Journal of Entomology

ISSN 1812-5670

Academic Journals Inc.

www.academicjournals.com

Journal of Entomology 8 (4): 384-390, 2011
ISSN 1812-5670 / DOI: 10.3923/je.2011.384.390

Preliminary Study to Investigate the Optimum Parameters of using Hematoporphyrin IX to Control Flesh Fly (*Parasarcophaga argyrostoma*)

[1]Tarek A. El-Tayeb, [2]Mayada M. Gharib and [2]Afaf M. Al-Gendy
[1]National Institute of Laser Enhanced Sciences, Cairo University, Giza, Egypt
[2]Department of Zoology, Faculty of Science, Fayoum University, Fayoum, Egypt

Corresponding Author: Tarek A. El-Tayeb, National Institute of Laser Enhanced Sciences, Cairo University, Giza, Egypt

ABSTRACT

Gastric, intestinal and nasal myiasis are animal diseases caused by flesh fly (*Parasarcophaga argyrostoma*). This study aims to cut the cycle of flesh fly at the adult stage to reduce the animal infection and control these diseases using sunlight and photosensitizer (Hematoporphyrin IX). This class of compounds is environmentally friendly with a high photosensitizing activity towards biological systems. It is approved for medical use in the photodynamic therapy of tumors and other diseases. Hematoporphyrin IX becomes toxic only when it is activated by sunlight. *Parasarcophaga argyrostoma* is readily attracted by sugar bait containing hematoporphyrin amounts in the small range (treated flies). Moreover, it appears to consume enough of the bait to allow the Hematoporphyrin IX (HP) to exert its phototoxic action when the insect is exposed to direct sunlight. The treated and untreated flies were dissected for midgut histological studies. The results of testing hematoporphyrin IX (HP) as a photoinsecticide is presented. 10^{-2} M L^{-1} HP caused the highest mortality (83%) and 1935 W m^{-2} sunlight intensity was enough to cause 96% mortality of HP treated flies. The results revealed that HP appears to be very active against *Parasarcophaga argyrostoma*. HP showed high ability to accumulate inside the insect organs The histological studies of *P. argyrostoma* alimentary canal showed high extent of alimentary canal tissue damage as a function of sunlight exposure times after incubation with 10^{-2} M L^{-1} HP concentaration. This study concluded the highest efficiency of HP to induce reduction of *Parasarcophaga argyrostoma* population, which can be used as a novel modality of its control.

Key words: *Parasarcophaga argyrostoma*, flesh fly, hematoporphyrin IX, control, Photosensitization, photodynamic

INTRODUCTION

The flesh fly, *Parasarcophaga argyrostoma* is abundant in Egypt and it has some medical and veterinary importance since it is of particular interest owing to its significant role in causing gastric, intestinal and nasal myiasis and invading various tissues of man and animals (facultative parasites) and often leading to series consequences. It normally lives in decomposing matter and may be attracted to foul wounds for larvae position. Larvae have been found in sore wounds and natural openings of man (Gardiner *et al*., 1983). The efficient control of these insects has long been the goal of workers in the field of medical entomology.

Chemical treatments create problems by leaving undesirable residues in food and there are public fears about chemical residues on safety of users and the environment, as well as the development of resistance to these insecticides. Thus in 1996, the Food Quality Protection Act limited the use of many pesticides, particularly those that share common toxicological mechanisms such as organophosphate, carbamate insecticides and triazine herbicides (Lyon and Newton, 1999). Thus, it is becoming clear that alternative pest management tools are needed, which will be less hazardous to human, non target animals and the environment, in the same time these alternative tools must be used in the field application with minimum cost. In this context, sunlight-activated photo-pesticides represent a possible alternative to traditional chemical compounds. The use of photochemical processes as a tool to control the population of several types of insects has been repeatedly examined in both laboratory experiments and field studies (Ben Amor and Jori, 2000). At the cellular level, most photosensitizers are able to induce apoptotic cell death (Luksiene, 2003).

This work aims to use the HP as a non-toxic and nonmutagenic photopesticide to control *P. argyrostoma* and choose the optimum parameters that can be used in the field application to have high percentage of mortalities of flies with maximum safety level and minimum cost.

