Effect of a novel photopesticide on some biological aspects of milkweed bug *Spilostethus pandurus* (Scopoli), with reference to ultrastructural changes in the midgut

H.A.S. Elelimy¹, H.H. Awad¹* & T.A. El Tayeb²

¹Entomology Department, Faculty of Science, Cairo University, Cairo, Egypt  
²National Institute of Laser Enhanced Sciences (NILES), Cairo University, Cairo, Egypt

The Hemipteran bug *Spilostethus pandurus* is a serious pest infesting plant seeds. Haematoporhyrin (HPF) was successful as a novel trend for *S. pandurus* control in the field due to its accumulation inside their organs after incubation and exposure to sunlight in the summer season. The survival rate of nymphs decreased with increasing HPF concentration. A concentration of $1 \times 10^{-3}$ M/l HPF increased the mortality of the first nymphal instar by 6.06- and 3.9-fold after 2 and 3 days as compared to the control, it produced the highest mortality that remained nearly constant throughout most of the 16 days of development. All biological parameters were affected, showing increased nymphal mortality, decreased number of deposited eggs by females and decreased percentage of egg hatchability. Light and electron microscopic studies on midgut regions (mg1–mg4) of adults resulting from treated nymphs revealed severe disorganisation and disintegration of cells and the presence of a great number of vacuoles. The basement membrane was detached. The microvilli were destroyed in the apical part and the food discharged. In addition, cell lysis appeared from mg1 to mg4 and many of cell organelles disappeared. The appearance of autophagic lysosomes was evident together with looped, vacuolated or swollen mitochondria. It seems likely that the nuclear membrane was ruptured or detached and clumping of the chromatin occurred. The muscle fibre and trachea were malformed. The obtained results might reflect the potency of HPF as a novel photoinsecticide in milkweed bug control in an integrated pest management (IPM) programme.

**Key words**: milkweed bug, Lygaeidae, gut, TEM, porphyrins, photopesticide.

INTRODUCTION

The milkweed bug, *Spilostethus pandurus* (Hemiptera: Lygaeidae), is known to be highly polyphagous. In Egypt, there are numerous economic reports on *S. pandurus* as a pest which has been increasing constantly in recent years, damaging numerous crops, including sunflower seeds, watermelon seeds, squash seeds, cantaloupe seeds, peanuts, cotton, sorghum, sesame, lobia, tomato, eggplant, sugar-cane, okra, pecans, whole kidney seeds, wheat and cabbage (Seleem 2012). The wide use of chemical insecticides has disturbed both the harmful and beneficial insect populations and has led to the development of resistance to most of the insecticides used in conventional pest management programmes (Ishaaya & Kontsedalov 2005). This raised the need to search for safer compounds with reduced risks of environmental pollution and led to the development of photoinsecticides. The photosensitisers of natural origin like porphyrin derivatives present specific advantages over conventional insecticides as they have low environmental impact (Dondji *et al.* 2005). They can undergo a very efficient photo-excitation by sunlight, producing a high quantum yield of singlet oxygen. The photosensitiser causes apoptotic cell death (Luksiene 2003). The cytotoxicity induced by porphyrins in combination with light is due to a photodynamic process (Idrish Miah 2002; Luksiene 2003; Awad *et al.* 2008; El Tayeb *et al.* 2013).

It has been reported that the larval mortality of *Aedes caspius* after haematoporphyrin IX formulation (HPF) increased with the increase of porphyrin concentration ($1 \times 10^3$ M/l), while the HPF accumulation in the larval body reached its maximum after 12 h of incubation. However, the remaining porphyrin concentration decreased with time, reaching its minimum level at 15 h after HPF removal from the treatment medium (El Tayeb *et al.* 2013). Similar results were obtained by Awad *et al.* (2008), who studied the effect of porphyrin reduction as a function of elapsed time.

