Influence of five different larval control agents on oviposition of *Culex pipiens* L. (Diptera: Culicidae)

Muhammet Mustafa Akiner[1], Elcin Eksi[2]

[1]RTE University, Faculty of Arts and Sciences, Department of Biology, 53100 Fener, Rize, Turkey
[2]Department of Food Engineering, Graduate School of Science and Engineering, Hacettepe University, Ankara, Turkey

Corresponding author: akiner.m@gmail.com

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Abstract: The effects of five different and widely used mosquito control agents (monomolecular film [MMF], diflubenzuron, *Bacillus thuringiensis* var. *israelensis* [Bti], *Bacillus sphaericus* [Bs] and temephos) on *Culex pipiens* L. oviposition were studied in both field and laboratory conditions. Half of the standard application dosages were used for all five control agents. Results were evaluated weekly for three weeks. All tested mosquito populations (Serik, Avariz, Slab and field) showed different degrees of oviposition preferences on experimental and control cups and pools. *Culex pipiens* L. tended to lay a lower number of eggs for tests groups than the control group throughout the whole test duration. Oviposition preferences of all *Cx. pipiens* decreased in the second week (except temephos both Avariz and field test, MMF both control and field test, *Bs* both Serik and control) and increased again in the third week. The repellent effects of MMF and temephos on oviposition were found to be higher than for the other larvicides. Mosquito species use different chemical cues for finding an ovipositional site. Therefore MMF and temephos may help in future control efforts, albeit less than the diflubenzuron, *Bti* and *Bs* on mosquitoes and mosquito-borne disease epidemics. ***Journal of the European Mosquito Control Association*** **33: 5-9, 2015**

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Introduction

Many chemical compounds may serve as oviposition attractants or repellents for mosquitoes even when present in small quantities. Gravid female mosquitoes use a combination of physical cues (colour, temperature, vegetation) and chemical cues (acetylated monohydroxy and dihydroxy fatty acids, 1,3-diglycerides) to locate and identify suitable oviposition sites (Bentley & Day, 1989). Therefore knowledge of female preferences may help mosquito population studies and the choice of adapted control applications. Many larvicidal compounds are used for mosquito control and there has been little research on the effect of these compounds on the oviposition behaviour of gravid mosquitoes.

Mather and De Foliart (1983) reported that oviposition traps containing a very high concentration of EC formulations of chlorpyrifos were repellent to *Aedes triseriatus* (Say) ovipositing females, but granular formulations of chlorpyriphos and temephos did not exhibit any repellency. It was shown that malathion and temephos at relatively high concentrations were repellent to *Aedes aegypti* (L.) in oviposition traps (Moore, 1977). Verma (1986) reported that cypermethrin, fenvalarate, decamethrin and permethrin affect the oviposition patterns by showing repellent effect of *Anopheles stephensi* Liston, *Ae. aegypti* and *Culex quinquefasciatus* Say, and they showed repellent effects on oviposition at LC99 concentration. It was shown that oviposition traps treated with pellets containing the insect growth regulator (IGR) methoprene were more attractive to ovipositing *Ae. aegypti* in field conditions than traps that contained only tap water (Carroll, 1979). Different commercial products of *Bacillus thuringiensis* var *israelensis* (*Bti*) and *Bacillus sphaericus* (*Bs*) have been formulated against mosquito larvae since their biocidal properties were discovered. These two microbial agents, in particular *Bti*, have been used extensively as larvicides since the early 1990s. Zahiri & Mulla (2005) demonstrated the effects of *Bti* and *Bs* for *Cx. quinquefasciatus* oviposition. They noted that *Bti* suspensions received a lower number of egg rafts than that of the control. It was further noted that there was an inverse relationship between the number of egg rafts and concentrations of *Bti*. Similar extra larvicidal effects have been noted in neem products that affect the oviposition of mosquitoes (Su & Mulla, 1998). Suman et al. (2013) indicated that insect growth regulators showed ovicidal effect on *Aedes* and *Culex* mosquitoes. They also noted that freshly laid *Cx. pipiens* L. eggs were susceptible to diflubenzuron application

Culex pipiens is one of the most important arthropod-borne disease vectors and transmits different disease agents such as West Nile virus, Rift Valley Fever virus, and Bancroftian flariasis worms (Jinfu, 1999; Paul *et al.*, 2005; Balenghien *et al.*, 2008). *Culex pipiens* is distributed in the temperate Palearctic and north Nearctic areas, where many mosquito control programmes have been conducted against this species. Mosquito control programmes exist in Turkey, generally conducted by municipalities, the Ministry of Health and associations of the tourism investors in the coastal plain. Control operations rely on different larvicides and adulticides. Although biopesticides derived from bacterial toxins and some synthetic agents (e.g. insect growth regulator, monomolecular film) have been used in many areas as a larvicide, the usage of pyrethroid and organophosporate formulations for larval control was banned in 2009 by the Turkish Ministry of Health.

