordeiro et al. (2008); Hazelton et al. (2001) & Smitha and Rao, (2012). The Malpghian tubules of *Anticarsia gemmatalis* (Noctuidae) infected with nucleopolyhedrovirus, showed cell death due to oncosis and apoptosis. These may be activated by depletion of energy reserves and accumulation of marker proteins, respectively (Cordeiro *et al*., 2008). The Malpighian tubules of house cricket, *Acheta domesticus* exposed to dibutyryl cAMP showed cytoplasmic vacuolation after 30 sec post stimulation (Hazelton et al. 2001). Histological changes in the Malpighian tubules of silkworm exposed to selenium were expressed in the degeneration of the cells along with their nuclei (Smitha and Rao, 2012). The present data also showed that the *H. brevipes* CH_2CL_2 extract caused a marked decrease in the protein content of the larval midgut cells. This may be due to the structural damage of the epithelial cell membrane of the treated individuals. This result is confirmed by Sousa et al. 1(993); Sakr (2007); Khosravi et al. (2010) and Rawi et al. (2011).

The insecticidal activities of the *H. brevipes* CH2CL2extract toward *S. littoralis* larvae may be due to the secondary metabolites persist in this extract. This plant is known to produce a range of terpinoids, flavonoids and pyrons (Deng, 2010 & Sakr et al., 2013).

CONCLUSION

From data previously published by the author, *H. brevipes* dichloromethane extract known to impair the normal growth and development of *S. littoralis* larvae causing death to theses larvae. *H.*

brevipes dichloromethane extract in the current study caused significant antifeeding activity against the 5th larval instar of *S. littoralis* in a dose-dependent manner. The antifeedants are friendly to the environments because they directly work on the target insects. The effect of this extract extends to the larval midgut and Malpighian tubules epithelial cells. Extensive destruction of the midgut epithelium and Malpighian tubule's architecture was observed in the larvae fed treated castor bean leave with *H. brevipes* CH_2CL_2 extract. The destruction in these organs could be responsible for the larval death reported by Sakr et al. (2013). These effects may be due to the alkaloids, flavonoid and pyron compounds persist in *H. brevipes* extract. Therefore, the target organ for this toxic material is the midgut and Malpighian tubules as indicated by their high sensitivity toward *H. brevipes* extract. Thus, *H. brevipes* CH_2CL_2 extract could be incorporated effectively as a bio-insecticide in the control program for *S. littoralis.*

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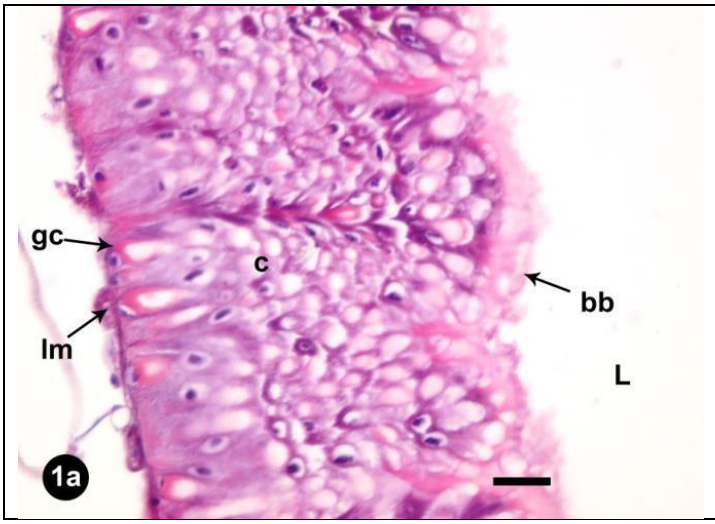
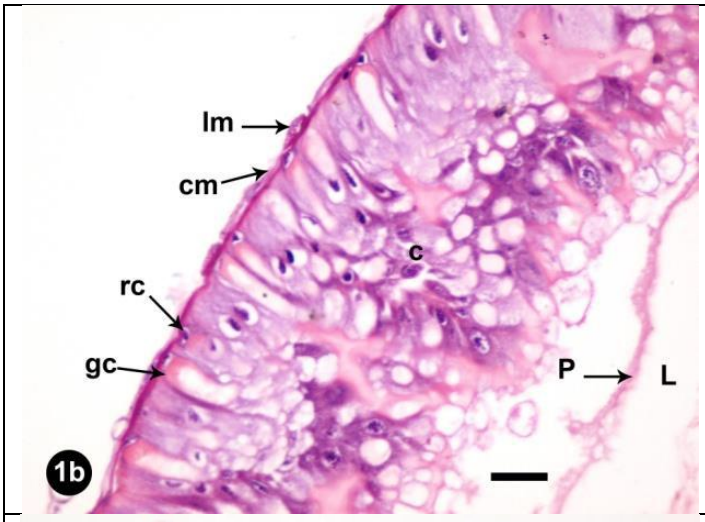


