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THE DELAYED EFFECTS OF CERTAIN CHEMICAL AND MICROBIAL INSECTICIDES ON *CULEX PIPIENS* (L.) COMPLEX LARVAE UNDER LABORATORY CONDITIONS

El-Khodary, A.S.; F.A. Abou-Attia; S.M.I. Metwally and H.M. Hassan

Economic Entomology Department, Faculty of Agriculture, Kafr El-Sheikh, Tanta University, Egypt

ABSTRACT

This work was conducted to study the effect of two chemical insecticides (chlorpyriphos and methomyl) and the microbial insecticides (biofly and dipel) on *C. pipiens* larvae during three generations (F_1, F_3 and F_5) with two exposure time (24 or 48 hours, plus continuous exposure until pupation). Meanwhile, their effect on some biological aspects of *Culex pipiens* (L.) by exposing the 3rd larval instar to sublethal concentration ($L.C._{10}$) under laboratory conditions.

Results indicated that the differences between the means of both larval, pupal mortalities, pupation and adult emergence of *C. pipiens* for chloropyiphos and mothomyl were highly significant. The interaction effects between chemical insecticides, and both generation & exposure time were highly significant for larval mortality and percentage of pupation, respectively. While no significant differences occurred between the tested generations or exposure time on pupal mortality and adult emergence.

In case of microbial insecticides, the difference between means of both larval, pupal mortalities, pupation and adult emergence for dipel and biofly were highly significant. The tested generations had significant effect on larval mortality and percentage of pupation, while no significant effect on either the pupal mortality or adult emergence was recorded. Exposure time was effective and was found to have high significant effect on previous aspects of *C. pipiens*. The interaction between chemical and microbial insecticides indicated that microbial insecticides were the more toxic against *C. pipiens* (L.).

The number of eggs per female and the percentage of hatchability were significantly reduced by chlorpyriphos and methomyl application, while no significant diferences between both compounds was recorded. Also, there were no significant differences among the tested generations on egg-deposition and hatchability percentage.

Biofly and dipel were found reduce egg-deposition and hatchability percentage significantly. Remarkable differences were found among the tested generations and both egg-deposition and hatchability percentage. The interaction between the chemical and microbial insecticides proved that, chemical compounds were significantly effective on reducing the mean number of eggs laid by *C. pipiens* females. While no significant differences were recorded on hatchability percentage.

INTRODUCTION

In recent years, microbial pesticides applications have gained priority for pest control, since chemical pesticides developed pest resistance and caused environmental hazards. In particular, microbial insecticides proved to be highly efficient against mosquitoes. The effects of these biocides on some biological aspects of *Culex pipiens* (L.) were studied. Karch *et al*. (1990)

applied *Bacillus sphaericus* against *C. pipiens* larvae. Saleh *et al*. (1990b) indicated that egg production and hatchability of *C. pipiens* survived from larvae treated with *B. thuringiensis* was significantly reduced in the first generation, and reductions in pupation and adult emergence were obtained. When larvae of *C. quinquefasciatus* and *Aedes aegypti* were infected by the fungus *Aspergillus parasiticus*, its fecundity and life-span were found to reduce (Nankumusana, 1985). Sandhu *et al*. (1993) found that the fungi; *Beauveria bassiana*, *Metarhizium anisopliae* and *Aspergillus flavus* were pathogenic to the second larval instar of *C. pipiens* with 50% mortality, and other authors confirmed that results; Kumar *et al*. (1995) and Thiery *et al.* (1997). El-Khodary *et al*. (1983) found that selection pressure of methomyl reduced the number of produced eggs in the three tested generations of *C. pipiens*. sublethal doses of fenoxycarb reduced egg hatchability and the survival of different stages of *C. pipiens* (Mohsen and Zayia 1995).

The present study was carried out to investigate the effects of sublethal concentration (LC_{10}) of chlorpyriphos and methomyl (Chemical insecticides) also, dipel-2x and biofly (microbial insecticides) on some biological aspects of *Culex pipiens* immature stages. The latent effect of these compounds on pupation, adult emergence, and fecundity were also considered.

