A study on the dynamics of *Aedes caspius* larval uptake and release of novel haematoporphyrin

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Haematoporphyrin efficiency on *Aedes caspius* increased the larval mortality with the increase of haematoporphyrin formulation (HPF) concentration (1×10^3 M/l). Larval mortality increased with increase the solar simulator light irradiances (350–650 W/m^2) and exposure times (45 min). Dynamics of HPF accumulation and release as a function of time feeding and consumption was investigated using confocal laser scanning microscopy (CLSM). HPF accumulation in the larval body reached its maximum level after incubation for 12 h. Remaining HPF concentration decreased as the time elapsed reaching its minimal level after 15 h of HPF removal from the treatment medium.

Key words: mosquitoes, porphyrin, solar irradiances, CLSM.

INTRODUCTION

Rift Valley Fever (RVF) is a viral pathogen that affects primarily domestic livestock, but can be passed to human causing fever. It is spread by infected mosquitoes, and among the principal mosquito vectors are *Aedes caspius* or *Culex* spp. Approximately 1 % of human sufferers die from the disease (Meegan *et al.* 1979), while in livestock the mortality level is significantly higher (Clements *et al.* 2007). *A. caspius* is a prominent pest widely distributed in the Palaearctic region where its larvae are primarily halophilic, with occasional occurrences in fresh water (Horsfall 1955; Gad *et al.* 1987).

The widespread use of insecticides has disturbed the parasitoid and predator populations and also led to an increase in the development of resistance to most of the insecticides used in conventional programmes (Ghosh *et al*. 2004; Ishaaya & Kontsedalov 2005). This problem can be solved by using safer compounds which have reduced environmental pollution.

The photosensitizers of natural origin like porphyrin derivatives present specific advantageous features such as low environmental impact (Dondji *et al*. 2005).

Porphyrins are promising photoinsecticides, because they absorb essentially most of the wavelengths of the solar spectrum. Hence, they can undergo a very efficient photoexcitation by sunlight producing a high quantum yield of singlet oxygen generated by energy transfer from excited triplet-state porphyrin into triplet ground state of oxygen in the cell system taking into consideration the chemical structure and the nature of the microenvironment in complex biological systems which may affect singlet oxygen quantum yield. The cytotoxicity induced by porphyrins in combination with light is due to photodynamic process (Idrish Miah 2002; Awad *et al.* 2008).

El-Tayeb *et al.* (2011) showed that the haematoporphyrin (HP) was successful as a novel trend for the control of *Parasarcophaga argyrostoma* in the field due to their accumulation inside the insect organs after incubation and exposure to sunlight.

The use of photosensitizers as a tool to control harmful insects has been examined in both laboratory and field studies (Yoho *et al.* 1976; Heitz 1987; Ben Amor *et al.* 1998; Abdel-Kader *et al*. 2008). The HP activities were tested in dipterous larvae and were applied also on *Aedes* sp. (Pimprikar *et al*. 1979; Pimprikar & Georghiou 1980).

In the present study, the HP which proved its photosensitization efficiency in laboratory studies was tested in a commercial formulation of HPF at different concentrations, to study its lethal effect on *Aedes caspius* larvae after adjusting the light fluence rate at different intensities and light exposure periods. Also, this study used the confocal laser scanning microscopy (CLSM) technology to evaluate the extent of HPF uptake and release by *A. caspius* larvae. The data obtained in this work give new insights on the success of this formula for field application to control *A. caspius*.

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MATERIAL AND METHODS

Insects

A pure strain of *A. caspius* larvae (third instar) was obtained from the *Aedes* Rearing Laboratory, Faculty of Science, Ain Shams University, Cairo, Egypt.

Porphyric insecticides preparation (HPF)

The haematoporphyrin formulation (HPF) is a preliminary formulation suggested for future field study. It was prepared by mixing 1.25 mg of Hematoporphyrin IX (HP) powder (Sigma-Aldrich) with 0.5 kg cane sugar (used as carrier and inert material) and autolysed yeast (used as mosquito larvae attraction agent). Stock solution was prepared by dissolving HPF stock powder in distilled water and the concentration of stock solution was determined by using a Perkin Elmer Spectrophotometer Lambda 40 in which the absorbance of hematoporphyrin IX was determined and its concentration was calculated using Beer Lambert law. Different HPF concentrations (1×10^{-3}, 1×10^{-4}, 1×10^{-5}, 1×10^{-6} and 1×10^{-7} M/l) were prepared by stock solution dilution using distilled water.