Fig.1a-b: Transverse section of the midgut of untreated (control) *S. littoralis* 5th instar larva, stained with H/E. Scale bar = 25µm. bb=brush border, c=



columnner cell, cm= circular muscles, gc= goblet cell, L= lumen, lm= longitudinal muscles, gc= goblet cell, P=peritrophic membrane, rc= regenerative cell.

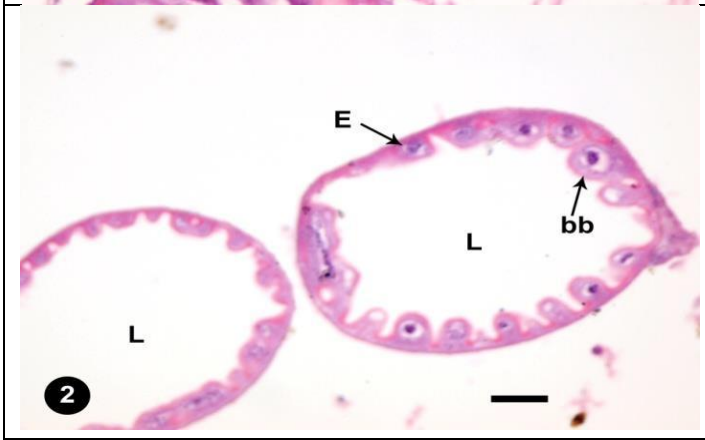
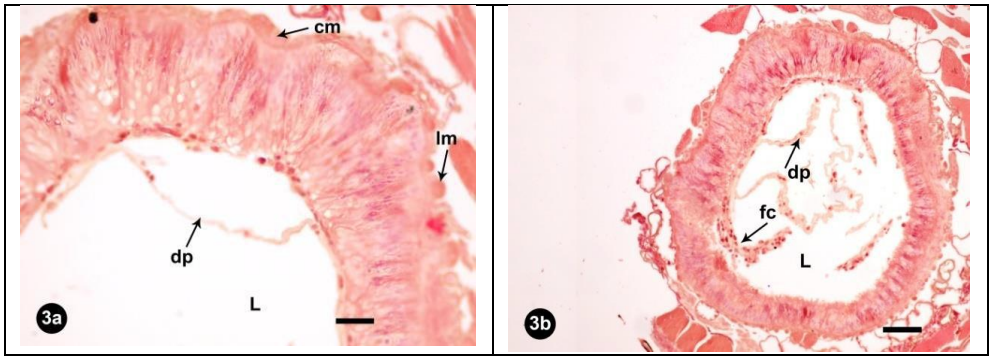


Fig.2: Transverse section of the Malpighian tubules of untreated (control) *S. littoralis* 5th instar larva, stained with H/E. Scale bar =25µm. bb=brush border, E= epithelial cell, L= lumen.



REFERENCES

Adel, M. M., El-Hawary, F. M., Abdel-Azize, N. F., Sammour, E. A., 2010. Some physiological, biochemical and histopathological effects of *Artemisia monosperma* against the cotton leafworm *Spodoptera littoralis*. Archive of phytopathology and plant protection, 43 (11):1098-111.

Adel, M. M and Sammour, E. A., 2012. Effect of sub-lethal dose of natural compound of *Medicago sativa* L. (Leguminaceae) on the hind gut and fatbody of *Spodoptera littoralis*. J. Applied Science Research, 8:1398-1408.

Aguirre, C., Valdes-Rodriguez, S., Mendoza-Hernandez, G., Rojo-Dominguez, A. and Blanco-Labra, A., 2004. A novel 8.7 kda protein inhibitor from chan seed (*Hyptis suaveolens* L.) inhibit proteases from the larger grain borer *Prostephanus truncates* (Coleoptera: Bostrichidae). Comparative Biochemistry and Physsiology (B):81-89.

Beyenbach, K.W., Skaer, H. And Dow, J.A.T., 2010. The developmental, molecular and transport biology of Malpighian tubules. Annu. Rev. Entomol. 55:351-374.**c.f. Pal, R. and Kumar,k., 2014.**Malpighian tubules of pharate adult during pupal-adult development in flesh fly, *Sarcophaga ruficornis* Fab. (Diptera: Sarcophagidae). J. Basic and Applied Zoology. Doi. 10.1016/j.jobaz.2014.06.002.