Our study aimed to determine the oviposition responses of two different *Cx. pipiens* populations in laboratory and field conditions to commercial formulations of monomolecular film, diflubenzuron, temephos, *Bti* and *Bs*.

Materials and Methods

Mosquito populations

Two different colonised populations of *Cx. pipiens* biotype pipiens were used for tests. The egg rafts and adult females of these populations were collected from Avariz village (Thrace region) and Serik town (Mediterranean region) in Turkey, while a *Cx. quinquefasciatus* Slab strain was used as a control.

Insecticides

All larvicides (*Bti*: VectoBac® 12 AS; *Bs*: VectoLex® WDG; temephos: Abate® 500 EC; monomolecular film: Aqnique MMF®; diflubenzuron: Dudim®) were obtained from KUYAB (Kundu Tourism Investors Association, Antalya).

Laboratory experiments

Tests were conducted in 40cm x40cm x40cm cages housed in a photoperiod (LD 14:10) and temperature (27 ± 20°C) controlled insectary. Since many researchers reported that the high dose application showed repellent effects, we chose half of the standard recommended (label) application dosages to test low dose effects on oviposition. Five cups (6 cm diameter and 4 cm depth) containing larvicides and one control cup containing 70 ml distilled water were placed in the cages. Each bioassay was performed by using two replicates for each mosquito strain and tests were replicated over the two successive months. Twenty-five gravid (4-5 days post bloodmeal) females were placed in each test cage. The number of egg rafts and the number of eggs in each cup were counted daily throughout 21 days.

Field experiment

Experiments to assess the response of gravid female mosquitoes to insecticide treated water were conducted in a 2x2 m semi-artificial pool prepared by digging in an open field. The semi-artificial pool was filled with tap water four days before the tests. All larvicides were directly applied with a hand sprayer one day before the test. The *Culex* sp. egg rafts were counted daily during the 21-day period. Field experiments were replicated in the two successive months. Egg rafts were identified as *Cx. pipiens* after hatchling in the laboratory and tested for autogeny to attribute to either biotype *pipiens* or *molestus*. Twenty adults (3 day old) were randomly chosen from all cohorts for testing autogeny and kept in new cages. Adults had access to 10% sucrose solution from a cotton wick and did not ensure a blood feed for first cohort; second cohort could feed on blood. Containers filled with deionized water were placed inside the cages for oviposition.

All data collected were summed and analysed weekly. A chi square test was used to statistically characterise the test results (Statistica® 8.0). An oviposition activity indexs (OAI) was calculated by using the formula: OAI = (NTreatment − NControl)/(NTreatment + NControl) in which N is the number of egg rafts in each treatment. This index is only a coarse measurement of whether or not the females are influenced by a particular treatment. The OAI varies from -1 to 1 with 0 indicating no response (Kramer & Mulla, 1979).

Results

All first cohorts tested for autogenic behaviour without blood feeding did not lay eggs during the 20 days. Second cohorts laid eggs 4-5 days after a blood meal. Thus, due to the absence of autogeny, all strains were considered as belonging to *Cx. pipiens* biotype *pipiens*.

Detailed data on oviposition preferences for both laboratory and field tests are given in Table 1. Egg-laying in MMF- and temephos-treated cups was lower than in *Bti*-, *Bs*- and diflubenzuron-treated cups. All strains laid nearly the same amount of eggs except MMF treated cups, but showed significantly lower production levels than the control group ($p<0.05$). The eggs laid in all cups (both treated and control) decreased during the second week and increased during the third week. The MMF-treated cup showed fewer eggs for all weeks compared to the other treated and control cups.