MATERIALS AND METHODS

This study was done as a part of Master thesis in the period of 2008-2010 in the national Institute of Laser Enhanced Sciences (NILES), Cairo University and Faculty of Sciences, Fayoum University, Egypt.

***P. argyrostoma* laboratory colonization:** A large glass vessel containing a few pieces of decaying meat was left in the open air for three days and a few drops of water were added whenever the meat showed any signs of dryness. In this condition the meat is a good larviposition site for wild flesh flies. The vessel was then transferred to the laboratory with its top covered with muslin. When the deposited larvae were fully developed and ready to pupate, they were transferred into muslin cages where adults emerged. The emerging adults were identified and kept separately.

A stock culture was maintained under laboratory conditions from which the different stages needed for the work experiments were taken.

The breeding stock of adults was maintained in 50×50×80 cm muslin cages. On emergence, the adults were supplied with sugar, milk and water as food. Sugar and milk were supplied in the form of a concentrated solution in a ratio of 1:2 or dried in the form of granulated sugar mixed with milk powder in the proportion of 1:2. Water was supplied by dipping a piece of cotton, as a wick, in a bottle filled with water. The meat was introduced in a petri-dish, as a larviposition medium and was changed daily. Both fresh and previously frozen meat attracted gravid females for larviposition. The cages were examined once a day in the morning for the newly deposited larvae. The sugar milk and water were checked and the meat was renewed whenever necessary.

Preparation of hematoporphyrin stock solution (Treated bait): Each normal bait was prepared by dissolving 100 g sugar in 70 mL distilled water. The treated bait was prepared by dissolving 1.7 g of hematoporphyrin (from porphyrin products (logan, utah) in 23 mL sugar solution and 2 mL of sodium hydroxide solution (0.1 M). The stock solution was left on magnetic stirrer for 2 h. The final solution was adjusted to pH = 6.5-7. The final HP concentration was measured by spectrophotometer (Perkin Elmer, LAMBDA 40). The stock solution was used within

two weeks because it is stable for a few weeks when kept in dark at 4°C (Thameur *et al.*, 1998). According to Beer' s Lambert law the measured absorbance is being correlated to the concentration of the solution. Diluted solutions were prepared by taking aliquots of the stock solution and added to the normal bait.

Estimation of HP concentrations inside the insect body: After feeding on HP, the flies were frozen then homogenized with 5 mL dist. H_2O using homogenizer. The suspension thus obtained was centrifuged for 25 min at room temperature and at 13000 rpm. The pellet was discarded, while known aliquots of supernatant were measured by reading the absorbance in sulfuric acid using spectrophotometer. The HP concentration was determined from Beer's Lambert law using absorbency 423000 in sulfuric acid. The results were expressed as moles L^{-1} of HP recovered by fly.

Exposure studies: The flies were exposed in transparent cages (7×5×11 cm). The sunlight fluence rate was measured by Eldonet WinDose 2000 dosimeter (Real Time Co., Germany). The actual fluence rate was accounted through the average of the different intensities during the exposure time.

Dark experiment: In this experiment, the flies groups were left for one week in the dark and each group was supplied by one of HP treated bait (10^{-2}, 10^{-3} and 10^{-4} ML^{-1}). The behaviour of the flies and percentage of fly mortality were monitored.

Histological studies: Three groups of flies (10 flies/each) were separated from the rearing cage. All groups were supplied with 10^{-2} M L^{-1} HP treated bait and left in the dark for 12 h as a feeding period. Each group was exposed to sunlight for one of the exposure times 2, 4 and 9 h. The died flies were dissected to get the mid gut. The alimentary canal of flies was removed and fixed using Bouin's, then dehydrated by using ascending series of ethanol. The samples were cleared by xylol, after that infiltrated and imbedded in paraffin wax. Thin sections of midgut were prepared using the microtome, mounted on glass slides and treated with Haematoxylin and Eosin stains to investigate the extent of HP effect on the level of flies' tissues.