Irradiation source

An ORIEL solar simulator (1000 W Xenon Lamp with variable power supply) was used for artificial light exposure. The fluence rate was measured using ELDONET dosimeter. The exposed insect containers were surrounded by an ice jacket to avoid temperature increase during light exposure.

Survival studies

Nearly 200 larvae were placed in each of 16 (500 ml) glass beakers; five beakers were treated with different HPF concentrations (1×10^{-3} to 1×10^{-7} M/l) and were left in the dark for 6 h then were exposed to 350 W/m^2 solar simulator light for 30 min.

Two beakers were treated with 1×10^{-5} M/l HPF and were left in dark for 6 h then exposed to different solar simulator light irradiance (450 and 650 W/m^2) for 30 min.

Three beakers were treated with 1×10^{-5} M/l HPF and were left in dark for 6 h then were exposed to 350 W/m^2 solar simulator light for different exposure times (15, 30 and 45 min). Another three dark control beakers (larvae were treated with highest HPF concentration, 10^{-3} M/l, without light exposure) and other light control beakers, (larvae exposed to light without HPF treatment), were done during this study.

The experiment was replicated three times. The mortality of mosquito larvae was recorded during and after light exposure time and corrected according to the equation of Abbott (1925) and the table correction of Hally (1952).

Dynamic of HPF larval uptake and release

HPF uptake. One beaker of 200 larvae was treated with 1×10^{-5} M/l HPF. Fifty larvae were taken after 1, 3, 9, and 12 h incubation periods. Each larval group of each incubation time was left in the tissue tek solution (Sakura Finetek, Japan) for one hour. Each larva was mounted on a microscopic slide using anti-fading mounting medium for CLSM (LSM 510, Carl Zeiss, Germany) examination (Bacallao & Steltzer 1989; Berod *et al.* 1981; El-Tayeb 2008).

The concentrations of HPF inside the tissues of *Aedes* larvae were determined by measuring the fluorescence intensity after excitation with a wavelength of 488 nm, emitted by the internal Argon Laser of LSM 510. Signal emission was detected using an external spectrometer coupled to the LSM 510.

HPF release. One beaker of 200 larvae was treated with 1×10^{-5} M/l HPF and was left in the dark. After 6 h of dark incubation period the larvae were transferred to HPF-free water. Fifty larvae were separated after 1, 3, 9, and 12 h. Each larval group was prepared for CLSM examination by the same method mentioned above.

Statistics

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

RESULTS AND DISCUSSION

Survival studies

Figure 1 shows the effect of different concentrations of the HPF on the percentage of larval mortality after exposure to solar simulator irradiance of 350 W/m^2 for 30 min. The percentage mortality during light exposure was 53 %, 44 % and 20 % at the HPF concentrations of 1×10^{-5}, 1×10^{-6} and 1×10^{-7} M/l, respectively; while in the case

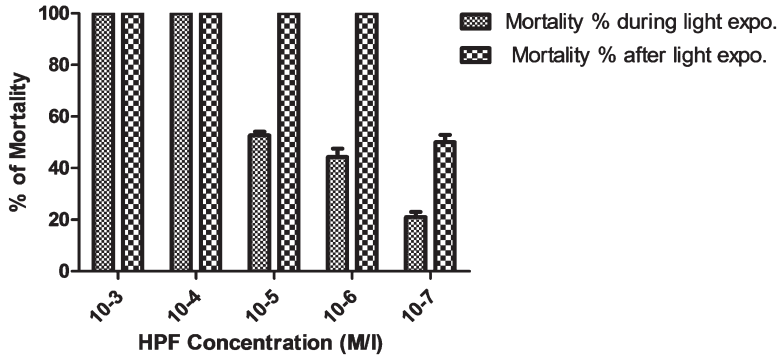

Fig. 1. The effect of HPF concentrations on the percentage mortality of *Aedes caspius* larvae after exposure to solar simulator irradiance of 350 W/m^2 for 30 min.