MATERIALS AND METHODS

a- Laboratory culture :

Larvae of *Culex pipiens* (L.) were collected from Kafr El-Sheikh region and reared in the laboratory for three successive generations to avoid any contamination with insecticides. Pupated larvae were transferred into cages filled with tap water until adult emergence. Emerging females were fed on pigeon's blood and males on 10% sugar solution.

b- Chemical and microbial insecticides :

Four materials were used to fined its effect on *C. pipiens* and they are as follows :

1. Methomyl: (Lannate 90%). A sample of wettable powder (900 gm a.i./kg).
2. Chlorpyriphos: (Dursban 4 EC). A sample of emulsifiable concentrate (480 gm., a.i./Liter).
3. Dipel-2x: A sample of wettable powder. Active ingredient: *Bacillus thuringiensis* subspecies *Kurstaki*, 32,000 International Units of Potency per Milligram (6.4% a.i.) was supplied by Abbott Laboratories. Chemical and Agricultural products Division. North Chicago. IL 60064, USA.
4. Biofly: A liquid formulation of the fungus *Beauveria bassiana* each cubic centimeter contains 3×10^6 conidia. It was provided by Nasr Fertilizers and Biocides Co. El-Sadat City, Egypt.

c- Effect of tested compounds with sub-lethal concentrations on the larvae of *Culex pipiens* (L.):

Each tested insecticide was dissolved in tap-water to make a stock solution or suspension from which the considered concentrations (LC_{10}) were prepared. A number of 300 third instar larvae (3 reps. × 100 larvae each) were immersed in the stock of chemical insecticides for 24 hrs and another 300 larvae were immersed for 48 hrs. for microbial insecticides. After exposure, the larvae were transferred to jars filled with tap water to complete their development. Adult emergence was calculated by counting and removing of pupal exuviae.

In another test, *C. pipiens* larvae were exposed to LC10 concentration until pupation. Percentages of: accumulated larval mortality, pupation, accumulated pupal mortality and adult emergence were calculated.

d- The latent effect of the four used compounds on the fecundity of *Culex pipiens*:

Larvae were reared until pupation under the chemical compound action. In another procedure larvae were exposed to the aqueous solutions of microbial insecticides (LC_{10}) for 48 hours, then they were transferred to clean water to complete their development until pupation and adult emergence. The tested generations were F_1 and F_5. Each test was replicated three times. Each replicate composed of 25 females and 25 males. Petri-dishes filled with tap water were placed inside the adult cages for egg raft oviposition. The hatched larvae were fed on a small amount of equal parts of dried yeast, fine dried bread and unfatty milk. The total number of eggs and hatched eggs, were determined for each treatment under the controlled conditions of 26 ± 2°C and 75% R.H.

The obtained data were statistically analyzed using the "MSTAT" statistical package on a computer (Freed, 1986). Percentage data were transformed to Arcsine. Combined analysis for each experiment was carried out to obtain the effect of chemical and microbial pesticides. The mean values were compared, according to Duncan's Multiple Range Test (DMRT) (Duncan, 1955).

RESULTS AND DISCUSSION

A- Effect of chloropyriphos, methomyl and their interaction with number of generations and exposure time on the larvae of *Culex pipiens* (L.) complex:

1. Larval mortality :

Results presented in Table (1) revealed highly significant differences in larval mortalities among the effects of chloropyriphos (28.51%) and methomyl (24.21%) and control (5.22%). Larval mortality in F_3 generation (18.61%) was statistically the same in F_5 generation (23.52%), while in F_1 generation it was the least (15.80%). Exposure time affected the larval mortality significantly, being lower (16.17%) during 24 hr exposure and higher (22.45%) during continuous exposure. Insecticide-generation interaction and insecticide-exposure time interaction gave highly significant effects on larval mortality, while other interactions were not significant.

2. Pupal mortality :

Application with both chlorpyriphos and methomyl significantly resulted in higher pupal mortality (12.03 and 9.97%, respectively) than control (2.22%). Pupal moralities were statistically the same in different tested generations and exposure time. The other interactions were not significant.