*Author for correspondence. E-mail: hananawad19@yahoo.com
on the percentage of survival of *C. pипiens* larvae. Moreover, El-Tayeb (2008) using confocal laser scanning microscopy studied the dynamics of haematoporphyrin HP accumulation in different organs of house flies and concluded that the changes in mortality rates during and after light exposure were related to the accumulation of HP inside the different insect organs. Thus, the aim of the present study was to test the lethal effects of a commercial formulation of HP, HPF, at different concentrations on the cellular structure of the midgut regions of the milkweed bug *S. pandurus* by means of light and electron microscopy. In addition, the effect of different concentrations of HPF on several biological parameters was investigated in order to obtain the optimum HPF concentration that can be used in field applications.

MATERIAL AND METHODS

**Insect**

The laboratory colony of *S. Pandurus* was obtained from a colony raised in the Department of Entomology, Cairo University, for several years and maintained on sunflower seeds and water as described by Awad et al. (2013).

**Preparation of haematoporphyrin stock solution**

The haematoporphyrin formulation (HPF) was prepared by mixing 0.067 mg of haematoporphyrin IX (HP) powder (Logan, Utah, U.S.A.), sugar solution, and 1 ml 0.2 M NaOH and 9 ml water. The mixture was stirred with a magnetic stirrer for 3 h. The final solution was adjusted to pH 6.5–7.0. The HPF concentrations were analysed by a spectrophotometer (Perkin Elmer, LAMBDA 40) according to Lambert Beer’s law using absorbency of 423000 in H2SO4 (0.2 M). The stock solution was kept in the dark at 4 °C and used within two weeks. Different HPF concentrations (1 × 10⁻³, 1 × 10⁻⁴ and 1 × 10⁻⁵ M/l) were prepared by dilution of the stock solution with distilled water.

**Application of HPF**

Three groups of sunflower seeds, each dipped in HPF concentrations, 1 × 10⁻³, 1 × 10⁻⁴ and 1 × 10⁻⁵ M/l, for few seconds were air dried and given as food for the target insects. Each treated group of seeds was offered to three subgroups each of 30 newly hatched nymphs that were put separately into a jar, supplied with water and covered. Another group of sunflower seeds was dipped in water and offered to two groups each consisting of three subgroups of 30 newly hatched nymphs each. The two groups served as control, one group was kept in the dark during the experiment while the other group was exposed to 14:10 L:D cycle. All groups of HPF-treated nymphs were incubated 24 h in the dark; then they were exposed to sunlight as an irradiation source from 09:00 to 12:00 during July and August. The exposed insect containers were surrounded by an ice jacket to avoid temperature increase during light exposure (Awad et al. 2008). The nymphs were examined daily; the percentage of nymph mortality was recorded in comparison with the control tests (dark control and light control). The HPF concentration of 1 × 10⁻³ M/l was selected for further midgut ultrastructural and biological studies.

**Biological study**

Ten groups each consisting of three subgroups of 30 newly moulted fifth instar nymphs were placed separately into three 250-ml beakers. Each group was supplied with sunflower seeds as food supply and water then covered to prevent nymphs from escaping. One group (150 nymphs) was fed on seeds treated by HPF concentration (1 × 10⁻³ M/l), while the other (150 nymphs) served as control and was fed on untreated seeds. The treated and control nymphs were left to continue their life-span for studying different biological aspects. Ten pairs of newly emerged adults resulting from the treated nymphs, as previously mentioned, were separated and one male and one female were placed into each of 10, 250-ml beakers and supplied with sunflower seeds, water and cotton rolls for egg deposition. The preoviposition period was recorded daily. The adults were observed daily for percentage mortality, and oviposition. The number of eggs laid per female (fecundity) was counted and the number of eggs hatching (fertility) was recorded. The incubation period was also recorded. All the experiments were conducted under regulated laboratory conditions in the summer season, from June to September, at 30 ± 2 °C, 60 ± 5 % RH and 14L:10D cycle. The experiments were replicated three times for treated and control groups under laboratory conditions.

