**THE INTERNATIONAL CONFERENCE ON BIOLOGICAL SCIENCES VOL. 1-PART2, 7-8 MAY 2000**

 **Proc. I.C.B.S., 1 (2) 2000: 123-132.**

**THE COMBINED EFFECT OF THE PHOTO-INSECTICIDE HEMATOPORPHYRIN AND THE INSECT GROWTH REGULATOR DIMILIN ON *Culex pipiens* LARVAE.**

**By**

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### ABSTRACT

 The present study deals with the compatibility of the hematoporphyrin and dimilin against *Culex pipiens* larvae. The early fourth and second larval instars of *C. pipiens* were pre treated with dimilin for 24, 48 and 72 hours or prolonged time ( i.e. till pupation) then exposed to the hematoporphyrin for 24 hours before irradiation and vice versa. The larvae were kept in darkness during treatment then were exposed to artificial light ( 380-400Wm\_2). It was found to produce higher mortality than if either of them was used alone. In addition the interaction between the hematoporphyrin and dimilin was also more synergistic in the younger instar than in the older fourth instars. Combination of the hematoporphyrin and dimilin provided acceptable control levels when dimilin was applied with 24 hours before the application of the hematoporphyrin before the irradiation. The obtained results showed the possibility of using the hematoporphyrin and dimilin in combinations for controlling *Culex pipiens* larvae.

**INTRODUCTION**

 The use of the photoinsecticide in insect control has received much recent attention due to its advantage over the tradional methods , as reported by, Yaho, *et al*. ( 1976), Heitz (1987), Lenke, *et al.* (1987), Ben Amor, *et al.* (1998) and Salama, *et al.* (1998). The advantageous of utilization of the photoinsecticide include low cost and the possibility of use for insect control management. This photoinsecticide can be directly administered in aqueous solution and in association with attractive; its photophysical, photosensitizing properties have been determined in a variety of media and have been shown to be particularly efficient, Jori (1985). The relatively high water solubility of hematoporphyrin, the ascertained lack of photomutagenic activity, Jori and Spikes (1983) and its wide spread clinical use as a phototherapeutic agent against solid tumours and other diseases, Jori (1986) and Brown (1997), although some potential hazards and limitation need to be considered, Heitz (1987), Yoho, *et al.* (1976), Lenke, *et al.* (1987). Photoinsecticide is also very rapidly photobleached upon exposure to UV or visible light, Jori and Spikes (1983).

# There is a shortage of literatures dealing with the combined effect of the hematoporphyrin and the dimilin against *Culex pipiens* larvae. However most of literatures are dealing with those treated with dimilin with other additives or hematoporphyrin with other additives too. The phototoxicity of the hematoporphyrin is based on its ability for generating singlet oxygen in presence of light. This cause severs damage to the cell membrane and resulted in cell death. However, it`s poorly stable in presence of light.

# In this study, the combined effects of the hematoporphyrin ( photoinsecticide) and the dimilin ( insect growth regulator) were studied against *Culex pipiens larvae.*

**MATERIALS AND METHODS**

 **Chemicals**

 All the chemicals used were analytical grade obtained from Sigma, Aldrich and Fluka (England). (England). Stock solutions of Hematoporphyrin were prepared by dissolving known amount of Hematoporphyrin in the minimal amount of 0.1M Na OH and then neutralizing by addition of 36% HCL. The Hematoporphyrin concentration in the final solution was determined by absorption spectrophotometry, using ε =423000 M-1 cm-1 at 401.5nm. The solutions of Hematoporphyrin were stable for 4 weeks at 4oC in darkness.

 Dimilin ( 25%WP) was used. All different concentrations were made using distilled water.

#  **Experimental models**

 The *C. pipiens* larvae was obtained from Blades Biological Nottingham and colonized in the Department of life Sciences; Nottingham Trent University, Clifton Lane, Nottingham, and NG11 8NS, UK.. The assessment of toxicity was based on the mortality of the tested larvae after irradiation. The early fourth and second larval instars of *C. pipiens* were exposed for 24 hours to different concentrations of hematoporpyrin and/or the dimilin in distilled water. Another groups of larvae were left without any treatments, or treated with hemtoporphyrin without irradiation, or exposed only to light without any treatment and served as control. Four replicates of the desired concentrations of each compound were prepared , in 250 ml glass beaker. Each beaker received 25 larvae of second or early fourth larval instars according to the experiment carried out. The percentages of mortality were plotted against the tested concentrations and the LC50 and the LC25 values were determined graphically. Mortality percentages were corrected by Abbott’s formula (1925), if the mortality in control exceeds 5%. These LC25 values were chosen for further studies.

