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PHOTOSENSITIZING EFFECTS OF HEMATOPORPHYRIN DIHYDROCHLORIDE AGAINST THE FLESH FLY *PARASARCOPHAGA ARGYROSTOMA* (DIPTERA: SARCOPHAGIDAE)

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ABSTRACT

There is an urgent need to use environmentally safe pesticides, which do not sneak into human food or animals and then back at him and damage the environment. The photosensitizers, such like hematoporphyrin dihydrochloride (HpD), a nontoxic chemical has an ability to harm the target pest when mixed with suitable baits. When exposed to light, the ingested HpD triggers the destruction of the midgut and inhibits the defensive enzymes, viz acetylcholine esterase (AchE), superoxide dismutase (SOD), catalase (CAT) and glutathione-S-transferase (GST). In the current study, HpD was investigated as photoinsecticide against 3rd larval instar and adults of a myiatic fly, *Parasarcophaga argyrostoma* (Robineau-Desvoidy) (Sarcophagidae: Diptera). Third instar larvae and adults were fed baits containing 5×10^{-2}, 5×10^{-3}, 5×10^{-4}, 5×10^{-5}, and 5×10^{-6} ppm of HpD. Mortality ratios were recorded at different post treatment sunlight exposure periods. The biochemical study of the impact of one median concentration, 5×10^{-4} ppm, proved its ability to cause damage that may be beyond repair for some defensive enzymes. In addition, fatal histopathological changes were detected in the midgut epithelium. The study showed that, HpD could be an excellent pesticide against the larvae and adults of the myiatic flies.

Key Words: acetylcholine esterase, antioxidant enzymes, midgut, hematoporphyrin dihydrochloride, *Parasarcophaga argyrostoma,* photosensitizer.

RESUMEN

Existe una necesidad urgente de utilizar pesticidas ecológicamente seguros, que no se filtren dentro alimentos para humanos o animales. Los fotosensibilizadores, como dihidrocloruro de hematoporfirina (HPD), tienen la capacidad de dañar a la plaga de enfoque de estudio cuando se mezcla con los cebos adecuados. Cuando se expone a la luz, el HpD ingerido provoca la destrucción del intestino medio e inhibe las enzimas defensivas, como las enzimas antioxidantes y de desintoxicación. En el presente estudio, se investigó HpD como un photoinsecticida contra el tercer estadio larval y adultos de una mosca miática, *Parasarcophaga argyrostoma* (Robineau-Desvoidy) (Sarcophagidae: Diptera). Para investigar los efectos de fotodinámicos de HpD, larvas del tercer estadio y adultos fueron tratados con diluciones en serie de 5×10^{-2}, 5×10^{-3}, 5×10^{-4}, 5×10^{-5} y 5×10^{-6} ppm. Se registró la tasa de mortalidad en diferentes períodos de exposición a la luz de sol pos-tratamiento. El estudio bioquímico del impacto de una concentración mediana, 5×10^{-4} ppm, demostró su capacidad de causar daños que pueden estar más allá de la reparación de algunas enzimas defensivas. Además, se detectaron cambios histopatológicos fatales en el epitelio del intestino medio. El estudio mostró que, HpD podría ser una excelente pesticida contra larvas y adultos de moscas miática.

Palabras Clave: dihidrocloruro de hematoporfirina, *Parasarcophaga argyrostoma*, efectos fotosensibilizantes, enzimas antioxidantes, acetilcolina esterasa, intestino medio

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The flesh fly *Parasarcophaga argyrostoma* (Robineau-Desvoidy) (Sarcophagidae: Diptera) is involved in myiasis. Their maggots gain entry through wounds or normal openings of animals or humans. This flesh fly has been reported several times as an agent of human cutaneous wounds and eye myiasis (Burgess &Spraggs 1992; Razmjou et al. 2007; Gómez-Hoyos et al. 2012). Chemical control of the myiatic flies like *P. argyrostoma* by traditional insecticides is difficult because of the larvae being protected inside wounds or bodies, and the high mobility of the adults. Alternative pest management tools are needed that are less hazardous to human, animals, and the environment. Therefore, sunlight activated pesticides, based on photosensitizer compounds (nontoxic photoactive organic) and which could be used as effective bioinsecticides, represent a possible alternative to traditional chemical insecticides (Filiberti et al. 2009). When ingested with food, these compounds accumulate within the insect pest; subsequently, following exposure to visible light, lethal photochemical reactions cause death of the target pest.