**Electron microscopy (TEM)**

Two-day-old adults resulting from first instar nymphs treated with HPF at concentration of 1 × 10⁻³ M/l, were used. They were dissected to remove
the midgut regions and placed in a prefixation solution (2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3) for 2 h at 4°C. The midgut regions were rinsed overnight at 4°C in 0.1 M phosphate buffer. The samples were postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer, pH 7.3 for 1 h at 4°C and then washed in phosphate buffer pH 7.3 for 1 h. Then dehydration was carried out in a graded series of alcohol (50–90% for 15 min and 100% for 1 h) and in pure acetone for 30 min. This was followed by infiltration in 2:1 acetone:Epon mixture overnight and 1:2 acetone:Epon mixture for 3 h then in pure resin overnight to ensure proper Epon penetration. The samples were embedded in Epon followed by polymerisation for 20 h at 70°C. Thin sections (60–90 nm) were cut with a glass knife using a RMC Inc. microtome, and collected on colloid grids. Ultra-thin sections were stained for 15 min with a saturated uranyl acetate solution and counterstained in lead citrate for 20 min (Reynolds 1963). Semi-thin sections were stained with toluidine blue (1%) and observed under a Cambridge light microscope. Ultra-thin sections were examined in a Joel JEM-1200 EX II transmission electron microscope (EM unit, Faculty of Science, Ain-Shams University).

**Statistical analysis**

All data were expressed as mean ± standard error of the mean (S.E.M.). The means of number of eggs/female in treated and control insects were compared using Independent Student’s \( t \)-tests. Also the number of hatched eggs of treated and control insects were compared using Independent Student’s \( t \)-tests. Comparisons between the means of mortality of nymphs treated with different concentrations of HPF (10\(^{-3}\), 10\(^{-4}\) and 10\(^{-5}\) M/l) and those of control nymphs were carried out using one-way analysis of variance (ANOVA) followed by Duncan’s post hoc test when the \( P \) value was statistically significant. Significance was determined at \( P < 0.05 \). All statistical analyses were done using SPSS (Statistical Package for Social Sciences, version 16).

**RESULTS AND DISCUSSION**

**Biological parameters**

Figure 1 shows that the nymphal mortality of *S. pandurus* in the control instars ranged from 0.00 after 1, 7, 10, 12, 15 d to 1.00 after 16 days the nymphal mortality increased by increasing the concentration of HPF from 1 × 10\(^{-5}\) to 1 × 10\(^{-4}\) to 1 × 10\(^{-3}\) M/l. After 2 and 3 days of feeding on

![Fig. 1. Mortality of different nymphal instars of *Spilostethus pandurus* fed on sunflower seeds treated with HPF at different concentrations.](image-url)
seeds treated with HPF at concentration of $1 \times 10^{-3}$ M/l the mortality of the first nymphal instar increased by 6.06- and 3.9-fold as compared to the control, respectively. Moreover, on the eighth and ninth day during the second nymphal instar, the mortality was 5.03 and 2.5 times that of the control. The third nymphal instar showed increased mortality by 1-, 3.5- and 2-fold as compared to the control on the 10th, 11th and 12th day, respectively. The maximum mortality was recorded in the fourth nymphal instar after 13 days of feeding on treated seeds, being 9.09-fold the control value. The fifth nymphal instar illustrated that the mortality had increased 2-fold compared to the control value on the 15th day. The first nymphal instar fed on seeds treated with a concentration of $1 \times 10^{-4}$ M/l HPF showed increased nymphal mortality by 3.03-, 6.06- and 4.5-fold on the second, third and fourth day as compared to the control value, respectively. On the fifth, sixth and eighth day during the second nymphal instar, the mortality was nearly 2, 3 and 2 times that of the control, respectively. The third nymphal instar on the 11th day showed decreased nymphal mortality that was half that of the control value. However, on the 11th day the mortality decreased to half that of the control. The maximum mortality was recorded in the fourth nymphal instar after 14th day being 6.06 times the control value. In the present study, the concentration of $1 \times 10^{-3}$ M/l HPF was chosen as it produced the highest nymphal mortality that remained nearly constant throughout most of the 16th day of development.