Bonhag, P.F. 1955. Histochemical studies of the ovarian nurse cells tissues and oocytes of the milkweed bug, *Oncopeltus fasciatus* Dallas. 1-Cytology: nucleic acid and carbohydrates. J. Morph., 96: 381-440.

Conti, B., Canale, A., Cioni, P.L., Flamini, G. and Rifici, A., 2011. *Hyptis suaveolens* and *Hyptis spicigera* (Lamiaceae) essential oils: qualitative analysis, contact toxicity and repellent activity against *Sitophilus granaries* (L.) (Coleoptera: Dryophthoridae). J. Pest Sci., 84: 219-228.

Corderio, B.A., Tiburcio, V.H., Hallwass, M., Paes, H.C., Ribeiro, B.M., Bao, S.N., 2008. Structureal and ultrastructural alteration of Malpighian tubules of *Antcarsia gemmatalis* (Hubner) (Lepidoptera: Noctuidae) larvae infected with different *Anticarsia germmatalis* multiple nucleopolyhedrovirus (AgMNPV) recombinant viruses. J. Inverteb. Pathol., 98, 7-19

Deng, Y., 2010. Bioactive Constituents of Two Medicinal Plants from Indonesia. Ph.D. Thesis in Pharmacy, Ohio State University, USA.

Dowd,P.F., Berhow, M.A. AND Johnson, E. T., 2011. Differential activity of multiple saponins against omnivorous insects with varying feeding preferences. J. Chem Ecol., 37:443-449.

Fermino, F., Conte, H., Falco, J.R., 2010. Analysis of nucleus activity in Malpighian tubules of *Diatraea saccharalis* (Fabricius) (Lepidoptera: Crambidae) larvae by critical electrolyte concentration. Neotropical Entomology, 39 (4): 568-571.

Gatehouse, A.M.R., Barbieri, L., Stirpe, F. and Croy, R.R.D., 1990. Effects of ribosome inactivating proteins on insect development - differences between Lepidoptera and Coleoptera. Entomol. Exp. Appl., 54:43-51.

Ghribi, D., Mesrati,A.L., Boukedi, H., Helleuch, M., Chaabouni, E.S., Tounsi, S., 2012. The impact of the *Bacillus subtilis* SPB1 biosurfactant on the midgut histology of *Spodoptera littoralis (Lepidoptera: Noctuidae)* and determination of its putative receptor. J Invertebr Pathol., 109 (2): 183-186.

Guerrero, G., Malo, E.A., Coll, J. and Quero, C., 2014. Semiochemical and natural product-based approaches to control *Spodoptera* spp. (Lepidoptera: Noctuidae). J. Pest Sci., 87:213-237.

Haouas, D., Flamini, G., Halima-Kamel, M.B. and Hamouda, M.H.B., 2010. Feeding perturbation and toxic activity of five *Chrysanthemum* species crude extracts against *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). Crop Protection, 29:992-997.

Hazelton, S.R., Felgenhauer, B.E., Spring, J.H, 2001. Ultrastructural changes in the Malpighian tubules of the house cricket, *Acheeta domesticus,* at the onset of diuresis: A time study. J. Inverteb Pathol., 247(1), 80-92.

Hu, X., Chen, L., Yang, R., Xiang, X., We, X., 2012. Molecular characterization of a peritrophic membrane protein from the silkworm, *Bombyx mori*. Mol Biol Rep, DOI 10.1007/S11033-012-2151-5.

Khosravi, R., Sendi, J.J., Ghadamyari, M., 2010. Effect of *Artemisia annua L.* on deterrence and nutritional efficiency of leser mulberry pyralid *Glyohodes pylolais* Walker (Lepidoptera: Pyralidae).J. Plant Protec. Res., 50 (4):421-428.

Lehane, M.J., Billingsly, P.F., 1996. Biology of Insect Midgut. Chapman &Hall, 486p.

Lehane, M.J., 1997. Pertrophic matrix structure and function. Annu. Rev. Entol., 42:525-550.

Martine, S.S., Van Emden, H.F., 2001. Growth disruption, abnormalities and mortality of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) caused by Azadirachtin. Neotropical Entomology, 30 (1):113-125.

Nuringtyas, T.R., Verpoorte, R., Van Oers, M.M., Leiss, K.A., 2014. Toxicity of pyrrolizidine alkaloids to *Spodoptera exigua* using insect cell lines and injection bioassays. J. Chem. Ecol., 40(6):609-616.