3. Pupation :

Chlorpyriphos was more significantly potent against *C. pipiens* larvae than methomyl, resulting in 67.67 % and 71.78% pupation, respectively, compared to 94.78% pupation for control. The three considered generations showed highly significant differences in between pupation percentage, despite that F_3 and F_5 were statistically the same. On the other hand, pupation was significantly decreased when the larvae were exposed continuously to the insecticides (75.11%) compared to 81.04%) pupation for 24 hr exposure. Interactions between chemicals and the number of the tested generation and between chemical and exposure time had highly significant effects on pupation, while other interactions had not.

4. Adult emergence :

Both chlorpyriphos and methomyl had highly significant effects on adult emergence, being 85.98% and 88.00%, respectively when compared to 97.77% for the control. Generations and exposure time had no significant effect on adult emergence. Also, interactions among the three tested parameters were not significant.

B- Effect of biofly, dipel and their interaction with number of generation and exposure time on the larvae of *Culex pipiens* (L.) complex:

1. Larval mortality :

Results recorded in Table (2) indicated that the difference between means of larval mortalities of dipel (69.82%) and biofly (68.78%) were highly significant if compared with control (5.22%). The number of the tested generations had significant effect on that character. Application of microbial insecticides showed high susceptibility in the fifth generation (51.41%) followed by the third one (47.74%), however, the first generation (44.67%) was still the lowest susceptible one. Data showed that there was highly significant differences between both exposure times in this character, as the highest average of larval mortality was found in larvae continuously exposed to microbial insecticides (59.51%). The interaction between the tested microbial insecticides and both number of generations & exposure time, was highly significant for larval mortality. The other interactions did not affect larval mortality significantly.

2. Pupal mortality :

Data presented in Table (2) revealed highly significant differences in pupal mortality due to microbial applications; 20.47% for biofly, 4.98% for dipel, when compared to control, 2.22%. Generations were not affected by microbial applications. However, continuous exposure of *C. pipiens* larvae to

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microbial insecticides resulted in 7.86% pupal mortality, compared to 10.59% in case of 48 hrs exposure. All interactions proved to have insignificant effects on pupal mortality, however microbial insecticide-exposure time interaction which gave highly significant effects.

3. Pupation :

The effect of bio-insecticides on pupation was significantly low in case of Biolfly (29.50%) and dipel (28.56%) compared to the control (94.78%). Tested generations had significant effects on pupation; 54.06, 50.89 and 47.89% for F_1, F_3 and F_5 generations, respectively. Also, exposure time had a highly significant effect on pupation; being lower (40.0%) in continuous exposure, and higher (61.89) in 48 hr exposure. Interactions between microbial compounds and the number of tested generation, and between microbial and exposure time were highly significant, however the others were not significant

4. Adult emergence :

The microbial insecticides were found to have highly significant effects on adult emergence of *Culex pipiens* pupae Table (2). Application of dipel significantly produced the low value of adult emergence (44.01%) followed by biofly (77.75%), when compared with control (97.78%). The statistical analysis showed no significant differences between tested generations in their effects on adult emergence. Highly significant differences were obtained between both time of exposure for adults emergence. Also, adult emergence was highly significant affected by interaction between microbial insecticides and exposure time. The other interactions did not significantly affected adult emergence.

C. A comparison between chemical and microbial insecticides on some biological aspects of *C. pipiens* (L.) complex :

Results in Table (3) revealed that microbial insecticides were more efficient against *C. pipiens* than chemical insecticides. The continuous exposure of mosquito larvae to microbial compounds gave the best results, and the highest larval mortality was obtained (59.51%), moreover the least pupation (40.0%) and least adult emergence (58.22%). Comparatively, chemical insecticides were less efficient than microbial insecticides in impairing biological aspects of *C. pipiens* either in 24 hr or continuous exposure. Highly significant differences were calculated among chemical and microbial tested compounds.

Table 3: Comparison between effects of chemical and microbial insecticides on some biological aspects of *Culex pipiens*.