Data in Table 1 show that emergence of $S.\ pandurus$ males and females from the last nymphal instar emerged at the same time from the normal and the surviving nymphal instars resulting from HPF-treated first instar. An abnormal emergence of adults was noticed at nearly the same percentage in both control and treated recording 1 % and 1.3 %, respectively; the wings were slightly shrunken and remained folded. Sometimes, these treated bugs remained inside the exuvia and died. The percentage of adult emergence from HPF-treated first nymphal instar was decreased to 0.5 times that of the control. The mean longevity of both males and females was significantly decreased recording 10 ± 0.27 day in adults emerging from HPF-treated first nymphal instar while that of the control ones recorded 11.2 ± 0.27 days. The preoviposition period increased significantly by 2.2-fold in adults emerged from HPF-treated first nymphal instar compared to that of the control ones. The number of eggs laid by one female (fecundity) and number of egg hatching in days were significantly decreased in females emerging from HPF-treated first nymphal instar compared to the control by 1.3- and 2.5-fold, respectively. The mean duration time of eggs resulting from females emerged from HPF-treated first nymphal instar showed a significant decrease compared to the control by 1.7-fold. In spite of the

Table 1. Biological parameters of $Spilostethus\ pandurus$ surviving from first nymphal instar fed on sunflower seeds treated with $1 \times 10^{-3}$ M/l HPF.

<table>
<thead>
<tr>
<th></th>
<th>Percentage of adult emergence</th>
<th>Longevity of adults (days) Mean ± S.E.</th>
<th>Preoviposition period (days) Mean ± S.E</th>
<th>No. of eggs per female Mean ± S.E</th>
<th>Number of eggs hatched Mean ± S.E</th>
<th>Incubation period of eggs (days) Mean ± S.E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>88 %</td>
<td>11.2 ± 0.27</td>
<td>3.9 ± 0.146</td>
<td>65.58 ± 6.35</td>
<td>40.69 ± 3.98</td>
<td>9.33 ± 0.26</td>
</tr>
<tr>
<td>n = 12 (3–5)</td>
<td></td>
<td></td>
<td>(3–5)</td>
<td>n = 26</td>
<td>n = 26</td>
<td></td>
</tr>
<tr>
<td>Treated</td>
<td>44 %</td>
<td>10 ± 0.27*</td>
<td>8.6 ± 0.147*</td>
<td>49.70 ± 6.91</td>
<td>16.17 ± 3.30*</td>
<td>5.48 ± 0.52*</td>
</tr>
<tr>
<td>$1 \times 10^{-3}$ M/l HPF</td>
<td>n = 8</td>
<td></td>
<td>(7–10)</td>
<td>n = 24</td>
<td>n = 22</td>
<td></td>
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*The mean difference is significant at the $P < 0.05$ level.
recorded economic status of the milkweed bug in Egypt as it is registered as a pest of sunflower seeds, our information about it is weak and fragmentary and little attention has been paid to it by previous workers. Seleem (2012) concluded that the hot weather of the summer in Egypt was more favourable for adult *S. pandurus* development than the cold winter. Some authors studied the biology of the mealy bug *Phenacoccus solenopsis* under controlled laboratory conditions. They illustrated that the development from immature stages to adult stage was greater for males (18.7 ± 0.9 days) than females (13.2 ± 1.8 days) and also that the reproductive period lasted 30.2 days (Vennila et al. 2010). The egg stage of *S. pandurus* ranged from 7 to 10 days under laboratory conditions (Kugelberg 1973). The mean incubation period of *S. pandurus* eggs was short in summer, 3.9 ± 0.146 days, but longer in the cold winter, 8.4 ± 0.201 days, as mentioned by Awad et al. (2013). However, the increased longevity was associated with a prolonged rate of sexual maturation and low mean daily fecundity and the lifespan increased during winter season and decreased during summer season, with the males living longer than the females (El-Shazly 1996). During summer, Awad et al. (2013) found that the eggs laid by each *S. pandurus* females exceeded that laid during winter recording 96.4 ± 1.94 eggs and 63.9 ± 3.84 eggs, respectively. Also in a bisexual population, the females lived longer in winter than in summer (28.27 ± 0.33 days and 17.15 ± 0.54 days, respectively) as compared to the males (27 ± 0.17 day and 12.44 ± 0.19 days, respectively).