**Joint action of The Hematoporphyrin and the dimilin mixture against *Culex pipiens* larvae.**

 The Hematoporphyrin and dimilin were mixed in equal volumes at concentrations equivalent to the LC25 values. The expected mortalities of the mixtures were calculated by the summation of the expected mortalities of the toxicant used in the mixture.

The equation of Sun and Johnson (1960) was used as follows:

**Co toxicity Factor** =100 x Observed % mortality- Expected % mortality

 Expected % mortality

This factor differentiates the results into three categories as follows:

A positive factor of 20 or more is considered as indicating potentiation.

A negative factor of –20 or more indicating antagonism.

The intermediate values between –20 and +2o indicating additive effect.

It must be noted that, all these experiments were conducted at room temperature (20±5 oC).

### Irradiation

####  The artificial light source used was a Philips UVA lamp (HP3148/A, half body, 8TL09 lamps of 40W each, from Philips Electronics, Croydon) with an average output of 6.8x105W cm-2 UVA and 6.1x103 Wcm-2UVB. The light intensity of the lamp was measured using a Glen Spectral Radiometer, model 1680B. The irradiation dose used was 7783 Jm-2.

**The statistical analysis**

The statistical analysis was performed using t-test.

 **RESULTS**

Results of the present experiments are shown in the tables (1, 2, 3, 4 , 5, 6 and 7) and graphically illustrated in figures (1, 2,3,4, 5,6 and 7). The obtained data indicated that the larvicidal activity of hematoporphyrin and dimilin was relatively high to the 2nd larval instar followed by 4th larval instar as shown in the table (1, 2, 3&4) and in figures ( 1, 2,3 &4). Also the data showed high level of activity of both hematoporphyrin and the dimilin against *C. pipiens* larvae at the different time of exposure based on the obtained LC50 . The response is dose- dependent , i. e. the larval mortality was increased with the increase of concentration. The obtained data indicate also the following:

- There is a significant increase in larval mortality due to the combined effect of the hematoporphyrin and the dimilin when they were used simultaneously ( p<0.05).

- The mixture of both hematoporphyrin and dimilin gave potentiation effect with both larval instars after exposure to 24&48 hours and additive effect with increase of time of exposure ( i. e. 72 hour).

- A significant higher mortality was obtained with second larval instar than in fourth instar as indicated from Table (5).

- Higher significant mortality resulted from pre- treating the larvae with dimilin then that pre-treating with hematoporphyrin for the fourth and second larval instars ( p< 0.05).

- The effect of pre- treating of larvae with dimilin indicating a potentiation effect as in Table (6).

-The effect of pre-treating larvae with hematoporphyrin indicating additive effect as in Table (6).

- The combined effect of hematoporphyrin with dimilin for prolonged time of exposure indicating only additive effect Table (7).

DISCUSSION

 The obtained data indicated the following:

 1-Hematoporphyrin induce high level of knockdown action in a short time to the treated *C. pipiens* larvae. 11 mM/ ml resulted in 100% mortality after one hour of irradiation with UV light. The difference in efficacy between hematoporphyrin and dimilin may be due to the different modes and sites of action of the two larvicides. However, both hematoporphyrin and dimilin are considered as a larvicidal agent. The obtained result was found to be in agreement with that of Ben Amor *et al*. (1998) who pointed out that porphyrins may represent a class of useful photoinsecticidal agents in particular, hematoporphyrin

 appeared to be very active against at least two fly species, namely *Ceratitis capitata* and *Bactrocera oleae* which are known to induce severe damage in various agricultural areas worldwide. The obtained larvicidal action of dimilin is in agreement with that of McKague and Pridmore ( 1978) who concluded that the ingestion of Juvenile hormone or insect growth regulator be larvae of the rain bows trout disrupt the normal process of cuticle depostion. They also stated that the higher doses greatly reduced emergence of adults. Also this result is in accordance with the findings of other workers Jakob, ( 1972), Mulla *et al.*( 1975) and Baker *et al.* ( 1997).