Character	Chemical insecticides		Microbial insecticides		Sig.
	24 hours	Continuous	48 hours	Continuous	
Larval mortality (%)	16.17 d	22.45 c	36.37 b	59.51 a	**
Pupal mortality (%)	7.09 c	9.06 ab	10.59 a	7.86 b	**
Pupation (%)	81.04 a	75.11 b	61.89 c	40.0 d	**
Adult emergence (%)	91.55 a	89.61 b	88.14 b	58.22 c	**

**** indicate P<0.01. In a row, means designated the same letter for each trait are not significantly different at 5% level according to DMRT (1955).**

D- Effect of chemical and microbial insecticides on females fecundity of *Culex pipiens*:

Results recorded in Table (4) indicated that the chemical insecticides showed negative effect on egg deposition and had highly significant effects comparable to the control. Methomyl (87.63%) and chlorphyriphos (85.95%) were obivously effective by decreasing egg hatchability. In the mean time no significant differences were recorded between the effect of the two chemical insecticides on egg hatchability. Also generation factor showed a similar results on egg hatchability and no significant differences were found between them.

Table 4:Effect of chemical insecticides and tested generations on fecundity of *Culex pipiens* females.

Character	Chemical insecticides (A)				Generations (B)			Interaction AB
	Control	Chloro-pyriphos	Methomyl	Sig.	F_1	F_5	Sig.	
Deposited egg	105.25 a	63.50 b	74.40 b	**	88.30	73.80	N.S	N.S
Hatchability %	96.91 a	85.95 b	87.63 b	**	91.45	88.87	N.S	N.S

**** and N.S. indicate P<0.01 and not significant, respectively.**
In a row, means designated the same letter for each trait are not significantly different at 5% level according to DMRT (1955).

Data in Table (5) confirmed that biofly caused a significant reducible effect on egg deposition (79.29 eggs) comparable to the control (105.25 eggs). Dipel have the lowest effect on fecundity of adult females (88.73 eggs) and there were no significant differences between its effect on egg deposition. Dipel was found to be not significantly different from control treatments.

Table 5: Effect of microbial insecticides and tested generations on fecundity of *Culex pipiens* females.

Character	Microbial insecticides (A)				Generations (B)			Interaction AB
	Control	Biofly	Dipel	Sig.	F_1	F_5	Sig.	
Deposited egg	105.25 a	79.29 b	88.73 ab	**	98.25 a	83.93 b	*	N.S
Hatchability %	96.91 a	91.81 b	88.91 b	**	94.69 a	90.40 b	*	N.S

***,** and N.S indicate P<0.05, P<0.01 and not significant, respectively.**
In a row, means designated the same letter for each trait are not significantly different at 5% level according to DMRT (1955).

The tested generations proved to have a significant effect on egg-depositon process Table (5). Application of microbial insecticides for both generations F_1, F_5 resulted 98.25 and 83.93 eggs, respectively, with significant difference. The interaction between microbial insecticides and the number of tested generation were found to have no significant on egg deposition of *Culex pipiens.* Microbial insecticides were found to reduce egg-hatchability of *C. pipiens* and highly significant differences were recorded between their effects on egg-hatchability and control treatment. The lowest percentage of egg-hatchability resulted from exposing larvae of the fifth generation to microbial insecticides (90.40%). Hatchability indicated no

significant affectes by the interaction between microbial insecticides and the number of generation tested.

Chemical insecticides were found to be the most effective compounds on egg deposition (81.05 eggs) followed by microbial insecticides (91.11 eggs) with significant differences between them Table (6). No significant differences were recorded between chemical and microbial insecticides on egg-hatchability.

Table 6:Comparison between effects of chemical and microbial insecticides on fecundity of *Culex pipiens* females.

Character	Chemical insecticides	Microbial insecticides	Sig.
Deposited eggs	81.05 b	91.11 a	*
Hatchability %	90.16	89.55	N.S

***and N.S indicate P<0.05 and not significant, respectively.**
In a row, means designated the same letter for each trait are not significantly different at 5% level according to DMRT (1955).

