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A Semi-field Study on the Effect of Novel Hematoporphyrin Formula on the Control of *Culex pipiens* Larvae

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ABSTRACT

The enhancement of the insecticidal activity of hematoporphyrin HP by a novel formula HPF was studied. The efficiency of hematoporphyrin IX (HP) and hematoporphyrin formula (HPF) was tested at concentrations of 1 x 10^{-5} and 1 x 10^{-6} M on *Culex pipiens* field strains in a semi-field experiment. A significant increase of larval mortality was noted after 5 days of application. The concentration of 1 x 10^{-5} M of HPF gave the same results of HP at the concentration of 1 x 10^{-4} M on the larval mortality after 5 days of application. Thus, a clear synergistic effect occurred due to the incorporation of sugar and other additives to the HP. Since the efficiency of HP and HPF as larvicidal substances on *C. pipiens* was evident. Their possible biochemical change on the body content of the larvae, carbohydrates, proteins and lipids, was also tested. However, it was found that the effect of HP and HPF on the larvae content of carbohydrates, proteins and lipids has no significant contribution to the larval mortality due to the damage of larval organelles as a result of the high oxidative stress caused by the photosensitize effect.

Key Wordes: *Culex;* Porphyrin; Control; Biochemistry

INTRODUCTION

Mosquitoes cause environmental nuisance and are vectors of many human diseases. Three genera *Culex, Anopheles* and *Aedes* include the main vector of major human pathogens responsible for malaria, yellow fever, dengue and encephalitis. The use of photochemical processes as a tool to control the population of several types of insects has been examined in both laboratory and field studies (Yoho *et al.,* 1976; Heitz, 1987).

Compounds of plant origin have been isolated, identified and studied as phototoxins against a wide range of pests including insects, fungi and weeds; among the main classes studied to this time is chlorophyll (Rebeiz *et al.,* 1990). This fact attracted the attention of Ben Amor *et al.* (1998a b) for the study of porphyric pesticides. Hematoporphyrin (HP) activities were tested towards *Ceratitis capitata,* which are known to cause heavy losses to crops. The preliminary trials with HP in field caged apricot trees were performed and gave very promising results; an almost 60% mortality for *C. capitata* against a 13% mortality of control flies that had received no HP. Extensive studies have been conducted about HP/visible light on dipterous larvae (Pimprikar & Georghiou, 1980). In Egypt, El-Tayeb (2003) reported that HP can be used as a photosensitizing agent for potentially controlling *Culex pipiens* larvae, which have medical and veterinary importance in laboratory studies. The author showed the porphyric toxicity consequences of the porphyrins among the various compartments of insect tissue and cells as well as the distribution in the various anatomical sites. They emphasized the importance of the site of action of photoactivable substances in determining the mortality of larvae. This had also been underlined by another author (Robinson, 1983). The porphyric photoinsecticides have low environmental impact and a negligible risk for human health, as the biosynthesis of abnormally large concentrations of the photosensitizer's porphyrins and their derivatives can be induced in some insects through adminstration of their metabolic precursor (Ben Amor, 1998a). Porphyrins appear to be particularly promising photoinsecticides because they absorb essentially all the wavelengths of the solar spectrum. Hence, they can undergo a very efficient photoexcitation by sunlight; moreover, many porphyrins are known to be powerful photodynamic sensitizers being characterized by a high quantum yield for generation of singlet oxygen, a cytotoxic oxygen derivative (Jori, 1985).

In this study, a new approach to the design of insecticides will be described. The HP which proved its photosensitization efficiency on laboratory studies was tested in commercial formula HPF and in the pure form HP in a semi-field study to control mosquito larvae. Also this study revealed the role of carbohydrates, proteins and lipids

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concentration changes on mosquito larval mortality during photosensitization process.

MATERIALS AND METHODS

Experimental insects. The field strain mosquito (*Culex pipiens*) larvae were collected in sufficient numbers from a typical shallow accumulation of water in Abo-Rawash at Giza district. This water had scattered areas of exposed mud and muddy sloping shore line. This water is polluted with decaying vegetable debris, human and animal excreta, other rubbish and piles of decaying refuge, defective latrines and septic tanks. Some microbial flora beside snails and a few numbers of *Eristalis* larvae were also present; all of which provided ideal habitats for *Culex* species. The collected larvae were placed in two glass tanks, each 200 L capacity, filled with the above described standing water and helin the roof of the National Institute of Laser Enhanced Sciences at Cairo University before starting the semi field experiments.