 2- High doses is needed to achieve the best control levels, however, the joint action improve such situation.

 3-The present study confirms the additive or synergistic (potentiation effect) interaction between the hematoporphyyrin and dimlin against *C. pipiens* larvae. These results confirm the findings of Sokolova and Ganushkina (1982) who concluded that in certain condition the effect of combinations between some *Bacillus thuringiensis* var. *israelensis* H-14 formulations and the growth regulator Methoprene was higher than when they were used separately , and that their effectiveness depend on the kind of preparation of Bactimos used, the dosage applied and the species composition of the mosquito treated.

 Our results clearly indicated that the synergistic effect might be due to the following:

 1-The decrease in tolerance of the *Culex pipiens* larvae to the rapid insecticidal action of hematoporphyrin which can be correlated with its mode of photo inducing irreversible damage to biological systems.

 2-Both hemotoporphyrin and dimilin may enhance the activity of each other against the tested larvae.

 In summary, the obtained results confirmed the insecticidal efficiency of the combined effect of the photoinsecticide hematoporphyrin and the insect growth regulator dimilin against *Culex pipiens* larvae, thus it may be recommended the usage of the tested toxicants in control management strategy to enhance the activity , reduce costs and reduce the pesticide impact in the environment.

# **ACKNOWLEDGEMENTS**

The authoress wishes to thank Drs S. Ahmad, S. Kirk and C. Terrellnield at Nottingham Trent University, Faculty of Science & Mathematics for the facilities they offered during my fellowship time. The Ministry of Higher Education in Egypt financially supported this work.

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Table (1): Susceptibility of the early 4th instar of *C. pipiens* larvae to photoinsecticide hematoporphyrin at different time of exposure ( 24,48 & 72 hours) & after irradiation with 380-400 W/M2 eartificial lights.

|  |  |
| --- | --- |
| Concentration( mM / ml ) | Averages of corrected mortality percent |
| 24h | 48h | 72h |
| X ± SE | X ± SE | X ± SE |
| 10 | 90.12 ± 1.12 | 94.51 ± 0.25 | 98.51 ± 0.21 |
| 7 | 58.26 ± 2.11 | 85.30 ± 0.90 | 94.9 0± 0.40 |
| 5 | 29.11 ± 0.78 | 74.11 ± 0.25 | 88.23 ± 0.67 |
| 2.5 | 5.34 ± 0.74 | 51.74 ± 0.64 | 73.10 ± 0.73 |
| 1 | 0 | 11.10 ± 0.32 | 31.10 ± 0.11 |
| LC50 | 5.9 | 2.6 | 1.4 |

Table (2): Susceptibility of the early 2nd instar of *C. pipiens* larvae to photoinsecticide hematoporphyrin at different time of exposure ( 24, 48 & 72 hours) & after irradiation with 380-400 W/M2 eartificial lights.

|  |  |
| --- | --- |
| Concentration( mM / ml ) | Averages of corrected mortality percent |
| 24h | 48h | 72h |
| X ± SE | X ± SE | X ± SE |
| 10 | 100 |  |  |
| 7 | 88.12 ± .13 | 98.10 ± 0.60 | 100 |
| 5 | 84.31 ± 0.40 | 93.1 ± 0.51 | 96.1 ± 0.7 |
| 2.5 | 61.5 ± 0.44 | 82.1 ± 0.54 | 97.1 ± 0.83 |
| 1 | 61.0 ± 1.0 | 91.10 ± 0.60 | 90.10 ± 0.98 |
| 0.1 | 5.1 ± 1.30 | 10 ± 1.4 | 20.0 ± 0.75 |
| 0.5 | 0 | 10.12 ± 2.40 | 19.1 ± 0.27 |
| LC50 | 1.6 | 0.84 | 0.38 |