Statistical analysis: The statistical package for social science (SPSS version 17.0) program were used for statistical evaluation and analysis of variance (frequently abbreviated ANOVA) (Miller and Miller, 1988).

RESULTS AND DISCUSSION

In this study, Hematoporphyrin IX was tested as a novel modality pesticide against one of the medical insects, *Parasarcophaga argyrostoma*. The results revealed the optimum parameters which induced the highest efficiency of HP in controlling HP treated *Parasarcophaga argyrostoma* using direct sunlight.

During the application of our experimental protocol, we found that there are many parameters, which can modulate the efficiency of HP as a photoinsecticide against *P. argyrostoma*. These parameters are HP concentrations, fluence rates, exposure times and incubation periods.

Figure 1 showed that *P. argyrostoma* was more affected by the highest concentration (10^{-2} M L^{-1}) of HP giving average mortality of 83% and the mortality of 10^{-3} M L^{-1} of HP was 50%. This means that the most efficient HP concentration for controlling of *P. argyrostoma* is 10^{-2} M L^{-1} which is different from the most efficient concentration used to control *Musca domestica*

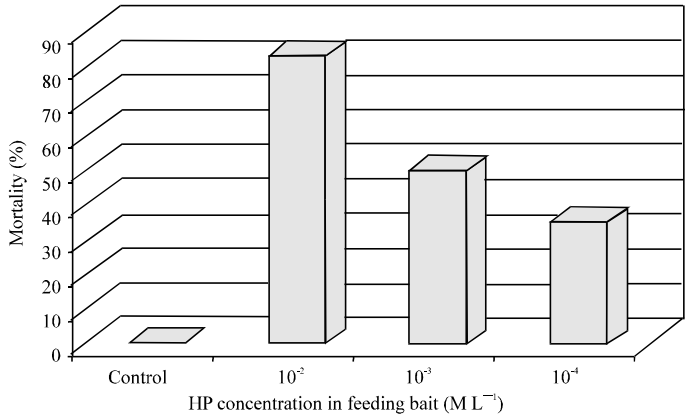

Fig. 1: Effect of different HP concentrations (10^{-2}, 10^{-3} and 10^{-4} M L^{-1}) on the % of mortality of *P. argyrostoma* (20 flies/concentration/replicate) exposed to natural sunlight (fluence rate: 236.50 Wm^{-2}) for 9 h immediately after incubation with HP for 12 h

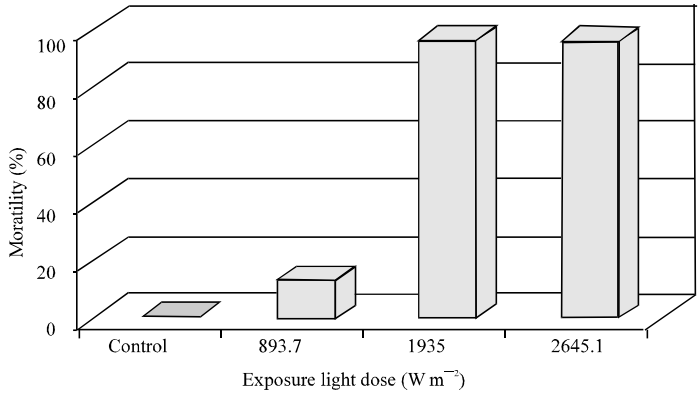

Fig. 2: Effect of different sunlight doses (893.7, 1935 and 2645.1 Wm^{-2}) on % mortality of *P. argyrostoma* (20 flies/dose/replicate) which had been exposed to 10^{-2} M L^{-1} HP and then exposed to sunlight for 3, 6 and 9 h

(10^{-3} M L^{-1}) and *Culex pipines* (10^{-5} M L^{-1}) studied in the previous work (El-Tayeb, 1999). This effect can be ascribed to the role played by the integument and the size of flesh fly. *P. argyrostoma* has most darkely pigmented body, heaviest integument and greatest size (Khoobdel *et al*., 2008) than *M. domestica* and *C. pipiens*. All these characters of the body of *P. argyrostoma* allow to transmit light dose less than lighter integument so the results of photodynamic reaction in case of *P. argyrostoma* is slower than in case of *M. domestica* and *C. pipines*.