Porphyric insecticides preparation. Hematoporphyrin IX (HP), obtained from porphyrines products (Logan, UT), was used in this work as received. No further treatment was employed. The HP concentrations was prepared by diluting a known volume of the aqueous solution into a known excess of 0.25 M H_2SO_4 and reading the absorbance at 401.5 nm (Mascanzoni *et al*., 1987; Ben Amor *et al.*, 2000). The pH of the stock solution of the used photosensitizer was adjusted to be 6.5-7. The absorption spectra of porphyrin were carried out with a Perkin Elmer Lambda 40 double beam spectrophotometer. The value of the flounce rates of the natural sun measured by a three channel eldonet dosimeter (Real Time Company Germany). Three different concentrations (10^{-4}, 10^{-5}, 10^{-6} M) of HP were prepared from stock solution, which was prepared by dissolving a known amount of porphyrines in the minimal amount of 0.1 M NaOH and then neutralizing by drop wise addition of 0.1 M HCl. The HP concentration in the final solution was determined by absorption spectrophotometer. The solution of HP was stable for some weeks at 4°C.

Hematoporphyrin formula (HPF) was prepared by mixing 2.5 mg of HP powder, from porphyrines products (Logan, UT) with 1 kg sugar and other additives homogenized. The homogenates were tested by dissolving constant weights of the mixture and measuring the concentration of HP in each using Beers Lambert law. According to this concentration, the diluted concentrations (10^{-5} & 10^{-6} M) were prepared.

Larvicidal test. The experimental larvae were divided into 3 groups: two treated groups, one placed in the dark condition and the other placed in light condition and a control group; 8 glass tanks (50 L capacity), filled with the previously described standing water were used. Nearly 2000 larvae were placed in each glass tank and tested for each concentration of HP and HPF. Each concentration of the porphyric insecticides was added (10^{-4}, 10^{-5} & 10^{-6} M for HP & 10^{-5} & 10^{-6} M for HPF) and placed under light. A glass tank dark-placed with 10^{-4} concentration of HPF and another two glass tanks, one dark-placed with porphyrin 10^{-4} HPF and another one light-placed without porphyrin acted as control larvae. The experiment was replicated two times. The mortality of mosquito larvae was recorded during day time (3, 9 & 18 h of sunrise) and corrected according to Abbott's equation (1925) and Hally (1952) table correction.

$$\text{Corrected mortality \%} = \frac{\text{Observed mortality \% - control mortality \% X 100}}{\text{100-control mortality \%}}$$

Determinations of total lipids, proteins and carbohydrates. The survived larvae in each treatment and control were obtained after 3, 9 and 18 h and starved for 6 h before carrying out the biochemical assays. Larvae were weighed and homogenized in 1 mL cold saline (0.9% NaCl). The homogenate was centrifuged at 10.000 rpm at 5°C for 10 min, at which the supernatant fractions being used for the determination of total proteins and total carbohydrates and the remaining pellets then extracted with chloroform and methanol (2:1) for the determination of total lipids. Total lipids were determined by the sulphophosphovanillin method (Knight *et al.,* 1972). The content of total proteins was determined with Folin-Ciocalteu technique of Lowry *et al.* (1951). Total carbohydrates were determined with the Anthrone method (Singh & Sinha, 1977). Each assay was replicated three times using a Schimadzu UV/visible spectrophotometer against a control blank (Teo *et al.*, 1990).

Statistical analysis. Data were subjected to statistical analysis using analysis of variance one way ANOVA (Snedecor & Cochran, 1967) and the least significant difference (LSD) test was used for mean separation at $P < 0.05$.

RESULTS AND DISCUSSION

In the field studies that conducted by El-Tayeb (2003) in Egypt, HP was examined for *C. pipiens* larvae lab strains, who found that HP concentrations of 10^{-4} and 10^{-5} M caused the highest mortality samples in the lab. This was the first trial using HP within a proposed novel form to control mosquito larvae. Hence, from the forgoing results, it can be concluded that there was a differences of this semi-field study. There is a similarity between the effect of HP in pure form and within the formula on the percentage of larval survival (Fig. 1 & 2). From the data it appeared that the HP concentration of 10^{-6} M caused decrease in larval survival by 100, 37.3 and 26.6% after1, 3 and 5 days, respectively in open environmental condition, while the mortality at 10^{-5} M was decreased by 100, 25.6 and 6% and at 10^{-4} M was 58.7, 34.3 and 0.7%, respectively. The light and dark controls, which were exposed to the similar experimental parameters had 87.7% and 99.5% larval survival after 3 days and 80 and 99.5% larval survival after 5 days in open environmental condition. This experiment was repeated with its all experimental conditions using HPF instead of HP (Fig. 2). The HP concentration within the HPF 10^{-6} M