### Ultrastructural study

#### Light microscopic studies

The midgut (mg) of *S. pandurus* is found just posterior to the oesophageal valve and is composed of four different regions, mg1–mg4 (Meguid et al. 2013). In the present study, the semi-thin sections of the untreated midgut regions (Fig. 2A–D) showed that the epithelial cells vary from short to long columnar cells. Some cells appear binucleated. The apical part of the cells from mg2–mg4 seemed darkly stained, covered with dense secretor products. The mg3 and mg4 were full of lipid globules covering the apices of the columnar cells and almost protruding into the lumen. The regenerative cells lie low beneath the epithelial cells. Meguid et al. (2013) found that the muscle layer was a very thin margin compared to the size of the cells. The midgut regions of the surviving adults obtained from HPF-treated nymphs at concentration of 1 × 10⁻³ M/l examined by light microscopy, in the present study, showed rapid and severe disorganisation and disintegration of the mid-gut cells mg1–mg4 with the presence of a great number of vacuoles (Fig. 2E–H). This is due to the disappearance of the boundaries between the cells. A few scattered groups of cells were sloughed off from their basement membrane. The columnar epithelial cells, discharged into the lumen, were clear when compared with those of the control. Lyses of the epithelial cells which appeared separated from each other and swelling of some cells were also evident. Moreover, there was an increase in vacuoles and deformation of epithelial cells. Rupture of most regenerative cells with the increase of their cavities was observed. The epithelial cells also showed bulbous protrusions from the apical plasma membrane and leakage of cell contents. The muscles were detached from the epithelium. The cracks in the cell cytoplasm probably occurred due to the loss of elasticity.

#### Electron microscopic studies

The ultrastructural survey of normal midgut regions of the milkweed bug *S. pandurus* adults (Fig. 3) showed that it is composed of a monolayer of epithelial cells and regenerative cells resting on a basement membrane. The luminal surface of the epithelial cells was thrown into numerous microvilli, filled with very minute fibrils (Fig. 3B–G). The apical cell membrane or microvillar border is dense and compact and the basal cell membrane forms a basal labyrinth. The basement membrane appears dense and amorphous. It is surrounded by a layer of muscle cells and trachea. The muscle layer and tracheal cell appeared ensheathed by connective tissue (Fig. 3H). The cytoplasm of the epithelial cells was packed with organelles, mainly mitochondria, rough endoplasmic reticulum, lysosomes and lipid droplets (Fig. 3A–F). The mitochondria were more numerous in the apical region of the cell under the microvillar border than in the basal region. They were cylindrical or cup-shaped having outer and inner membranes; the inner membrane was folded into cristae which were oriented mainly transversely. Two adjacent cells were connected laterally (Fig. 3B, C, D, G). The microvilli were uniform in the first region and blebs extruding from the microvillar border (arrow) were
observed (Fig. 3B). Cells were filled with large and numerous lipid globules mostly in the apical region and sometimes extruding into lumen. Large lipid globules appeared in the mg3 region which is the lipoid zone; lysosomes in mg2 were large in size and density and appeared as large autophagic lamellate structures (Fig. 3C). The microvillar border in the mg3 region was not as compact as in the previous regions (Fig. 3E, F). Some epithelial cells appeared binucleated in mg2, mg3 and mg4 (Fig. 3D, F, G). The cell nucleus in mg cells of *S. pandurus* appears spherical and in most cells is centrally located or just below the apical region. Most cells are binucleated, which could be due to high cell activity in which large quantities of nucleic acid moves in and out of the nucleus to generate synthesis and secretion of protein enzymes. The nucleoli have dense chromatin dispersed in clumps with batches near the nuclear membrane.