Pal, R. and Kumar, K., 2014. Malpighian tubules of pharate adult during pupal-adult development in flesh fly, *Sarcophaga ruficornis*

Fab. (Diptera: Sarcophagidae). J. Basic and Applied Zoology, 67 (1): 10-12. Doi. 10.1016/j.jobaz.2014.06.002.

Pavunraj, M., Muthu, C, Ignacimuthu, S., Janarthanan, S., Duraipandiyan, V., Raja, N. and Vimalraj, S., 2011. Antifeedant activity of a novel 6-(4,7-hydroxy-heptyl) quinine from the leaves of the milkweed *Pergularia daemia* on the cotton bollworm *Helichverpa armigera* (Hub.) and the tobacco armyworm *Spodoptera litura* (Fab). Phytoparasitica, 39:145-150.

Pavunraj, M, Baskar, K., Paulraj, M.G., Ignacimuthu, S. and Janarthanan, S., 2014 a. Phagodeterrence and insecticidal activity of *Hyptis suaveolens* (Poit.) against four important lepidopteran pests. Archives of phytopathology and plant protection, 47:113-121.

Pavunraj, M, Baskar, K., Janarthanan, S. and Arumugam, M., 2014b. Phytochemical effects of Spilanthes acmella (L.) Murr. Leaves on three economically important lepidopteran insect pests. J. Costal Life Medicine, 2(7):549-554.

Prado, M.A., Montueenga, L.M., Villaro, J.C., Etayo, J.C., Polak, J.M., Sema, M.P., 1992. A novel granular cell type of locust Malpighian tubules: Ultracstructureal and immunocytochemical study. Cell Tissue Res, 268:123-130.

Portero, A.G., Gonzalez-Coloma, A., Reina. M. and Diaz,C.E., 2012. Plant-defensive sesquiterpenoids from *Senecio* species with biopesticide potential. Phytochemistry Reviews, 11(4): 391-403.

Raja, N., Veyasankar, A., Venkatesan, J.S. and Ignacimuthu, S., 2005. Efficacy of *Hyptis suaveolens* against lepidopteran pests. Current Science, 88:220-222.

Rawi, S. M., Bakry, F.A., Al-Hazm, M. A., 2011. Biochemical and histopathological effect of formulated and non-formulated plant extracts on *Spodoptera littoralis* larvae. Inter. Research J. Plant Science, 2 (4): 107-118.

Santana-Meridas,O., Polissiou,M., Izquierdo-Melero, M.E., Astraka, K., Tarantilis, P.A., Herraiz-Penalver, D. and Sanchez-

Vioque, R., 2014. Polyphenol composition, antioxidant and bioplaguicide activities of the solid residue from hydrodistillation of *Rosmarinus officinalis* L. Industrial Crops and Products, 59:125-134.

Sakr, H.H., Abo-El-Mahasen, M.M., 2006. Biological and biochemical studies on *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), treated with *Artemisia monosperma* (Compositae) extracts. Proc. 4th *Int. Conf. Biol. Sci.*, Fac. Sci., Tanta Univ., Egypt: 359-366.

Sakr, H.H., 2007. Toxicity of *Streptomyces lavendulae* (Streptmycetaceae) culture filtrate to *Spodoptera littoralis* (Boisd) (Lepidoptera: Noctuidae). Egypt. J. Exp. Biol. (Zool.), 3: 55-61.

Sakr, H.H. and Hassab El-Nabi, S. E., 2007. Histological and nucleic acids alterations in *Spodoptera littoralis* (Boisd) (Lepidoptera: Noctuidae) induced by *Streptomyces lavendulae* (Streptmycetaceae) culture filtrate. Egypt. J. Exp. Biol. (Zool.), 9: 32-41.

Sakr, H.H., Roshdy, S.H. and El-Seedi, H.R., 2013. *Hyptis brevipes* (Lamiaceae) extracts strongly inhibit the growth and development of *Spodoptera littoralis* (Boisd.) larvae (Lepidoptera: Noctuidae). J. Applied Pharmaceutical Science, 10:83-88.

Salama, H.S., Dimetry,H.S., Salem, S.A., 1970. On the host preference and biology of the cotton leaf worm *Spodoptera littoralis.* Zeitung fur angewandte Entomlogie, 67: 261-266.

Simmonds, M.S.J.; Blaney,W.M.; Delle Monache,F. and Marini Bettolo, G.B., 1990. Insect antiffedant activity associated with compounds isolated from species of *Lonchocarpus* and *Tephrosia* .J. Chem. Ecol., 16(2):365-380.