Table (3): Biological activity of the insect growth regulator dimilin against 4th instar of *C. pipiens* larvae, at different time of exposure ( 24, 48 & 72 hours).

|  |  |
| --- | --- |
| Concentration( ppm ) | Averages of corrected mortality percent |
| 24h | 48h | 72h |
| X ± SE | X ± SE | X ± SE |
| 20 | 90.12 ± 1.12 | 95.51 ± 0.25 | 98.51 ± 0.21 |
| 10 | 58.26 ± 2.11 | 70.30 ± 0.90 | 86.9 ± 0.40 |
| 7 | 23.11 ± 0.78 | 49.11 ± 0.25 | 66.23 ± 0.67 |
| 5 | 15.34 ± 0.74 | 10.2 ± 0.64 | 46.1 ± 0.73 |
| 2.5 | 0.0 ± 0.0 | 8.10 ± 0.32 | 21.10 ± 0.076 |
| LC50 | 9.5 | 7.5 | 4.8 |

Table (4): Biological activity of the insect growth regulator dimilin against the 2nd instar of *C. pipiens* larvae, at different time of exposure ( 24, 48 & 72 hours).

|  |  |
| --- | --- |
| Concentration( ppm ) | Averages of corrected mortality percent |
| 24h | 48h | 72h |
| X ± SE | X ± SE | X ± SE |
| 20 | 100 |  |  |
| 10 | 95.12 ± 0.13 | 98.10 ± 0.60 | 100 |
| 7 | 89.31 ±0.40 | 96.1 ± 0.51 | 97.8 ±0.7 |
| 5 | 82.5 ± 0.44 | 93.1 ± 0.54 | 97. 1 ± 0.83 |
| 2.5 | 61.0 ± 1.0 | 81.10 ± 0.60 | 90.10 ± 0.98 |
| 1 | 35.1 ± 1.30 | 51.19± 1.40 | 79.0 ± 0.75 |
| 0.1 | 0 | 10.12± 2.40 | 19. 1± 0.27 |
| LC50 | 1.5 | 0.8 | 0.18 |

**The joint action of hematoporphyrin and dimilin against *C. pipiens* larvae.**

Table (5): The combined effect of hematoporphyrin ( H) and dimilin ( D) against *C. pipiens* larvae when used simultaneously in mixture for different time of exposure.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Co toxicityfactor | Averages of mortality % | ExposureTime(hour) | ConcentrationsUsed at LC25 | LarvalInstar |
| Observed | Expected | H + D(mM/ml)+ (ppm) |
| +22 | 61 | 50 | 24 | 4.3+6 | FourthInstar |
| +24.21 | 67.2 | 54.10 | 48 | 1.5+4.5 |
| +10.16 | 66.1 | 60 | 72 | 0.7+2.6 |
| +31.11 | 59 | 45 | 24 | 0.8+0.7 | SecondInstar |
| +23 | 65.55 | 53.3 | 48 | 0.38+0.2 |
| +8 | 66.74 | 66.5 | 72 | 0.14+0.05 |

Table (6): The combined effect of hematoporphyrin (H) and dimilin (D) against *C. pipiens* larvae when used pre-treated with either of them

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| CoToxicityfactor | Averages of mortality% | ExposureTime(hour) | ConcentrationsUsed at LC25 | Larval instar |
| Observed | Expected | H + D(mM/ml)+(ppm) |
| +11 | 49.75 | 44.65 | 24 | 4.3 followed by 6 | Forth instarPre-treatedwith (H) |
| +12 | 56.34 | 50.56 | 48 | 1.5 followed by 4.5 |
| +12 | 75.4 | 67.5 | 72 | 0.7 followed by 2.6 |
| +32 | 65.8 | 49.5 | 24 | 6 followed by 4.3 | Fourth instarPre-treatedWith (D) |
| +21 | 73.4 | 60.48 | 48 | 4.5 followed by 1.5 |
| +15 | 75.95 | 66.42 | 72 | 2.6 followed by 0.7 |
| +6 | 35.37 | 32.89 | 24 | 0.8 followed by 0.7 | Second instarPre-treatedWith (H) |
| +5 | 45.3 | 43.45 | 48 | 0.38 followed by 0.2 |
| +12 | 55.3 | 49.5 | 72 | 0.14 followed by 0.05 |
| +42 | 50.57 | 35.5 | 24 | 0.7 followed by 0.8 | Second instarPre-treatedWith (D) |
| +24 | 67.8 | 55.3 | 48 | 0.2 followed by 0.38 |
| +32 | 78.53 | 59.5 | 72 | 0.05 followed by 0.14 |