The midgut regions of surviving adults resulted from HPF-treated nymphs of *S. pandurus* at a concentration of $1 \times 10^{-3}$ M/l (Figs 4–7) examined by TEM showed that the contents of mg cells were discharged, the cytoplasm was extremely vacuolated and contained degenerated organelles, which appeared as cell debris. Moreover, the cell lysis appeared from mg1–mg4 and many of its organelles

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**Fig. 2.** Photomicrographs of the semi thin sections of midgut (mg) regions, from untreated control adults and surviving adults resulting from HPF-treated nymphs. **A–D,** first, second, third and fourth midgut regions from untreated control adults. **E–H,** first, second, third and fourth midgut regions from survived adults after HPF treatment. Showing cracks in the cell cytoplasm (line), binucleated (Bi N), columnar epithelial cells (Ep), lumen (Lu), lipid globule (lp), muscle layer (mu), nucleus (uniN), regenerative cells (Rc), vacuoles (v). Magnification: A, B, D, F, H = ×400; C, G = ×100.
disappeared with the appearance of dark residual bodies, autophagic lysosomes. The disorganisation of polymorphic mitochondria, looped, vacuolated and swollen, was also evident. Also the basement membrane between the two neighbouring cells was observed (mg1 and mg4) and sometimes appeared detached. The microvilli were destroyed, shrunken or curled, in the apical part and the food was discharged and many ruptured vacuoles were observed. The perimicrovillar membrane covered and folded the microvillar membrane (Fig. 4B arrow; Fig. 5B black...
The nuclear membrane was observed, one of the two adjacent nuclei showed ruptured areas in the nuclear membrane as observed in mg3 and mg4 (Figs 6B; 7C, D, E). In mg4 it may be suggested that the nuclear membrane was folded, and the surface area of the nucleus increased (Fig. 7C, D). Clumping of the chromatin body which migrates to the periphery of the nucleus was evident. It has been suggested that Golgi bodies were fragmented into small particles. The rough endoplasmic reticulum was also separated into narrow vascular structures without attachment to the nuclear membrane. The muscle cells revealed the degenerated sarcomeres and irregular vacuoles or gaps appeared. The basal lamina that surrounds the tracheae was detached and its nucleus became elongated and all the observed tracheal cells were collapsed.

Hematoporphyrin belongs to the group of photopesticides which are rapidly photo bleached.
upon exposure to UV or visible light. HPF appeared to accumulate in the cuticle, midgut, malpighian tubules, and adipose tissue as reported by several authors (Salama et al. 2002; El Tayeb et al. 2013; Abdel Kader & El Tayeb 2014). Similarly, several authors found vacuolation and disintegration of the epithelial and regenerative cells, as well as disappearance of muscularis and detachment of basement membranes in the midgut of Spodoptera exigua treated with diflubenzuron, malathion and cypermethrin (Younis et al. 2000; Bakr et al. 2008).

The midgut epithelium of S. pandurus is not in direct contact with the food bolus, due to the presence of the perimicrovillar membrane (PMM), which ensheathes the cells and sprouts from the base of the microvilli up to their tips extending into the gut lumen (Silva et al. 2004; Silva et al. 2007). The PMM is responsible for the absorption of nutrients in hemipterans (Silva et al. 2007; Meguid et al. 2013). On the other hand, the present study revealed that HPF caused many changes to the mitochondrial cristae which were partially or

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**Fig. 5.** Electron micrographs of epithelial cells from second midgut region of surviving adults resulting from HPF-treated nymphs of Spilostethus pandurus at concentration of $1 \times 10^{-3}$ M/l. A, showing basement membrane (bm), lipid globule (lp), lysosome (ly), vacuolated mitochondria (m), shrinkage of musculosa and detached muscle fibre (mu), uni- and binucleated two adjacent cells, nucleus (N), vacuoles (v), collapsed tracheole (tr). B, C, lipid globule (lp), lumen (Lu), microvilli (mv), perimicrovillar membrane (pm). Scale bars = 2 µm.