of 1×10^{-3} and 1×10^{-4} M/l 100 % larval mortality was recorded. Fig. 2 indicates the effect of different irradiance, from the solar simulator light, 350, 450, and 650 W/m^2, on the percentage of the larval mortality. The HPF concentration of 1×10^{-5} M/l caused 46.9 % of the larval survival when the larvae exposed to 350 W/m^2 solar simulator irradiance compared to the irradiances of 450 and 650 W/m^2 which caused 100 % of larval mortality. It is clear that mortality percentage of the larvae increases with increasing the irradiance dose from 350 to 450 W/m^2. Also it is clear that the range of irradiance which was chosen in this study matches the range of sunlight irradiance in the different climatic seasons in Egypt. This result agrees with that reported by Abdel-Kader *et al.* (2000) who found that the effect of HP on *Culex pipiens* larvae survival in the sunny seasons was more effective than in other seasons. Similar results were obtained by El-Tayeb (1999) and Abdel-Kader *et al.* (2008) in *Musca domestica.* This may be attributed to the fact that the accumulated number of photons absorbed by the target will increase with increasing irradiances causing an increase in the number of excited photosensitizer molecules. This reaction was followed by a concomitant increase in the amount of singlet oxygen produced by the photochemical reaction (Abdel-Kader et al. 2008).

Figures 1, 2 and 3 show a clear direct effect on the larval mortality, which may be due to a sudden shock on the nervous and tracheal systems or due to the indirect starvation effect, which may be due to the damaged epithelial cells of the gut (Awad *et al.* 2008; El-Tayeb 2008). The HPF concentration of 1×10^{-5} M/l caused a clear increase in larval mortality by 79 % and 100 % during light exposure after 15 and 30 min from irradiation to 450 W/m^2, respectively. The percentage of larval mortality increased significantly with the increase in the exposure time ($P \leq 0.05$). Similarly, Awad *et al.* (2008) reported a significant increase in the mortality of *C. pipiens* larvae treated with HPF as a result of the high oxidative stress caused by the photosensitizer effect. Kress *et al.* (2001) analysed the fluores-

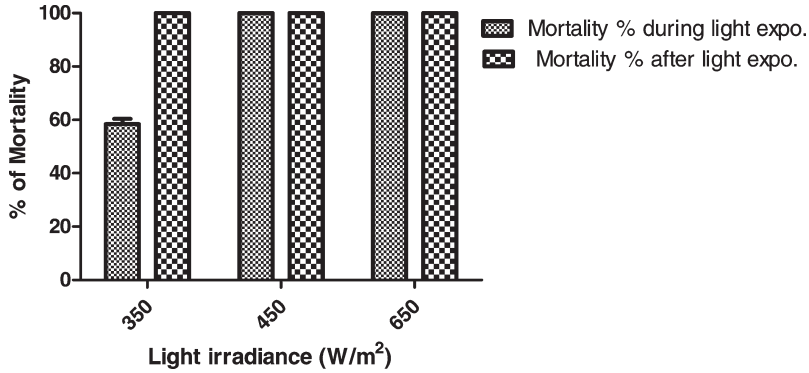

Fig. 2. The effect of different solar simulator fluence rates on the percentage mortality of *Aedes caspius* larvae treated with 1×10^{-5} M/l HPF concentration and exposure for 30 min to solar simulator irradiance.

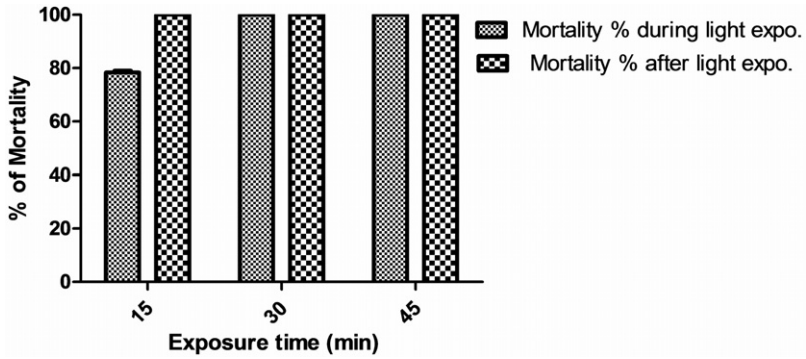

Fig. 3. The effect of different exposure times of solar simulator light (450 W/m^2) on the percentage mortality of *Aedes caspius* larvae treated with 1 × 10^{-5} M/l HPF concentration.

cence characteristics of HP in mosquito larvae after two different incubation times. They suggested that HP seems to be a promising candidate to kill mosquito larvae by photosensitizing agents. The HP has been shown to interact with polar groups of the lipid layer where the hydrophobic porphyrin core is embedded into the lipid region of the bi-layer of treated insect cells (Jori & Spikes 1983). This behaviour is important as the photo-insecticide must be able to enter cells through the lipid membrane of the larvae; hence, the porphyrins are localized on the lipid phase (Jori 1985).