The sunlight dose (Fig. 2) plays an important role, in the effect of photosensitizer (HP) on the survival of *P. argyrostoma*. It is clear from this figure that the mortality percentage of the flies (13, 96, 96%) increases with increasing the fluence rate (893.7, 1935, 2645.1 W m^{-2}, respectively) of the light. This behavior is agree with the previous work (El-Tayeb, 1999; Luksiene *et al*., 2005) in which, they found that the effect of HP on *Liriomyza bryoniae*, *C. pipiens* larvae and *M. domestica* mortalities in the sunny seasons is more than the other seasons because; with increasing sunlight intensity the number of photons striking the target increases and so, the

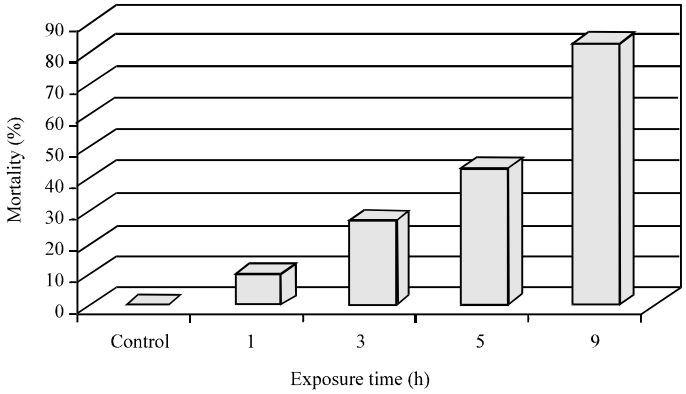

Fig. 3: Effect of different light exposure times (1, 3, 5 and 9 h) on % of mortality of *P. argyrostoma* (20 flies/each exposure time/replicate) which had been exposed to 10^{-2} M L^{-1} for 12 h. (fluence rate of natural sunlight: 236.50 W m^{-2})

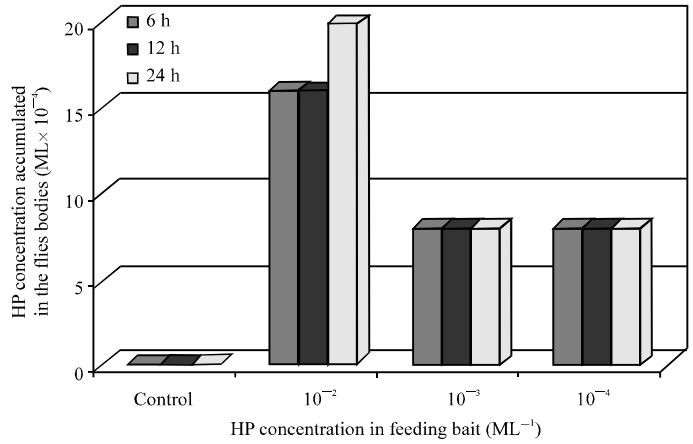

Fig. 4: Effect of different HP concentrations and incubation periods on the accumulation rates of HP in *P. argyrostoma* body (20 flies/concentration/replicate). The flies were exposed to the HP bait for 6,12 and 24 h

number of excited HP molecules will be increased followed by a concomitant increase of the amount of singlet oxygen produced by photochemical reaction. Also, this support Fondren *et al.* (1978) work in which they studied the light intensity as a critical parameter in the photodynamic toxicity of rose Bengal to the adult house fly, they showed that the accumulated number of photons needed to kill 50% of a population decreased as the intensity increased. This would interfere that; there is a regenerative capacity within the insect that is more efficiently overcome by photodynamic action as the light intensity increase.

The different light exposure times have significant differences on the percentage of survival of *P. argyrostoma*. It is clear from Fig. 3 that the percentage of mortality of flies (10, 26.66, 43.3 and 83%) increased with increasing light exposure time (1, 3, 5 and 9 h, respectively) after treatment with HP. The effective light exposure time in case of *P. argyrostoma* control using HP is lesser than the light exposure time of the other photopesticides because the half Lethal Time (LT50) for *P. argyrostoma* fed on 10^{-3} M L^{-1} was 9 h. While the other photopesticides like Phloxin B, LT50 = 13.31h and tetrachloro- fluorescein, LT50 = 31.48 h when the flies fed on 10^{-3} M L^{-1} (Fondren *et al.*, 1979).