Gravid females of the *Cx. pipiens pipiens* Avariz strain laid significantly fewer eggs in larvicide–treated cups than in the control during first week (Table 1; $p<0.05$). Gravid females tended to lay more eggs in distilled water than the water treated with larvicide in all weeks for Serik population ($p<0.05$). Females laid more eggs on *Bti*-treated water than in other treated cups during first week. The water treated with MMF and temephos was less preferred by gravid females which laid fewer (1.59 former and 4.80 latter per female) eggs than other treated water in the second week for Serik population.

Culex quinquefasciatus (control strain) tended to lay more eggs on distilled water than on larvicide-treated water (Table 1). Mean egg numbers laid on water samples containing *Bti* (n=31.83) and *Bs* (n=21.71) were higher than those on other larvicide–treated water in first week, but all treated cups produced a significantly lower number of eggs than the control group ($p<0.05$). While the second week results showed a decreasing trend for *Bti*, temephos and diflubenzuron-treated waters as well as distilled water only; *Bs* and MMF-treated waters displayed an increasing trend. Distilled water showed a higher number of eggs and also higher than those of the Avarız and Serik *Cx. pipiens* populations in the second week. All treated water showed more eggs during second week and nearly reached the same number for *Bti* (32.26) and *Bs* (21.76) samples during third week.

Oviposition patterns for field experiments were evaluated on the basis of numbers of egg rafts. The *Bti*-treated pool received more egg rafts than the other pools during the first week but also significantly less than the control group ($p<0.05$). During the second week, all test and control pools received fewer egg rafts than during the first week. During the third week, although MMF and temephos-treated pools yielded a higher number of egg rafts than during the first and second weeks, all other treated and control pools showed fewer egg rafts than during the first and second week (Table 1).

Oviposition response for all groups is shown as an oviposition activity index (OAI) (Table 2). All tested agents displayed repellent effects for the two *Cx. pipiens pipiens* populations in the laboratory and field. Although OAI varied and changed between populations and weeks, all tested agents showed repellent effects for *Cx. pipiens* oviposition. Repellent effect decreased gradually from the 1st week to 3rd week.

An additional adverse effect on the shape of egg rafts was noted. Egg rafts on *Bti* and *Bs* treated waters are generally shapeless and smaller than other treated waters (Figure 1).

Table 1: Oviposition response per female mosquito (mean number of eggs per female) of *Cx. pipiens*, strains Avarız and Serik and *Cx. quinquefasciatus* laboratory strain and open field experiment to water treated with five different larvicides, for 3 replicates

Strain	Time	Monomolecular Film (2l/ha)	Diflubenzuron (12.5g/ha)	Bti (250ml/ha)	Bs (100g/ha)	Temephos (125ml/ha)	Control
Cx. pipiens, Avarız	1st week	21	28.99	21.01	22.24	4.94	39.92
	2nd week	0.43	12.53	7.45	11.91	11.16	32.90
	3rd week	9.26	21.61	21.86	24.98	13.97	40.35
Cx. pipiens, Serik	1st week	5.38	14.38	30.88	9.72	9.19	49.80
	2nd week	1.59	8.94	10.90	10.53	4.80	40.95
	3rd week	9.18	17.33	19.94	19.68	12.47	38.38
Cx. quinquefasciatus, Control	1st week	0.94	17.05	31.83	21.71	9.56	66.25
	2nd week	3.72	6.62	10.05	32.94	7.78	41.79
	3rd week	6.02	14.30	32.26	21.76	11.7	46.80
Field Experiment[1] *Cx. pipiens*	1st week	2	11.5	17.5	12	5.5	26.5
	2nd week	4	10	13.5	8	6	24.5
	3rd week	6.5	9	9.5	7	8	17.5

Table 2: Oviposition activity indices for all tested *Cx. pipiens* populations and open field experiment