Porphyrins, a group of photosensitizers, appear to be particularly promising photoinsecticides because they absorb essentially all the wavelengths of the solar spectrum. They can undergo very efficient photoexcitation by sunlight (Jori 1985). Porphyrin derivatives was found effective for inducing a microbiocidal or larvicidal action in polluted waters (Fabris et al. 2012).The insecticidal activities of hematoporphyrin were tested against dipterans in different habitats, viz., terrestrial [*Ceratitis capitata* (Wiedemann) (Tephritidae) and *Bactrocera oleae* (Rossi) (Tephritidae) (Ben Amor et al. 1998a, b)], and aquatic [larvae of *Culex pipiens* L. (Culicidae) and *Aedes aegypti*L. (Culicidae) (Salama et al. 2002; Awad et al. 2008, Lucantoni et al. 2011)]. Moreover hematoporphyrin was effective against the leafminer fly, *Liriomyza bryoniae* Kaltenbach (Agromyzidae) (Luksiene et al. 2007). The success in using hematoporphyrins widely against various dipteran pests suggested an investigation on its effect on the adults of the myiatic fly *P. argyrostoma* (El-Tayeb et al. 2011). Hematoporphrin was even found to be effective against the snail, *Biomphalaria alexandrina* (El-Hommossany et al. 2012).

Hematoporphyrins are stress factors that induce the release of excessive reactive oxygen species (ROS), which cause severe damage in cells of the treated pest (Ben Amor 2000). To protect against the damage of ROS, insects have a network of antioxidant enzymes such like, SOD, CAT, GST and peroxidases (Wang et al. 2001; Weirich et al. 2002; Dubovskiy et al. 2008). AchE, in spite of its role in detoxifying insecticides, especially organophosphates in *P. argyrostoma* (Morallo & Martin 1967), was a target for the photosensitizers (Weiner et al. 2011).

The aim of our study was not only to evaluate the photoinsecticidal efficiency of HpD on 3rd instars and adults (males and females) of *P. argyrostoma*, but also to investigate its biochemical effects on the detoxification enzyme AchE and some major antioxidant enzymes, including SOD, CAT, and GST. In addition, we examined the histopathological changes in the mid gut cells in response to HpD treatment.

Materials and Methods

Insect Culture

Parasarcophaga argyrostoma adults were collected from a slaughtering house located at Assiut, Egypt. This species was cultured in an incubator at 25 °C ± 1 and 60% RH. The adults were fed on sugar solution. Minced beef meat was offered as a substrate for feeding and maggot laying.

Bioassay

HpD was purchased from Sigma-Aldrich. A stock solution (0.1 mol. Dm^{-3}) was prepared. For estimating the lethal concentration (LC_{50}), 5 serial dilutions were used against the 3rd instars and both male and female adults. The serial dilutions - based on preliminary experiments - were made with glycerol to obtain 5×10^{-2}, 5×10^{-3}, 5×10^{-4}, 5×10^{-5}, and 5×10^{-6} ppm. The solutions were supplied to adults in cotton pieces covered with solid sugar granules, and to 3rd instars as a mixture with minced meat as larval food. Five replicates of 15 insects per replicate were tested at each concentration. After feeding overnight in darkness, the treated insects were fed with HpD-free food and exposed to direct sunlight (equivalent to 810 W/m^2) for periods of 0.5, 1, 1.5, or 2 h. The mortality ratios were recorded and the LC_{50} values were calculated. In the control the 3rd instars and adults were treated with the same dilutions of HpD as previously mentioned, but kept on HpD- free food in darkness until the end of the experiment. Another control experiment was done in which untreated 3rd instars and adults were exposed to direct sunlight for the same periods as the treated insects.

Preparation of Samples for Biochemical Studies

Newly molted 3rd instars and newly emerged male and female adults were fed on food treated with 5×10^{-4} ppm and exposed to direct sun light for 45 min. The bioassay results showed that this concentration is the lowest concentration that induced considerable larval and adult mortalities (near 50%) at 60 min after light exposure. Insects that survived were homogenized in sodium phosphate buffer (pH 7). Homogenates were centri-

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fuged at 4,000 rpm for 15 min at 4 °C, and the supernatants were used for enzyme assays. In the control, the insects were fed treated food and kept in darkness until homogenization.