Dark experiments revealed that the hematoporphyrin had no toxic effect in absence of light hence there is no toxic effect on human and animals when it accumulated inside their bodies. This is agreeing with (Kappus *et al*., 1988) in which they tested the dark toxicity on human keratinocytes. Previous study declared the dark toxicity in some of other photopesticides. Creighton *et al*. (1980) reported the dark toxicity of rose bengal to the cabbage looper, the corn earworm and the pickleworm. James and Heitz (1987) showed that both phenylheptatriyne and alpha-terthienyl displayed ovicidal activity against the eggs of the fruit fly in the dark. The high carcinogenic potential of this class of compounds has kept them from being exploited as much as would be expected if there was no carcinogenic risk.

The most attractive feature of HP was investigated in this study is its ability to accumulate inside the biological tissues of flies by a rate which exceeds the excretion rate of flies, especially when the concentration and incubation time are long enough. The highest rate of accumulation was revealed in the flies which were fed on the highest HP concentration (10^{-2} M L^{-1}) in the all HP ncubation periods. The HP concentration accumulated in the flies bodies which were fed on 10^{-2} M L^{-1} HP bait was 20×10^{-6} M L^{-1} HP accumulated in the flies bodies. The HP feeding concentration of 10^{-8} and 10^{-4} M L^{-1} caused the same amounts of HP accumulation (8×10^{-6} M L^{-1} HP) inside the flies' bodies for the whole HP bait incubation periods

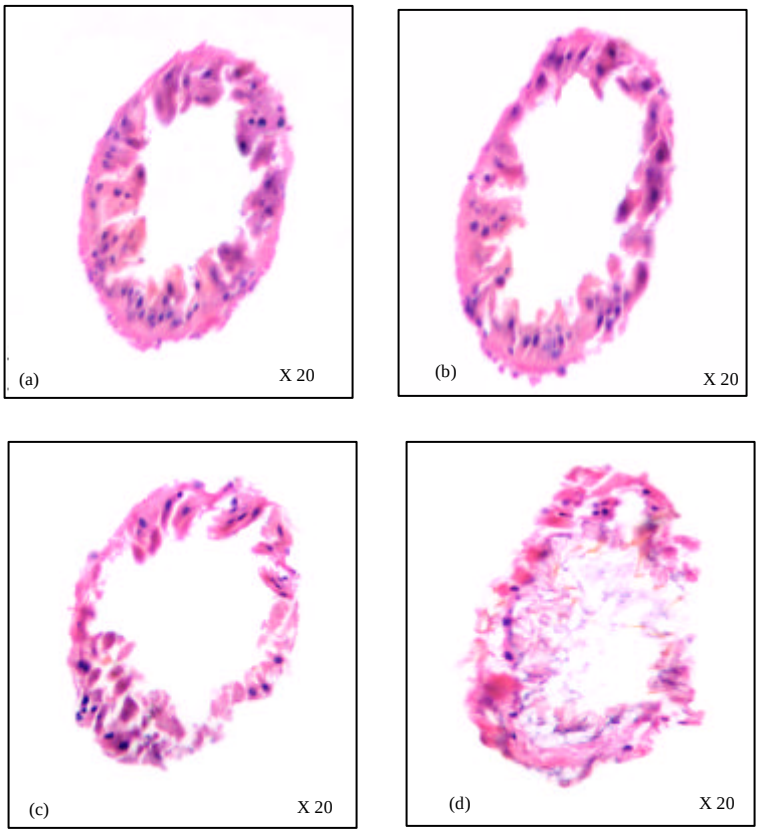

Fig. 5: Transverse sections of *P. argyrostoma* alimentary canal (midgut) showing: (a) Control flies (b) Flies treated with HP and exposed to sunlight for 2 h (c) Flies treated with HP and exposed to sunlight for 4 h and (d) Flies treated with HP and exposed to sunlight for 9 h

(Fig. 4). This may interprets the results of Fig.1 in which the highest mortality rate appeared with the highest HP concentration in the feeding medium. This agree with Awad *et al.* (2008) which tested the effect of HP as a photopestiside on Culex pipien larvae in a semi-field study.