	Time	Monomolecular Film (2l/ha)	Diflubenzuron (12,5g/ha)	Bti (250ml/ha)	Bs (100g/ha)	Temephos (125ml/ha)
Avarız	1st week	-0.95	-0.15	-0.31	-0.28	-0.77
	2nd week	-0.97	-0.44	-0.63	-0.46	-0.49
	3rd week	-0.62	-0.3	-0.29	-0.23	-0.48
Serik	1st week	-0.8	-0.55	-0.23	-0.67	-0.68
	2nd week	-0.92	-0.64	-0.57	-0.59	-0.78
	3rd week	-0.61	-0.37	-0.31	-0.32	-0.5
Control	1st week	-0.97	-0.59	-0.35	-0.5	-0.74
	2nd week	-0.83	-0.72	-0.61	-0.11	-0.68
	3rd week	-0.77	-0.53	-0.18	-0.36	-0.6
Field experiment	1st week	-0.85	-0.39	-0.2	-0.37	-0.65
	2nd week	-0.71	-0.6	-0.28	-0.5	-0.6
	3rd week	-0.45	-0.32	-0.29	-0.42	-0.37

Discussion and conclusion

All tested agents addressed in this study have been used in many areas of Turkey for mosquito control operations. MMF showed the highest repellency for all tested control agents. Kramer & Mulla (1979) described an OAI that varied between 1 to -1, where -1 indicated highest repellency effect for oviposition. MMF potentially affects all life stages of mosquitoes and is used in many areas individually or in combination with other larvicides. Physical pesticide properties and the impossibility of resistance development may favour usage of this larvicide in many areas (Becker *et al.*, 2003). Because this insecticide has side effects on active animals on the water surface, however, one should be careful with the use of this insecticide. In this study, we found repellent effects in both laboratory and field experiments and these results are consistent with previous reports (Nayar & Ali, 2003; Stark, 2005).

Insect growth regulators are comparatively safer for nontarget organisms (Mulla, 1995) and have been recommended for mosquito control (WHO, 2006). Diflubenzuron is a chitin synthesis inhibitor and is used in many areas for the control of mosquitoes and other nuisance organisms (Suman *et al.*, 2013). Our results revealed repellent effects for the ovipositon of *Cx. pipiens* strains in both laboratory and field experiments. This effect was similar to *Bti*- and *Bs*–treated water for the Avarız strain. Field experiments showed similarity between diflubenzuron and *Bs* (Table 2). Our results indicate that half of the label dose negatively affects *Cx. pipiens* oviposition. Some researchers indicated that newly laid eggs are more sensitive to the insect growth regulators than embryo-developed eggs (Miura et al., 1976; Vasuki, 1990; Suman et al., 2013). Aforementioned studies showed ovicidal and repellent effects for diflubenzuron and indicated extra potential effects for mosquito control in the pre-adult phase. In addition to aforementioned studies, our results showed the repellent effects on the mosquito oviposition.

Bti and *Bs* have been widely-used as larvicides since their availability on the market. Some researchers have recorded a lower number of egg rafts for *Bti*- and *Bs*-treated water than for control, and have reported an inverse relationship with the number of egg rafts and *Bti* concentrations with *Cx. quinquefasciatus* (Zahiri and Mulla, 2005; 2006). We observed the same pattern for *Bti*- and *Bs*-treated water with all tested *Culex* spp. populations during all weeks. Although adults chose *Bti*- and *Bs*-treated waters less frequently than the control cups, *Bti*- and *Bs*-treated cups yielded a higher number of egg rafts than all other cups treated with other larvicides in this study. OAI for *Bti* ranges from -0.18 to -0.63 (Table 2), indicating a lower *Bti* repellent effect for *Cx. pipiens* than for the other larvicides tested. The *Bs* effects on *Cx. pipiens* oviposition ranged between -0.11 to -0.67 which represent the second lowest negative effects for oviposition compared to the other larvicides used in this study (Table 2). Zahiri & Mulla, (2005) reported a relationship between reduction of egg rafts and adult mortality on treated surfaces. Zahiri & Mulla (2006) demonstrated another adverse effect on the shape and size of egg rafts. Our study suggests similar effect of *Bti* and *Bs* suspended waters. In contrast, *Aedes* species tend to lay more eggs in *Bti*-treated traps than in the control (Santos *et al.*, 2003; Stoops, 2005). Barbosa *et al.* (2010) showed a similar situation and reported increasing oviposition activity for *Bti* treated oviposition traps and noted extra effects for skatole or infusion added cups for oviposition.