Dynamics of HPF larval uptake and release

CLSM was used to clarify the distribution of HPF inside the organs and tissues of *A. caspius* larvae. It provides three sources of information; fluorescence images, fluorescence spectra and life time measurements; so it may help to study the dynamics of HPF accumulation and release as a function of time. This information helps in the determination of the suitable time for HPF application in the field study. On the other hand, it is possible to find a relation between the photosensitization efficiency of the HPF and the behaviour of the insect uptake. CLSM can also provide a relative estimation for the HPF concentration inside the treated larvae compared to the control larvae. From the CLSM images and their related spectra, it is clear that the amount of HPF in the thoracic, anterior and posterior abdominal regions of *A. caspius* larvae showed the maximum accumulation of HPF after incubation for 12 h (Figs 4, 5 and 6). This means that the larvae should be incubated with HPF for sufficient time before light exposure to obtain fast and high mortality. Also it is clear that HPF moves inside the alimentary canal from the thoracic region to the abdominal region. This movement could affect the accumulation rate because the fate of the porphyric insecticide will end when the larva defecates. The effect of the time elapsed after HPF removal from the medium of *A. caspius* larvae on the extent of the remaining HPF concentration may decrease due to active release of HPF from larval tissue. It is clear that the percentage of HPF decreases due to the tissue release process in the thoracic, anterior and posterior abdominal regions from *A. caspius* larvae showed the least amount, 50 %, of HPF accumulation remaining at 12 and 15 h after HPF is removed from the larval feeding (Figs 7, 8 and 9). This means that even after 15 h there is still some HPF in the larval bodies. This confirms the previous laboratory experiments in which the author studied the effect of HPF reduction as a function elapsed time on the percentage of the survival of *C. pipiens* larvae (Awad *et al.* 2008). CLSM images and their related spectra (Figs 7, 8 and 9) showed the dynamic of HPF release by *A. caspius* larvae has the same regime in all the larval parts (thorax; abdomen and posterior, respectively.) The remaining HPF amount after 12 and 15 h may be due to HPF particles adhering to the internal lining of the alimentary canal. This means that the larvae could not release this amount even after 15 h.

El-Tayeb (2008) used CLSM to study the behaviour of HP accumulation in different organs of house flies and explained the reasons of mortality rates during and after light exposure were related to the different accumulation dynamics of HP inside the different insect organs. These results support the investigation of the behaviour of HPF in the body of *A. caspius* larvae and explain the results of final effects of photosensitization processes on larval