The obtained results, concerning the fecundity of *C. pipiens*, comes in agreement with those obtained by El-Khodary *et al*. (1983) and Bayoumi *et al.* (1988) who indicated that methomyl reduced the number of produced eggs of *C. pipiens*. Similar results were obtained by Aguilera *et al*. (1995) on *C. quinquefasciatus* that indicated that the treated larvae with malathion egg production was reduced by 57% and 59% using LC_{30} and LC_{70}, respectively. They also indicated that egg production was slightly reduced by chlorpyriphos. However, the problem of insect resistance to insecticide applictions was investigated by Yebakima *et al.* (1995) stating that larval population of *C. pipiens* exhibited resistance between 6.4 and 51.4 against chloropyriphos.

Concerning microbial insecticides, Schnetter *et al*. (1983) indicated that larval susceptibility decreased between the 1st and 4th instar when larvae of *C. pipiens, Aedes vexans, A. sticticus, A. contans* and *A. rusticus* were treated with *B. thuringiensis* var. *israelensis*. Saleh *et al*. (1990a) found that larval instars, mosquito species, exposure time and temperature significantly influenced larvicidal activity of *B. thuringiensis* (H-14). As a conclusion, microbial insecticide, proved to be highly efficient against *Culex pipiens*. The continuous exposure of *C. pipiens* to the considered microbial insecticides resulted in high larval mortality, and reduced both pupation and adult emergence percentages.

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التأثير المتأخر لبعض المبيدات الكيماوية والحيوية على يرقات البعوض المنزلى *Culex pipiens* (L.) complex. تحت الظروف المعملية

أحمد سعد الدين الخضرى، فايز على أبوعطية، شوقى محمد ابراهيم متولى، هانى محمد حسن
قسم الحشرات الإقتصادية – كلية الزراعة بكفرالشيخ – جامعة طنطا

أجرى هذا البحث لدراسة التأثير المتأخر لبعض المبيدات الكيماوية (الكلوربيروفوس، الميثوميل) والمبيدات الميكروبية (البيوفلاى، الديبل) على ثلاثة اجيال مختبرة (الأول – الثالث – الخامس) وفترتان لتعريض اليرقات للمبيدات الكيماوية هما مدة 24 ساعة أو التعريض المستمر حتى التعذير، أما المبيدات الميكروبية فكانت فترة التعريض هى 48 ساعة أو التعريض المستمر على بعض الصفات البيولوجية للبعوضة المنزلية وذلك بمعاملة العمر اليرقى الثالث بالتركيزات غير المميته للمبيدات المختبرة (التركيز القاتل لـ 10% من الأفراد المعاملة).

وتم الحصول على النتائج التالية :

- أظهر التحليل الإحصائى أن مبيد الكلوربيروفوس كان أكثر المركبات سمية ضد الطور اليرقى للبعوضة المنزلية ويلية الميثوميل وظهرت أختلافات عالية المعنوية بينهما عند المقارنة بالكنترول. وكان التفاعل بين تأثير المبيدات الكيماوية وكل من الأجيال المختبرة (الأول – الثالث – الخامس) وفترة التعريض (24 ساعة – التعريض المستمر) عالى المعنوية على الموت اليرقى والتعذير. بينما لا توجد فروق معنوية بين كل من المبيدات الكيماوية والأجيال المختبرة وزمن التعريض فى التأثير على موت طور العذراء وخروج الحشرات الكاملة.
- وفيما يخص المبيدات الميكروبية، وجدت فروق عالية المعنوية بين متوسطات الموت اليرقى، موت العذارى، التعذير، خروج الحشرات الكاملة واستخدام المبيد البكتيرى (ديبل) والمبيد الفطرى (بيوفلاى) عند مقارنتها بالكنترول. كما أظهر التحليل تأثيراً عالى المعنوية للأجيال المختبرة (الأول – الثالث – الخامس) على الموت اليرقى والتعذير، بينما لم يظهر تأثير معنوى لهذه الأجيال على موت العذارى وخروج الحشرات الكاملة. أيضاً وجد تأثير عالى المعنوية لزمن التعريض على جميع الصفات البيولوجية المذكورة سابقاً.
- أوضح التحليل المقارن بين المبيدات الكيماوية والميكروبية أن المبيدات الميكروبية أكثر فعالية (سمية) ضد البعوضة المنزلية خاصة عند المعاملة المستمرة بالمبيد الحيوى.
- أظهرت النتائج انخفاض متوسط عدد البيض الموضوع لكل أنثى، نسبة فقس البيض معنوياً عند المعاملة بكل من الكلوربيروفوس، الميثوميل بالمقارنة بالكنترول، ولم تسجل فروق معنوية بين تأثير كل منهما على السلوك المدروس، كما لم يلاحظ وجود إختلافات معنوية بين الأجيال المختبرة على هذه الصفات.
- المبيدين الميكروبين: البيوفلاى، الديبل أديا الى انخفاض متوسط عدد البيض الموضوع ونسبة الفقس كما لوحظت فروق معنوية بينهما بالمقارنة بالكنترول. كما وجدت فروق عالية المعنوية بين الأجيال المختبرة (الأول – الخامس) وكل من وضع البيض ونسبة فقس البيض.
- أظهر التفاعل بين المبيدات الكيماوية والميكروبية أن المركبات الكيماوية لها تأثيراً معنويا فى خفض عدد البيض الموضوع لإناث البعوضة المنزلية، بينما أوضح التحليل المقارن عدم وجود اختلافات معنوية بين المبيدات الكيماوية والحيوية على نسبة فقس البيض للبعوضة المنزلية.
- ويوصى الباحثون باستخدام المبيدات الميكروبية التى أثبتت فعالية عالية ضد البعوضة المنزلية، حيث أدى التعريض المستمر الى زيادة نسبة موت يرقات الحشرة، وانخفاض كل من نسب التعذير وخروج الحشرات الكاملة.