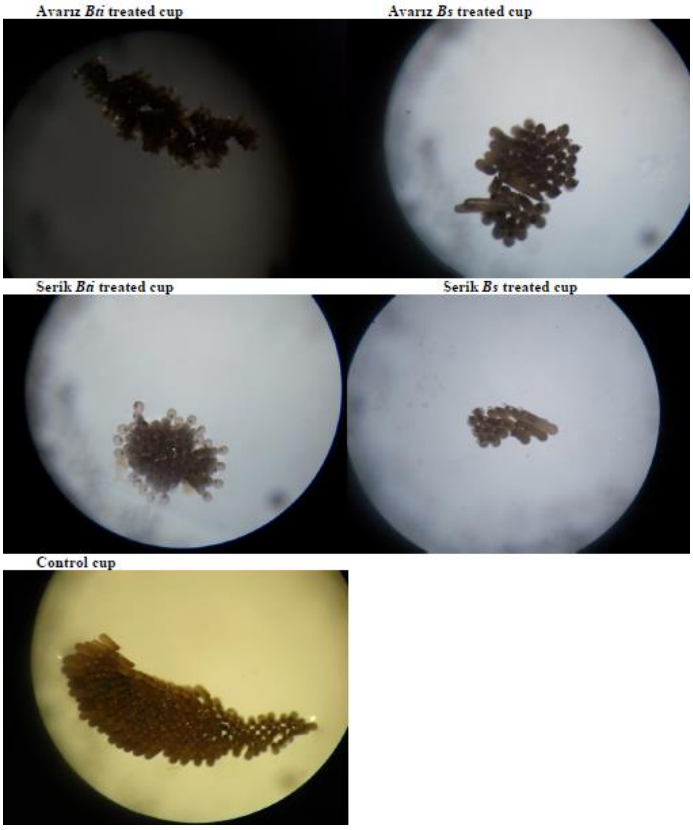

Figure 1: *Cx. pipiens* egg rafts in *Bti* and *Bs* treated cups and control cup

Temephos was widely used in Turkey until the ban in 2012 and resistance was reported in Turkey for some populations as in many areas in the world (Akiner *et al*., 2009; Grisales *et al*., 2013). Although resistance development reports have been documented in many areas, temephos is still preferred over mosquitocidal agents in many arthropod-borne disease endemic areas except in banned countries (e.g EU countries) (Grisales *et al*., 2013). In our experiments, temephos exhibits higher repellent effects than all other larvicides except MMF. OAI varied between -0.37 and -0.77. Higher repellent effects were generally found during the first week and lower repellent effects during third week (Table 2). Mather & De Foliarth (1983) reported an 80% reduction of oviposition in tree-hole oviposition sites for *Ae. triseriatus* after the application of Abate® (temephos) and Dursban® (chlorpyrifos-ethyl). Although our results indicate higher repellent effects for temephos, Ling *et al*. (2013) suggested that an attractant effects exist with an application of temephos for *Ae. aegypti* in Malaysia. Nazni *et al*. (2009) reported that oviposition rates for *Ae. albopictus* (Skuse) on temephos-treated ovitraps was lower than on *Bti*-treated ovitraps and control paddles. They also noted negative OAI for temephos-treated ovitraps during the six-week ovitrapping period. In contrast to this report, Canyon & Muller (2013) did not encounter any differences in ovipositional response between groups exposed to insecticide (malathion, temephos) presence and their control group.

In summary, all studied larvicides (MMF, diflubenzuron, *Bti*, *Bs* and temephos) showed repellent effects on *Cx. pipiens* oviposition. Different degrees of ovipositional response were found but all of them exhibited repellency for tested populations of *Cx. pipiens* and in the field. These larvicides have been used for a long time period in Turkey and the usage of temephos has been banned for at least two years. Best control practices in many areas have shifted to the use of environmentally friendly control agents e.g. *Bti*, and *Bs*. New studies have shown the additional effects on different stages of the mosquito's life cycle. Our results demonstrated some levels of repellency effects of MMF, diflubenzuron, *Bti*, *Bs* and temephos for *Cx. pipiens* population from Turkey, but many researchers have reported attractant effects and ineffectiveness of different agents (e.g. *Bti*, temephos, imidacloprid) on several *Aedes* species oviposition (Stoops 2004; Nazni *et al*. 2009; Antonio-Arreola *et al*. 2011). Mosquito species use different chemical cues for selecting an oviposition site. Therefore, more detailed studies on different control agents to investigate their effects on mosquito species in Turkey are needed. Extra advantages or disadvantages profiles may be helpful for selecting larvicides within future integrated mosquito control practices.

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