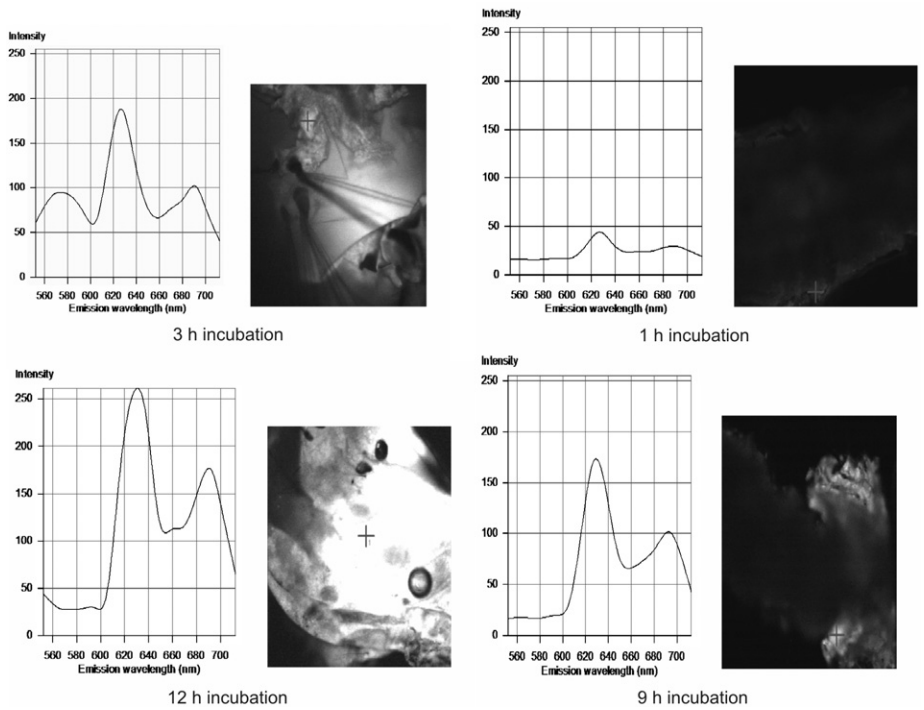

Fig. 4. CLSM images and spectra showing the extent of HPF accumulation in the thoracic region of *Aedes caspius* as a function of incubation time.

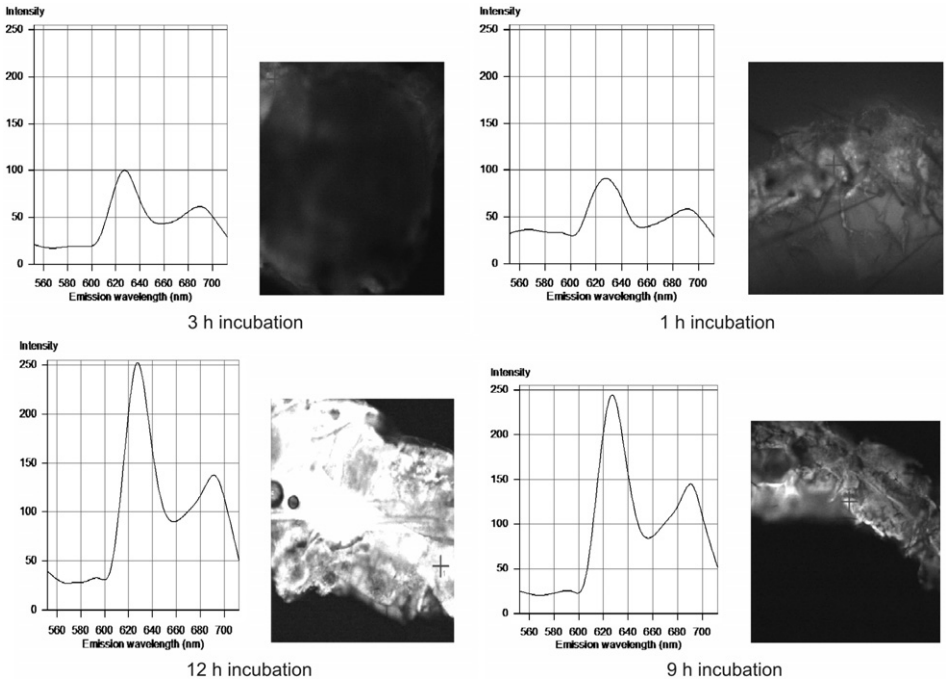

Fig. 5. CLSM images and spectra showing the extent of HPF accumulation in the abdominal region of *Aedes caspius* as a function incubation time.