Histological studies (Fig. 5 a-d) confirmed the results of Fig. 2 which support the previous interpretation of which related to the cause of flies death due to starvation. The damaged alimentary canal stopped to absorb the digested food to supply to the other body organs.

Results study of this work concluded that HP photosensitization process is a promising method for control of *P. argyrostoma*. Accordingly, it is possible to say that this study is available to wide field studies. The advantages of HP and the method of its application are enough to expect the success of this method in the field application.

REFERENCES

Awad, H.H., T.A. El-Tayeb, N.M. Abd El-Aziz and M.H. Abdelkader, 2008. A semi-field study on the effect of novel hematoporphyrin formula on the control of *Culex pipiens* larvae. J. Agric. Soc. Sci., 4: 85-88.

Ben Amor, T. and G. Jori, 2000. Sunlight-activated insecticides: Historical background and mechanisms of phototoxic activity. Insect Biochem. Mol. Biol., 30: 915-925.

Creighton, C.S., T.L. Mcfadden and J.M.J. Schalk, 1980. Toxicity of rose bengal to the cabbage looper, the corn earworm and the pickleworm. Feorgia Entomol. Soc., 15: 66-68.

El-Tayeb, T.A., 1999. The use of photoactivable pesticides for the control of potentially noxious insects. M.Sc. Thesis, National Institute of Laser Enhanced Sciences, Cairo University, Cairo.

Fondren, J.E., B.R. Norment and R. Hetiz, 1978. Light intensity as a critical parameter in the dye-sensitized photooxidation of the house fly, *Musca domestica*. Environ. Entolmol., 7: 891-894.

Fondren, J.E., B.R. Norment and R. Hetiz, 1979. Dye sensitized house fly toxicity produced as function of variable light sources. Environ. Entomol., 8: 432-436.

Gardiner, C.H., V.S. James and B.A. Valentine, 1983. Visceral myiasis caused by *Musca domestica* in a cat. J. Am. Vet. Med. Assoc., 182: 68-69.

James, H. and R. Heitz, 1987. Pesticide Composition and Method for Controlling the Oriental Fruit Fly. American Chemical Society, Washington, DC, pp: 19.

Kappus, H., C. Reinhold and M. Artuc, 1988. Photodynamic toxicity of hematoporphyrin derivatives to human keratinocytes in culture. Mol. Toxicol., 1: 295-299.

Khoobdel, M., N. Jonaidi and M.S. Rashti, 2008. Blowfly and flesh fly (Diptera: Cyclorrhpha) Fauna in Tehran, Iran J. Entomol., 5: 185-192.

Luksiene, P., 2003. Photodynamic therapy: Mechanism of action and ways to improve the efficiency of treatment. Medicina, 12: 1137-1150.

Luksiene, P., V. Buda and S. Radþiute, 2005. Effects of visible-light-activated hematoporphyrin dimethyl ether on the survival of leafminer *Liriomyza bryoniae*. EKOLOGIJA, 3: 17-21.

Lyon, G.D. and A.C. Newton, 1999. Implementation of Elicitor Medited Indused Resistance in Agriculture. In: Indused Plant Defenses Against Pathogens and Herbivores, Agrawal, A.A., S. Tuzun and E. Bent (Eds.). APS Press, St. Paul, MN, pp: 299-318.

Miller, J.C. and J.N. Miller, 1988. Statistics for Analytical Chemistry. John Wiley and Sons, New York.

Thameur, B.A., M. Trochin, L. Bortolotto, R. Verdiglion and G. Jori, 1998. Porphyrins and related compounds as photoactivatable insecticides. 1. Phototoxic activity of hematoporphyrin toward *Ceratitis capitata* and *Bactrocera oleae*. Photochem. Photobiol., 67: 206-211.