Table I. The concentrations of carbohydrates, proteins and lipids of HP treated and control samples after different sun light exposure times

Exposure	Carbohydrate			Lipids			Protein		
Time	Treated	Dark control	Light control	Treated	Dark control	Light control	Treated	Dark control	Light control
3 hours	332.5±42.30^{c}	48.87±23.20^{a}	67.37±1.18^{b}	2.47±0.42^{a}	2.03±0.5^{a}	1.73±0.21^{a}	143.95±8.55^{b}	246.23±13.51^{c}	242.45±0.83^{c}
9 hours	76±1.73^{b}	58.33±0.47^{a}	68.83±1.04^{b}	7.67±0.31^{c}	3.53±0.40^{b}	6.00±0.00^{c}	76.92±1.06^{a}	82.29±3.04^{a}	91.97±1.36^{a}
18 hours	13.17±0.29^{a}	19.5±0.01^{a}	21.9±0.17^{a}	3.93±0.25^{b}	5.53±0.31^{c}	2.77±0.06^{b}	151.39±8.50^{b}	170.43±0.75^{b}	177.07±0.6^{b}
F-value	143.69	2.285	254.289	196.04	54.412	955.5	103.33	315.17	177.456
p-value	0.000*	0.183	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*

All values are represented as Mean±S.D
= There is a significant difference between Insecticide by using one way ANOVA at p<0.05
The same letter means that no significant difference between each two times by using
Tukey HSD test at p<0.05

Table II. The concentrations of carbohydrates, proteins and lipids of HPF treated and control samples after different sun light exposure times

Exposure	Carbohydrate			Lipids			Protein		
time	Treated	Dark control	Light control	Treated	Dark control	Light control	Treated	Dark control	Light control
3 hours	144.77±5.27^{a}	48.87±23.20^{a}	67.37±1.18^{b}	2.1±0.66^{a}	2.03±0.5^{a}	1.73±0.21^{a}	148.69±3.75^{c}	246.23±13.51^{c}	242.45±0.83^{c}
9 hours	53.27±2.15^{b}	58.33±0.47^{a}	68.83±1.04^{b}	6.63±0.40^{b}	3.53±0.40^{b}	6.00±0.00^{c}	52.69±0.44^{a}	82.29±3.04^{a}	91.97±1.36^{a}
18 hours	39.57±0.50^{a}	19.5±0.01^{a}	21.9±0.17^{a}	2.77±0.7^{a}	5.53±0.31^{c}	2.77±0.06^{b}	90.33±0.28^{b}	170.43±0.75^{b}	177.07±0.6^{b}
F-value	90.42	2.285	254.289	49.62	54.412	955.5	147.42	315.17	177.456
p-value	0.000*	0.183	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*

All values are represented as Mean±S.D
* = There is a significant difference between Insecticide by using one way ANOVA at p<0.05
The same letter means that no significant difference between each two times by using
Tukey HSD test at p<0.05

caused a decrease in larval survival by 100, 26.6 and 7.3%, respectively after 1, 3 and 5 days in open environment, while this decrease at 10^{-5} M was 80, 60 and 0.8%, respectively. The HP concentration of 10^{-5} M in HPF caused nearly the same percentage of larval survival of the HP at the concentration 10^{-4} M (Fig. 1 & 2). This may be attributed to the formula content, that reflect the suitability of using the sugar as HP carrier in the commercial formula. Since sugars are known as feeding stimulants to some insects (El-Sharaby *et al.*, 1978; Awad, 1992), they generally affects the feeding response of the larvae, as they are gustatory stimulants affecting the feeding response of different insect pests and have shown promise in increasing the effectiveness of microbial diseases versus the tested larvae (Salama *et al.*, 1985).