Assays of Enzymes and Total Protein

The activity of AchE was measured according to Simpson et al.(1964), using acetylcholine bromide at a level of 6×10^{-3} as a substrate. The activity present in the homogenate was expressed as µg substrate hydrolyzed/insects/min.

The activity of SOD was determined by measuring the inhibition of autoxidation of epinephrine in pH 10.2 at 30 °C (Misra & Fridovich 1972). The values were expressed as U/min/mg protein.

The activity of CAT was determined basing on its ability to decompose H_2O_2 (Beers & Sizer 1952). The values were expressed as U/min/mg protein.

The activity of GST was determined using 1-chloro-2,4-dinitrobenzene as a substrate (Habig et al. 1974). The values were expressed as µmol/ gram body weight /min.

Total protein in the insect homogenates was determined by the commercially available kit obtained from Biodiagnostic, Egypt (Henry 1964).

Histopathological Studies

The following histological procedures were applied according to Drury & Wallington (1980). Third instars and adults that survived treatment with 5×10^{-2} ppm and exposure to light for 1.5 h were dissected. Their midguts were fixed in formal alcohol solution for about 16 h. Midguts of control insects (fed with 5×10^{-2} ppm but kept in darkness) were also dissected and these midguts were fixed in the same manner. The specimens were washed in 70% ethyl alcohol, dehydrated, cleared, sectioned and stained with Harris haematoxylin and eosin stains.

Statistical Analysis

The mean and standard error (SE) were estimated. The patterns of variations in percent mortality and results of enzyme assays were analyzed by one-way ANOVA. Mortalities of the control were corrected using Abbott's formula (Abbott 1925). The main effects of different concentrations of the photosensitizer compound and exposure periods to light on percent mortality and the effect of the photosensitizer compound on enzyme activity and their interactions were separated by the Tukey test and Dannett's T-test at the 0.05 level of significance. The variances between treatments were found to be homogeneous. SPSS package release 10 (SPSS Inc. 1998) was used in these statistical analyses.

Results

Photosensitization Process to Control *P. argyrostoma*

In the present study, a series of dilutions of the photoinsecticide HpD was prepared in order to study its lethal effects on the third instars and adult males and females of the flesh fly. Preliminary results showed that treatment of the controls of 3rd instar and adults with the HpD dilutions followed by darkness did not result in significant mortalities compared with the untreated group. The mortality ratios of the 3rd instar treated with different concentrations of HpD and exposed to sun light for variable periods (Table 1) show that HpD induces no mortality at 5×10^{-6} ppm. Further 5×10^{-5} ppm kills only about 3.3% of the treated 3rd instars after post treatment light exposure of 2 h. At 5×10^{-4} ppm HpD applied to 3rd instar, 56% mortality occurred after exposure to light for 1.5 h; and the mortality increased to 66.6% at a 2 h post treatment light exposure period. At 5×10^{-3} ppm, the mortality was 17.5% at a 1 h exposure period, and it increased to 96% at a 2 h post treatment light exposure period. The highest concentration of 5×10^{-2} ppm caused 87% mortality at a 1 h post treatment exposure to light. The mortality ratio in 3rd instar that have not been subjected to light after insecticide treatment was 9.2%, but when exposed to light for more than 1 h the larval death rate increased to 87 %.

Table 2 shows the effects of treatment with the various concentrations of HpD on the males. The 5×10^{-5} ppm treatment resulted in a substantial mortality ratio of 73.2% at 2 h post treatment sun light exposure. Treating the males with 5×10^{-4} ppm resulted in a dramatic increase of the mortality ratio to about 98% after 1.5 h post treatment exposure to sunlight. Still higher concentrations (5×10^{-3} and 5×10^{-2} ppm) caused dramatic increases in mortality ratios starting from 1 h of post treatment light exposure.