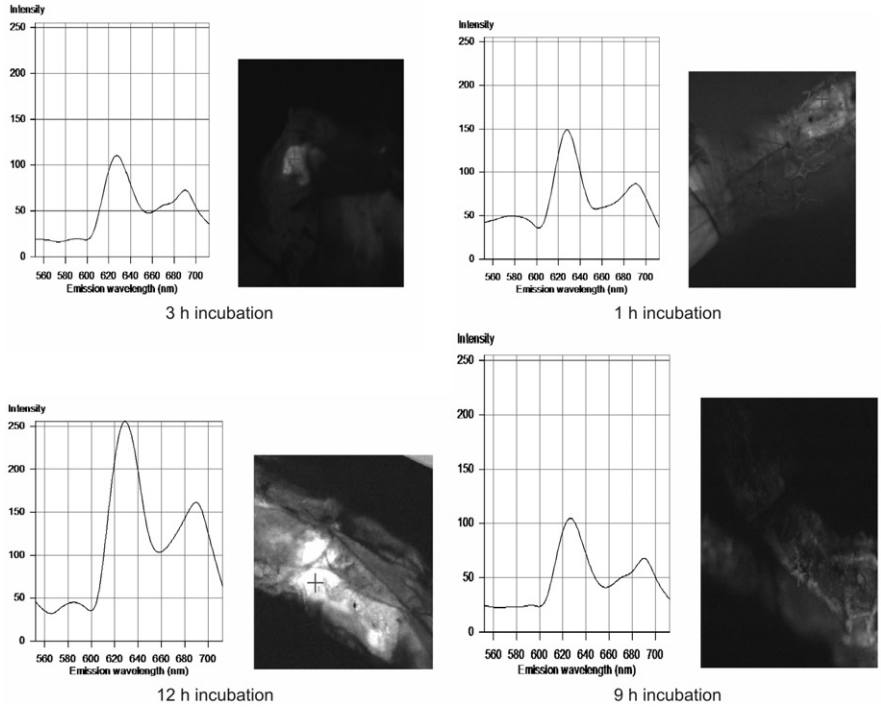

Fig. 6. CLSM images and spectra showing the extent of HPF accumulation in the posterior abdominal region of *Aedes caspius* as a function of incubation time.

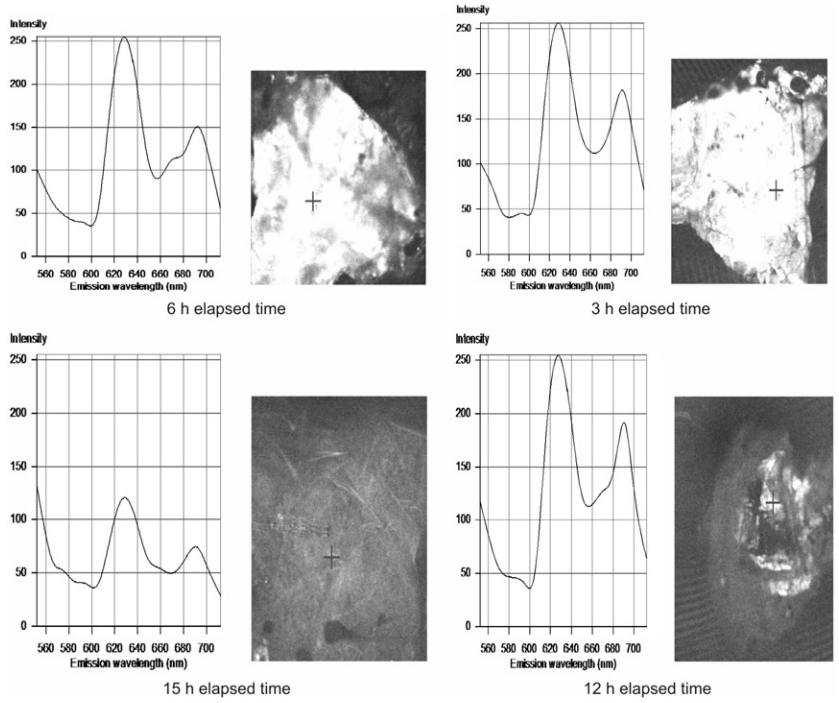

Fig. 7. CLSM images and spectra for the thoracic region of *Aedes caspius* larva showing the effect of time elapsed after HPF removal from feeding medium on the extent of remaining HPF concentration.