# Table (7): The combined effect of hematoporphyrin (H) and dimilin (D) against C. pipiens larvae when used for prolonged time of exposure (4 days for fourth instar and 9 days for second instar).

|  |  |  |  |
| --- | --- | --- | --- |
| CoToxicityfactor | Averages of mortality% | ConcentrationsUsed at LC25 | Larval instar |
| Observed | Expected | H + D(mM/ml)+(ppm) |
| +10 | 94.5 | 85.76 | 0.7 + 2.6 | Fourth instar |
| +9 | 100 | 92.45 | 0.1 + 0.05  | Second instar |

Fig (1): Comparative susceptibility of the 4th instar of *C. pipiens* to hematoporphyrin at different times after irradiation with UV light (LC50)

Fig (2): Comparative susceptibility of the 2nd instar of *C. pipiens* to hematoporphyrin at different times after irradiation with UV light (LC

fig (3): Biological activity of dimilin against the 4th larval instar of *C. pipiens*at at different time of exposure

fig (4): Biological activity of dimilin against the 2nd larval instar of *C. pipiens*at at different time of exposure

# Fig (5): The combined effect of hematoporphyin and dimilin against the 4th and the 2nd larval instars of *C. pipiens*larvae when simultaneously in mixture for different time of exposure

Fig (6): The combined effect of hematoporphyrin (H) and dimilin (D) against *C. pipiens larvae when pretreated with either of them*

Fig (7): The combined effect of hematoporphyrin and dimilin against *C.pipiens* larvae when used for prolonged time of exposure.

**التأثير المختلط لمادة الهيماتوبورفيرين المنشطة ضوئيا ومنظم النمو الحشري الديميلين علي برقات بعوضة *الكيولكس ببينز***

**إلهام محمد أحمد سلامة**

**قسم علم الحشرات - كلية علوم - جامعة الزقازيق فرع بنها**

 في دراسات معملية سابقة أثبت الهيماتوبورفيرين أنه أكثر تأثيرا علي الطور اليرقي الرابع ليرقة ***الكيولكس ببينز*** ، ويعتمد التأثير الضوئي السام لهذا المبيد الضوئي علي قدرته لإنتاج شوارد متأينة في وجود الضوء وهذا يحدث تأثيرا مدمرا علي جدار الخلية فيحدث موت الخلية ، ومع ذلك فهو ضعيف الثبات جدا في وجود الضوء وفي هذه الدراسة الحالية تم دراسة توافق كلا من الهيماتوبورفيرين والديميلين معا. تم معاملة بداية الطورين اليرقيين الثاني والرابع ***الكيولكس ببينز*** بواسطة الديميلين لفترات متباينة وهي 24.48.72 ساعة أو لمدة طويلة

( لحين ظهور العذاري ) ثم يتم تعريضهم للهيماتوبورفيرين لمدة 24 ساعة قبل التعرض للضوء أو العكس بالعكس. تظل اليرقات في الظلام لحين تعرضها للضوء الصناعي (400-380 وات لكل متر مربع). ووجد أن تأثيرهما معا يحدث نسبة أماتة أعلي من إستخدام كلا منهما منفردا ، بالإضافة إلى أن تأثيرهما المتداخل بين الهيماتوبورفيرين والديميلين كأنا أكثر إضافة في الطور اليرقي الصغير عن الطور اليرقي البالغ. أثبت التأثير المجتمع لهيماتوبورفيرين والديميلين أكثر قبولا عندما يتم التعرض للهيماتوبوفيرين بتأخير 24 ساعة قبل التعرض للضوء ومن هنا يتضح تماما من النتائج المتحصل عليها إمكانية إستخدام كلا من الهيماتوبورفيرين والديميلين مختلطين لمقاومة يرقة بعوضة ***الكيولكس ببينز.***