Table 3 displays the effects of treating the adult females with various HpD dilutions. The mortality ratio increased with increasing HpD concentration and exposure time to light after treatment. The concentration 5×10^{-5} ppm killed 56.6% of females at the 2 h of post treatment light exposure. The concentration 5×10^{-4} ppm raised the mortality ratio to 70.9% when the treated females were exposed to light for 1.5 h. Increasing the concentration to 5×10^{-3} ppm resulted in a 68.8% mortality ratio at 1 h of post treatment light exposure. Treating with the highest concentration (5×10^{-2} ppm) resulted in 74.6% mortality at 0.5 h of post treatment light exposure and then escalate dramatically with longer exposures to sunlight.

Effect of HpD on the Activity of AchE, SOD, CAT, and GST

The third instars, and male and female adults were treated with 5×10^{-4} ppm and exposed to

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TABLE 1. THE PHOTODYNAMIC EFFECT OF VARIOUS HEMATOPORPHYRIN DIHYDROCHLORIDE CONCENTRATIONS (PPM) ON THE MORTALITY RATIOS OF THE 3RD INSTARS OF *PARASARCOPHAGA ARGYROSTOMA* AT VARIOUS POST-TREATMENT PERIODS OF EXPOSURE (HOURS) TO DIRECT SUNLIGHT OF 810 W/M^2.

	Concentrations of hematoporphyrin dihydrochloride					
Sunlight Exposure Periods	0	5 × 10-6	5 × 10-5	5 × 10-4	5 × 10-3	5 × 10-2
2 h	0aA	0aA	3.31 ± 0.223bA	66.557 ± 0.238cB	96.39 ± 1.881dC	100dD
1.5 h	0aA	0aA	0aA	56.32 ± 0.437bB	73.877 ± 0.571cC	100dD
1 h	0 aA	0aA	0aA	0aA	17.54 ± 0.826bB	86.91 ± 0.588cC
0.5 h	0aA	0aA	0aA	0aA	0aA	12.207 ± 0.566bB
0 h	0.667 ± 0.667aA	0aA	0aA	0aA	0.683 ± 0.683aA	9.203 ± 1.047aB

Values of mortality ratios are expressed as mean ± SE (N =15 larvae; 5 replicates).
Different superscripts (small letters) within a column refer to significant difference ($P < 0.05$) among exposure periods.
Different superscripts within a raw (capital letters) refer to significant difference ($P < 0.05$) among HpD concentrations.

Table 2. The photodynamic effect of various hematoporphyrin dihydrochloride concentrations (ppm) on the mortality ratios of adult males of *Parasarcophaga argyrostoma* at different periods (in hours) of post-treatment exposure to direct sunlight (810 w/m^2).

Sunlight Exposure Periods	Concentrations of hematoporphyrin dihydrochloride					
	0	5×10^{-6}	5×10^{-5}	5×10^{-4}	5×10^{-3}	5×10^{-2}
2 h	2 ± 2bA	26.353 ± 0.151dB	73.23 ± 0.549eC	99.663 ± 0.284eD	100dD	100cD
1.5 h	1 ± 1bA	20.817 ± 0.649cB	26.903 ± 0.516dC	97.92 ± 0.0096dD	99.763 ± 0.232dD	100cD
1 h	0bA	13.83 ± 0.441bB	21.173 ± 0.178cC	50.147 ± 0.405cD	84.09 ± 0.47cE	100cF
0.5 h	0.3 ± 0.3bA	12.497 ± 0.304bB	13.127 ± 0.163bB	46.857 ± 0.608bC	54.267 ± 0.34bD	83.623 ± 0.474bE
0 h	7.33 ± 0.33aAB	6.5 ± 0.755aA	7.4 ± 0.603aAB	8.13 ± 0.186aAB	8.4 ± 0.006aAB	9.1 ± 0.01aB

Values of mortality ratios are expressed as mean ± SE (N =15 males; 5 replicates).
Different superscripts (small letters) within a column refer to significant difference ($P < 0.05$) among exposure periods.
Different superscripts within a row (capital letters) refer to significant difference ($P < 0.05$) among HpD concentrations.