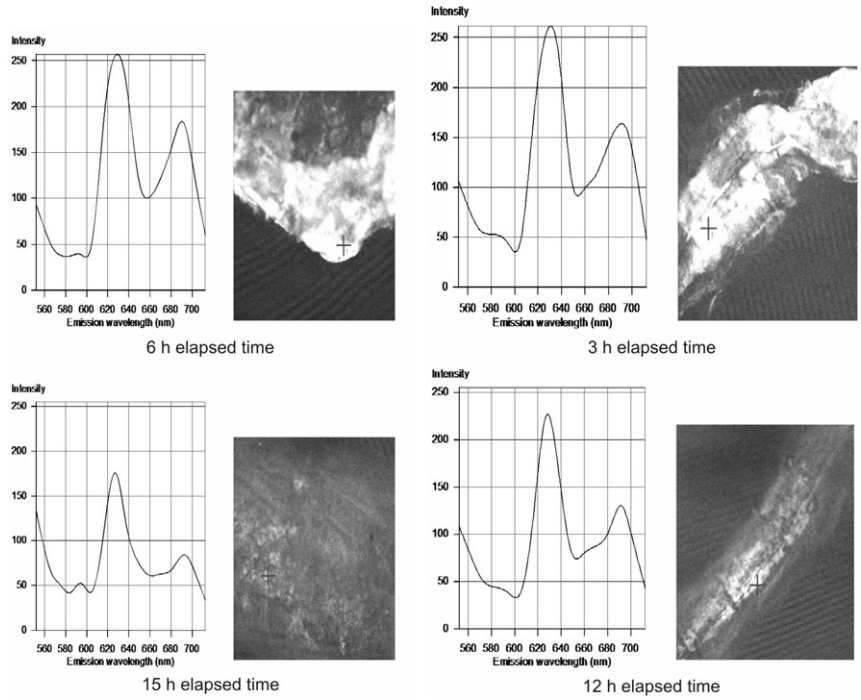

Fig. 8. CLSM images and spectra for the abdominal region of *Aedes caspius* larva showing the effect of time elapsed after HPF removal from feeding medium on the extent of remaining HPF concentration.

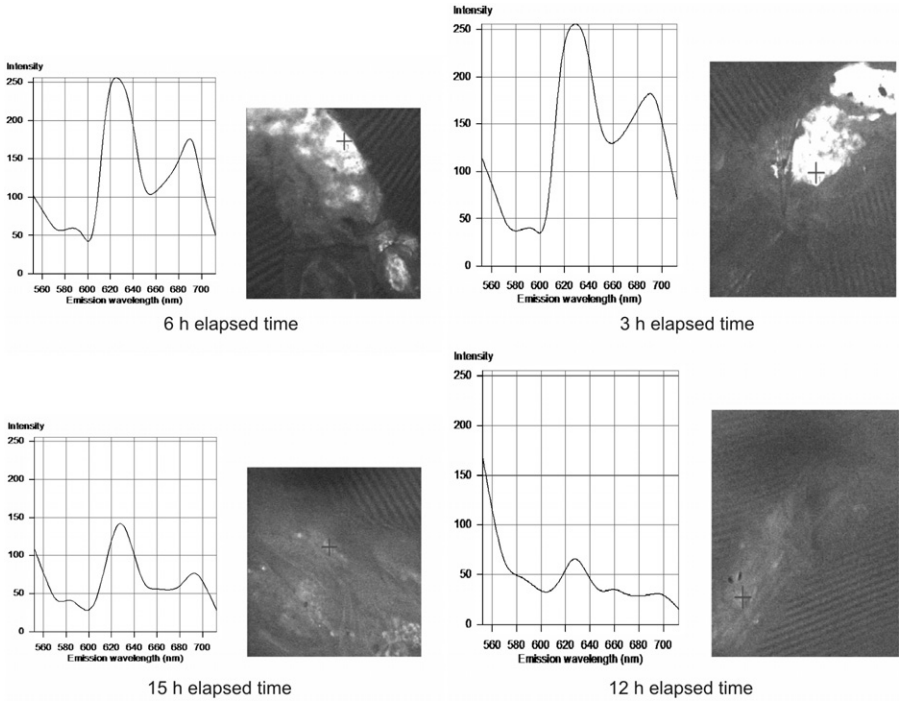

Fig. 9. CLSM images and spectra for the posterior part of *Aedes caspius* larva showing the effect of time elapsed after HPF removal from feeding medium on the extent of remaining HPF concentration.

mortality. At the cellular level, most photosensitizers are able to induce apoptotic cell death (Luksiene 2003). This result also agrees with Luksiene *et al.* (2005) and El-Tayeb *et al.* (2011), who illustrated the effect of HP on *Liriomyza. bryoniae, C. pipiens* and *M. domestica* larval mortality in the sunny seasons was more than the others, due to the increase of sunlight intensity and liberating number of photons striking the target cells. Hence, the number of excited HP molecules increases followed by increasing the amount of singlet oxygen produced by the photochemical reaction.

CONCLUSION

The data obtained demonstrate the influence of photodynamic insect control, and point out that HPF is a promising, very effective photoinsecticide against *A. caspius* larvae. Extensive studies are imperative to explore the effect of the concentrations used in this work on the beneficial aquatic organisms in the same habitats of *A. caspious* larvae. In addition, the use of the porphyric insecticides in new formulations incorporating larvicidal analogues for controlling mosquito larvae in Egypt, especially in areas of widespread abundance of this species, has a great economic importance due to their availability, safety, low cost and high efficiency. They can also be used on a wide scale together with the integrated pest management programme to overcome this pest in the field. It is very interesting to study the photochemical fluorescence emission profiles of HPF from the larval body tissues. This knowledge may open the way to a novel control strategy.

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