Smitha, S., Rao, A.V.B., 2012. Histopathological changes in Malpighian tubules of silkworm exposed to selenium. Am-Euras J. Toxicol. Sci., 4(2), 98-102.

Sousa, M.V., Morhy L., Richardson, M., Hilder V.A., Gatehouse, A. M. R., 1993. Effects of the cytolytic seed protein enterolobin on

coleopteran and lepidopteran insect larvae. Entomol. exp. Appl., 69:231-238.

Terra, W.R. 2001. The origin and functions of the insect peritrophic membrane and peritrophic gel. Arch. Insect Biochem. Physiol., 47:47-61.

Truitt, C.L., Wei, H., Pare, P.W., 2004. A plasma membrane protein from *Zea mays* binds with the herbivore elicitor volicitin. The plant cell 16:523-532.

Vatanparast, M., **Hosseininaveh, V., Ghadamyari, M., Sajjadian, S. M., 2012.** Pectinase and cellulose activity in the digestive system of the elm leaf beetle, *Xanthogaleruca luteola* Muller (Coleoptera: Chrysomelidae). J. Asia-Pacific Entomology, 15(4):555-561.

Weinhold, A., and Shaker, K. , 2011. Phaseoloidin, a homogentisic acid glucoside from *Nicotiana attenuate* trichomes, contributes to the plant's resistance against lepidopteran herbivores. J. Chem. Ecol., 37:1091-1098.

تأثير مستخلص ثنائى كلوروميثان لنبات هيبيتس بريفيبس *Hyptis brevipes* على التغذيه والتركيب النسيجى للمعى الوسطى وأنابيب ملبيجى ليرقات دودة ورق القطن الكبرى *Spodoptera littoralis*
(حرشفيه الاجنحه:نوكتيدى)

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أظهرت دراستنا السابقه أن مستخلص ثنائى كلوروميثان لنبات هيبيتس بريفيبس *Hyptis brevipes* (Lamiaceae) يثبط النمو والتطور ليرقات دودة ورق القطن الكبرى *Spodoptera littoralis* (حرشفيه الاجنحه،نوكتيدى) مسببا الموت لهذه اليرقات. وتهدف الدراسه الحاليه الى تقييم تاثير هذا المستخلص على التغذيه وكذلك على التركيب النسيجى للمعى االوسطى وانابيب ملبيجى ليرقات دودة ورق القطن الكبرى. وقد اجريت تجربه لتقييم نشاط هذا المستخلص كمانع للتغذيه باستخدام تركيزى ٢% و٤% ضد العمر اليرقى الخامس. وقد اظهرت النتائج ان المستخلص يثبط التغذيه بنسبه 76.7% ، 85.3% عند تغذيه اليرقات على ورق خروع معامل بتركيز ٢%، ٤% ،على التوالى. كذلك أجريت تجارب التقييم الحيوى للمستخلص بتغذية العمر اليرقى الثالث لمدة ثلاثه ايام متتاليه على تركيزين (٢%،٤%) من هذا المستخلص. وقد أظهرت النتائج حدوث عدة تغيرات نسيجيه فى كل من المعى الوسطى وانابيب ملبيجى، مع ظهور لعلامات الموت المبرمج للخلايا فى هذين العضوين. حيث أدت التغذيه على ورق معامل بتركيز ٤% الى حدوث تهتك فى الغشاء الحول غذائى، وامتلاء تجويف المعى الوسطى بمجموعه من الخلايا الطلائيه (ذات أنويه مميزه لظاهرة الموت المبرمج للخلايا) بداخل تجويف المعى الوسطى. وبالنسبه لانابيب ملبيجى فقد احدث المستخلص تدميرا ملحوظا للتركيب النسيجى لها بعد اربعه ايام من المعامله بتركيز ٤%. كذلك أدت التغذيه على المستخلص النباتى بهذا التركيز الى حدوث انخفاض ملحوظ فى المحتوى البروتينى لخلايا المعى الوسطى وخاصة فى السيتوبلازم لليرقات مقارنة باليرقات غير المعامله (الكنترول).

ولقد أوضحت هذه النتائج أن المعى الوسطى وانابيب ملبيجى حساسه للمركبات السامه الموجوده بهذا النبات ، مما يفسر رفض الحشرات التغذيه على اوراق الخروع المعامله بهذا المستخلص وكذلك التأثير المميت لهذا النبات على اليرقات المعامله به ، ويعزز من امكانيه استخدام هذا المستخلص فى برامج مكافحة دودة ورق القطن الكبرى.