TABLE 3. THE PHOTODYNAMIC EFFECT OF VARIOUS HEMATOPORPHYRIN DIHYDROCHLORIDE CONCENTRATIONS (PPM) ON THE MORTALITY RATIOS OF ADULT FEMALES OF *PARASARCOPHAGA ARGYROSTOMA* AT DIFFERENT PERIODS (IN HOURS) OF POST-TREATMENT EXPOSURE TO DIRECT SUNLIGHT (810 W/M^2).

	Concentrations of hematoporphyrin dihydrochloride					
Sunlight Exposure Periods	0	5×10^{-6}	5×10^{-5}	5×10^{-4}	5×10^{-3}	5×10^{-2}
2 h	0aA	23.103 ± 0.113dB	56.573 ± 1.365eC	77.95 ± 0.104eD	89.45 ± 0.278eE	98.7 ± 0.351dF
1.5 h	0aA	15.643 ± 0.224cB	20.647 ± 0.676dC	70.893 ± 0.673dD	82.25 ± 0.577dE	96.3 ± 1.205dF
1 h	0aA	8.38 ± 0.446bB	16.3 ± 0.169cC	42.327 ± 0.663cD	68.783 ± 0.394cE	91.783 ± 0.829cF
0.5 h	0aA	1.317 ± 0.662aA	9.367 ± 0.649bB	30.87 ± 0.567bC	42.083 ± 0.0083bD	74.617 ± 1.26bE
0 h	1 + 1aA	1 ± 0.577aA	0.667 ± 0.667aA	1.1 ± 0.1aA	2 ± 0.058aA	2.7 ± 0.3aA

Values of mortality ratios are expressed as mean ± SE (N =15 females; 5 replicates).
Different superscripts (small letters) within a column refer to significant difference ($P < 0.05$) among exposure periods.
Different superscripts within a raw (capital letters) refer to significant difference ($P < 0.05$) among HpD concentrations.

direct sunlight for 45 min. The survived insects were used for measuring the activity of AchE, SOD, CAT, and GST. Results in Fig. 1A show the effects of HpD on AchE in the 3rd instar larvae, males, and females. HpD caused reduction in the activity of AchE in all treated larvae and male and female adults. The activity of AchE decreased to 4.21, 3.32, and 10.27 µg substrate hydrolyzed/insect/min, which represent 47.571%, 44.09%, and 39.52% activity relative to the controls of the 3rd instars, males, and females respectively.

The effects of HpD on SOD are shown in Fig. 1B. HpD treatment decreased the activity of SOD to 25.62, 16.35, and 40.39 U/min/mg protein, which represent 59.60%, 35.94%, and 67.93% relative to the controls of the 3rd instars, males, and females respectively.

The activity of CAT (Fig. 1C) was reduced by HpD treatment to 3.3, 61.03, and 33.31 U/min/mg protein, which represent 31.98%, 75.06%, and 52.86% relative to the controls of the 3rd instars, males, and females respectively.

The activity of GST (Fig. 1D) was reduced by HpD treatment to 11.83, 5.92, and 6.40 µmol/gram body weight/min, which represent 39.92%, 46.12%, and 43.44% relative to the controls of the 3rd instars, males, and females respectively.

Histopathological Effects of HpD on the Midgut

The midguts of larvae (data not shown) and adults (Fig. 2) are each surrounded by an inner layer of circular muscle fibers (CMF) and outer layer of longitudinal muscle fibers. A single layer of more or less columnar epithelial cells followed by a peritrophic membrane lines the midgut lumen (Fig. 2A).Treating the larvae and adults with HpD at 5×10^{-2} ppm followed by post treatment exposure to light for 1.5 h caused severe damage to the mid gut epithelial cell layer. Detachment of the epithelium from the gut wall, followed by their collapse into the gut lumen was evident. The muscle layers atrophied and the peritrophic membrane completely disappeared (Fig. 2B).