**Fig. 6.** Electron micrographs of epithelial cells from third midgut region of surviving adults resulting from HPF-treated nymphs of Spilostethus pandurus at concentration of $1 \times 10^{-3}$ M/l. A, showing lateral cell membrane (LCM), lipid globule (lp), mitochondria (m), shrinkage and curled microvilli (mv), perimicrovillar membrane (pm). B, binucleated cell, nucleus (N), vacuoles (v). C, lipid globule (lp), destroyed microvilli (mv). D, disintegration of the cytoplasm and cell organelle, shrinkage of musculosa and detached muscle fibre (mu), vacuoles (v). Scale bars: A, B, C = 1 µm; D = 2 µm.
totally lost. Some mitochondria appeared swollen with irregular shapes while others appeared greatly elongated with prominent cristae. Also, the nuclear membrane was disrupted. In addition, the lysosomes and Golgi bodies were hypertrophied with different deformities. However, Golgi bodies where markedly affected and some of them had been fragmented into small particles. The secretor granules associated with the Golgi bodies disappeared. The limiting membranes of lysosomes were ruptured. The secondary lysosomes were accumulated in the nuclear region. Bradly et al. (2001) found similar results during the treatment of penultimate instar nymphs of *Schistocerca gregaria* with flufenoxuron. The present margination and nuclear chromatin seemed to be changed early leading to the cell death. Vacuole formation is a cellular defence mechanism against cytotoxins and prevents them from disrupting cellular metabolism and this represents the initial stage of disintegration. The rough endoplasmic reticulum then breaks down into separate vascular structures, and the Golgi body fragments into small particles. The present changes are in line with those observed...
by Bakr et al. (1997) in Culex pipiens with insect growth regulators.

Haemoglobin digestion occurs in the gut lumen producing heme which is a potent toxin and may catalyse reactive oxygen species formation (Deterding et al. 2004), leading to lipid, protein and DNA damage (Schmitt et al. 1993). In addition, heme can also be associated with phospholipid membranes, altering their structures and leading to cell disruption (Silva et al. 2007). As HPF is a derivative from haemoglobin, it may be proposed that HPF generates reactive oxygen species and these may attack the (PMM) leading to its destruction. On the other hand, the haemolymph volume decreases during photosensitisation and the haemocoeal fluids undergo rapid transfer from the body cavity to the alimentary canal and this behaviour is suggested to aid in cytotoxic mechanisms in PMM. Dijoux et al. (2006) confirmed phototoxicity and cytotoxicity in vitro using essential oils from Citrus aurantiun dulcis and Cymbopogon citrates.

From both the ultrastructural and biological studies, it may be suggested that malformation occurred in different midgut regions and this may underlie the high nymphal mortality and the decrease in the percentage of adult emergence and their longevity observed after treatment of S. pandurus with HPF.

The use of chemical insecticides leaves undesirable residues in the surrounding environments. The photopesticides are clear alternative pest management tools which are needed, and are friendly to the environment, with a minimum cost and low impact.

CONCLUSION

From the present data it may be concluded that HPF exhibited dangerous histopathological effects on the midgut regions of S. pandurus after feeding on sunflower seeds treated with HPF. Although these results confirm the insecticidal efficiency of haematoporphyrin, a photoactivated insecticide, against milkweed bugs, the mode of action needs to be investigated by further studies in future. Thus, its use may be recommended in pest control management strategies to reduce costs and reduce the pesticide impact in the environment. Also this study could help in integrated pest management programmes, IPM, to control this economically important bug in tropical and subtropical areas. Thus HPF is a safe measure that can protect the crops of economic importance such as sunflower plants and increase the oil yield.

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