It was important to investigate the effect of HPF and HP on the main mosquito larvae body contents, (carbohydrates, proteins & lipids). Tables I - III summarize the biochemical analysis of carbohydrates, proteins and lipids of living *C. pipiens* larvae treated with HP and HPF. These analyses were done after 3, 9 and 18 h to reveal the dynamic change in the concentrations of these biochemical compounds during photosensitization process. The data of these experiments illustrated the significance difference of the exposure times, (Table I & II) and the significance difference of HP and HPF effects (Table III). The carbohydrate content decreased significantly during the photosensitization process but this concentration did not decrease more than the minimum value in case of control larvae. This implied that carbohydrate change does not contribute in the mortality of mosquito larvae. Protein and lipid contents indicated a significant fluctuation in their

Table III. The concentrations of carbohydrates, proteins and lipids of HPF treated and control samples after different sun light exposure times

Insecticide	Carbohydrate Mean±S.D	Protein Mean±S.D	Lipids Mean±S.D
HP	76±1.73^{c}	76.92±1.06^{b}	7.67±0.31^{b}
HPF	53.27±2.15^{a}	52.69±0.44^{a}	6.63±0.40^{a}
Dark control	58.33±0.47	82.29±3.04	3.53±0.4
Light control	68.83±1.04^{b}	91.97±1.36^{c}	6.00±0.00^{a}
F-value	139.95	1120	24.82
p-value	0.000*	0.000*	0.001*

* = There is a significant difference between Insecticide by using one way ANOVA at p<0.05
The same letter means that no significant difference between each two Insecticides by using
Tukey HSD test at p<0.05

concentrations, which increased or decrease significantly with exposure times. This indicated that the larvae can compensate for any change in proteins and lipids during the photosensitization process. HPF revealed nearly the same effects of HP on the carbohydrate, proteins and lipids of exposed larvae (Table I & II). There was little difference between the control samples and treated ones for lipid concentration (Table III). In case of protein and carbohydrate concentration, it was found that there were significant differences between the treated and control samples but these differences were still in the safe limits for larvae living.

In conclusion, the effect of HP and HPF on the larvae content from the carbohydrates, proteins and lipids has no great effect on larval mortality, implying that the larval mortality was oxidative damage to organelles. This may indicate the safe application of these agents on the larger

Fig. 1. Effect of different HP concentrations on survival % of *C. pipiens* larvae exposed to natural sunlight in open environmental conditions

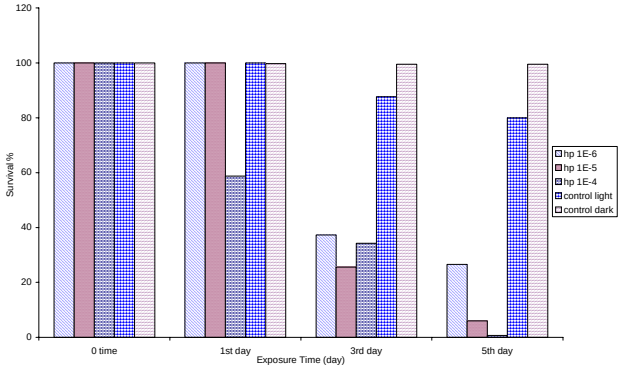
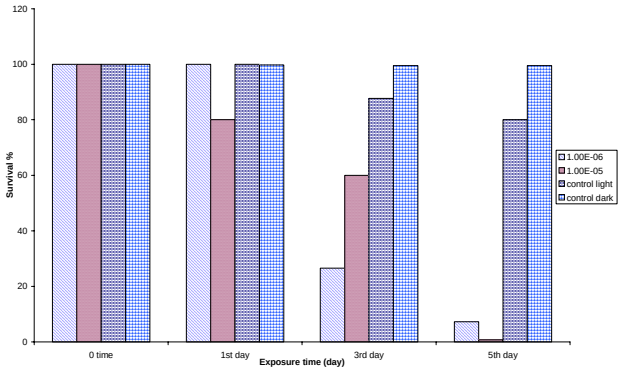


Fig. 2. Effect of different HPF concentrations on survival % of *C. pipiens* larvae exposed to natural sunlight in open environmenal conditions



animals like the fish and others. Extensive studies are imperative to explore the effect of the concentrations used in this work on the other benefit aquatic organisms in the same habitats of *C. pipiens* larvae samples. In addition, the use of these compounds in new formula in the form of larvicide's analogues, their application in controlling mosquito larvae in Egypt especially in Giza district has a great economic important due to their availability, safety, low cost, high efficiency. They can also be used on a wide scale together with the integrated pest management program to overcome this pest in the field. In addition, they do not cause the environmental pollution, which is the most dangerous drawback of chemical insecticides.

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