DISCUSSION

There is interest in the application of environmentally safe tools to control insect pests. Despite the successful use of conventional insecticides, they often show undesirable toxicity to non-target organisms, such as useful insects, fishes or mammals. In addition, insect resistance to traditional pesticides is on the sharp rise. Therefore, it is becoming clear that alternative pest management tools are needed. Sunlight activated pesticides represent a possible alternative to traditional chemical compounds (Ben Amor et al. 2000). The results described in this study point out that, the HpD concentration of 5×10^{-4} ppm required about

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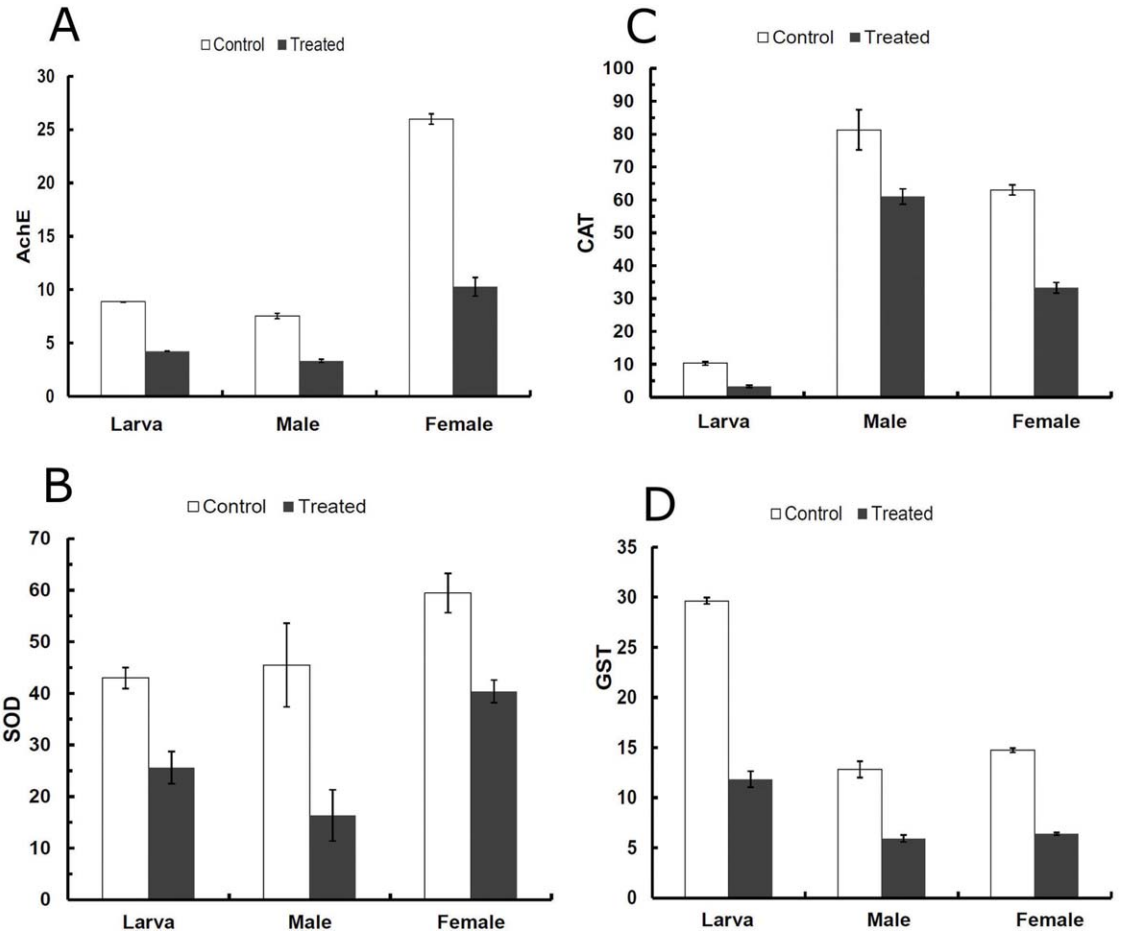


Fig. 1. The effect of 5×10^{-4} ppm of hematoporphyrin dihydrochloride on the activities of AchE(A), SOD (B), CAT (C) and GST(D) of 3rd instars, and male and female adults of *Parasarcophaga argyrostoma*. The post treatment exposure period to direct sunlight was for 45 min. Data shown are means ± SE (n = 15). SE indicated by bar.