Table 1. Effect of chemical insecticides, tested generations and exposure time on some biological aspects of *Culex pipiens* mosquito under laboratory conditions (26±2°C and 75% R.H.).

CHARACTER	Chemical insecticides (A)				Generations (B)				Exposure time (C)			Interaction			
	Control	Chloro-pyriphos	Methomyl	Sig.	F_1	F_3	F_5	Sig.	24 hours	Continuous	Sig.	AB	AC	BC	ABC
Larval mortality (%)	5.22 c	28.51 a	24.21 b	**	15.8b	18.61a	23.52a	**	16.17 b	22.45 a	**	**	**	N.S	N.S
Pupal mortality (%)	2.22 b	12.03 a	9.97 a	**	7.52	7.77	8.92	N.S	7.09	9.06	N.S	N.S	N.S	N.S	N.S
Pupation (%)	94.78 a	67.67 c	71.78 b	**	81.39a	78.17b	74.67b	**	81.04 a	75.11 b	**	**	**	N.S	N.S
Adult emergence (%)	97.77 a	85.98 b	88.00 b	**	91.20	90.52	90.03	N.S	91.55	89.61	N.S	N.S	N.S	N.S	N.S

**** and N.S indicate P<0.01 and not significant, respectively.**
In a row, means designated the same letter for each trait are not significantly different at 5% level according to DMRT (1955).

Table 2. Effect of microbial insecticides, tested generations and exposure time on some biological aspects of *Culex pipiens* mosquito under laboratory conditions (26±2°C and 75% R.H.).

CHARACTER	Microbial insecticides (A)				Generations (B)				Exposure time (C)			Interaction			
	Control	Biofly	Dipel	Sig.	F_1	F_3	F_5	Sig.	48 hours	Continuous	Sig.	AB	AC	BC	ABC
Larval mortality (%)	5.22 c	68.78 b	69.82 a	**	44.67 b	47.74ab	51.41a	*	36.37b	59.51a	**	**	**	N.S	N.S
Pupal mortality (%)	2.22 b	20.47 a	4.98 b	**	7.44	9.91	10.32	N.S	10.59a	7.86b	**	N.S	**	N.S	N.S
Pupation (%)	94.78 a	29.50 b	28.56 c	**	54.06 a	50.89ab	47.89b	*	61.89a	40.00b	**	**	**	N.S	N.S
Adult emergence (%)	97.78 a	77.75 b	44.01 c	**	74.97	72.25	72.31	N.S	88.14a	58.22b	**	N.S	**	N.S	N.S

***.** and N.S indicate P<0.05, P<0.01 and not significant, respectively.**
In a row, means designated the same letter for each trait are not significantly different at 5% level according to DMRT (1955).