1.5 h of post treatment light exposure to induce a considerable mortality ratio about 56% in the 3rd instars of *P. argyrostoma*. The concentrations 5×10^{-6} and 5×10^{-5} ppm were too low to induce larval mortality at most post treatment light exposure periods used in this study. The LC_{50} values of HpD on 3rd instars in all post treatment light exposure periods were much higher than those of adult *P. argyrostoma* males and females. The significant decrease in the LC_{50} of the 3rd instars related to increased post treatment light exposure periods may be due to the transparency of the larval cuticle compared to the adult cuticle. HpD had no obvious toxic effect on the larvae of *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae) with post treatment light exposure (Abd El-Naby 2002). This was attributed to the dark pigmentation of the larval body. However, the photoinsecticide, 5-aminolevulinic acid, exhibited obvious toxicity to all stages of the well sclerotized grasshopper *Oxyachinensis* Thunberg (Orthoptera: Acrididae) (Mei-ling et al. 2011). Adult males of *P. argyrostoma* were found to be more sensitive than females (Table 4). The reason could be due to higher accumulation of HpD in the male bodies than in the females. In contrast, females of *L. bryoniae* leafminers, for behavioral reasons, were found to be more sensitive than males to hematoporphyrin dimethyl ether and other photosensitizers like acridine orange, aminolevulinic acid and methylene blue (Luksiene et al. 2007).

Generally, the effect of HpD on *P. argyrostoma* was concentration and post treatment light exposure period dependent. The present results showed that the efficiency of photodynamic sensitizers as insecticidal agents is affected by a variety of experimental parameters; and the first factor being photosensitizer concentration. The photoinsecticidal effect steadily increased with increasing the concentration of the photosensi-

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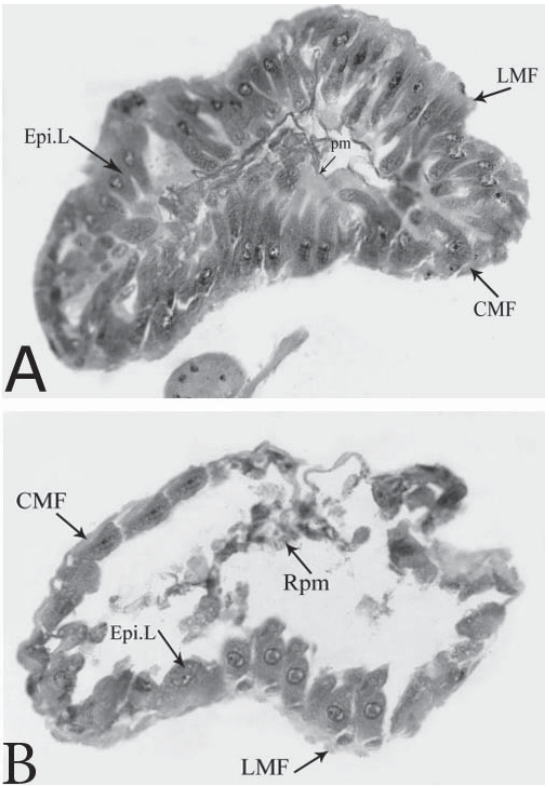


Fig. 2. Histological structure of midgut of adult treated with 5×10^{-2} ppm hematoporphyrin dihydrochloride and (A) kept in darkness during post treatment, (B) exposed to direct sun light for 1.5 h post treatment. The histopathological changes in the midgut include, detachment and collapse of columnar epithelial cell layer (EpiL), atrophy of inner circular (CMF) and outer longitudinal muscle fibers(LMF) and destruction of peritrophic membrane (Pm). Magnification × 20.

tizing agent in the bait. The second factor was the duration of the post treatment light exposure period. The rate of the photosensitized killing of insects appeared to increase with prolongation of post treatment exposure to light. Such conclusions are likely to be of general validity since essentially identical results were obtained with porphyrins against several insects including *C. capitata*, *B. oleae*, *Stomoxyscal citrans* (L.) (Diptera: Muscidae) (Ben Amor et al. 1998a), the ciliate *Colpoda inflata* (Kassab et al. 2002), and *A. aegypti* (Karunaratne et al. 2005). The actions of other photosensitizers, including methylene blue, rose bengal, and rhodamines were similarly affected (Ben Amor et al. 2000).

In the present study a significant decrease in the detoxification enzyme AchE activity was found after application of HpD at 5×10^{-4} ppm. The reason for the inhibition of AchE in response to HpD treatment could be the release of ROS because similar results were obtained with different photosensitizers. The photosensitizer, rose bengal or meso-tetra (N-methyl-4-pyridyl) porphyrintetratosylate, generated singlet oxygen, which acts as a strong oxidizing agent of specific amino acids like tryptophan found in the active site of AchE (Michaeli & Feitelson 1994). Methylene blue, which also generates singlet oxygen, caused irreversible inactivation of AchE of the Pacific electric ray, *Torpedo californica* (Weiner et al. 2011). However, no significant effect was detected in the activity of the detoxification enzymes, general esterases, in all stages of the locust *O. chinensis* treated with the photoinsecticide, 5-aminolevulinic acid (Mei-ling et al. 2011). A direct correlation between hematoporphyrin IX and ROS generation in larvae of *C. capitata* was found (Pujol-Lereis et al. 2010). Our results suggest that HpD may generate ROS, which, in turn, may cause inactivation of AchE in *P. argyrostoma*. The interaction of HpD with AChE of *P. argyrostoma* needs more investigation.

In the present study, HpD treatment resulted in significant decrease in activities of the antioxidant enzymes SOD, CAT, and GST of *P. argyrostoma*. The reason for this decrease may be the generation of ROS, which overwhelms the antioxidant defenses. HpD induced oxidative stress, which led to the generation of free radicals and alterations in antioxidants enzymes in plant cells (Riou et al. 2014). Porphyrin inactivated catalase through the production of singlet oxygen, which caused amino acid damage (Hirakawa et al. 2013). Following UV-A radiation of male and female *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) adults, the antioxidant enzyme genes of CuZnSOD, CAT and GST and their expression profiles (except MnSOD in females) were

TABLE 4. LC_{50} VALUES FOR 3RD INSTARS, ADULT FEMALES AND ADULT MALES OF *PARASARCOPHAGA ARGYROSTOMA* TREATED WITH HEMATOPORPHYRIN DIHYDROCHLORIDE AND EXPOSED TO DIFFERENT PERIODS (IN HOURS) OF DIRECT SUNLIGHT (810 W/M^2).

Sunlight Exposure Periods	3rd larval instar	Males	Females
0.5 h	3.2×10^{-2}	1.3×10^{-5}	1.7×10^{-4}
1 h	2.58×10^{-3}	1.09×10^{-5}	6.85×10^{-5}
1.5 h	1.06×10^{-4}	1.005×10^{-6}	1.65×10^{-5}
2 h	2.02×10^{-5}	3.82×10^{-7}	4.6×10^{-7}

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significantly upregulated at certain time points (from 30 to 90 min) but declined later (Wang et al. 2014). High-intensity environmental stress also suppressed the activity of the antioxidant enzymes (Heck et al. 2003; Polte & Tyrrell 2004).

The success of the HpD at 5×10^{-4} ppm in inhibiting AchE and the antioxidant enzymes of the 3rd instars and adults of *P. argyrostoma*, indicates strong oxidative damage by ROS production. It is worth to mention that hematoporphyrin IX and hematoporphyrin formula did not induce significant changes in total proteins, lipids and carbohydrates in larvae of *C. pipiens* (Awad et al. 2008).

Some attempts have been made to protect insects against photodynamic action by the administration of antioxidizing agents, such as carotenes, tocopherol and ascorbic acid (Robinson & Beatson 1985). In all cases, no appreciable protection was obtained, even though these compounds are powerful inhibitors of photooxidative reactions in vitro. The lack of photoprotection in insects confers a special advantage to the photosensitizing compounds as substantially irresistible insecticides.

The midgut wall appears to be the primary photodamaged site leading to feeding inhibition (Ben Amor et al. 1998a). The collapse of the epithelial cell layer resulting from HpD treatment resembles that observed with the histopathological effects of novel, meso-substituted cationic porphyrin (Lucatoni et al. 2011) and the natural compound pellitorine on the midgut of *A. aegypti* (Perumalsamy et al. 2013). The longitudinal and circular muscle layers of the mid gut were severely atrophied (Fig. 2b). Similar muscular damage occurred in larvae of *C. pipiens* treated with hematoporphyrin (Salama et al. 2002) and in *C. capitata* treated with the Phloxine B (Berni et al. 2003). It is worth noting that, similar histopathologies do not necessarily imply the same mode of action.

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