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Field efficacy and non-target effects of temephos granules against *Culex pipiens* (Diptera: Culicidae) and microorganisms in septic tanks, Republic of Korea

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Abstract

A granular formulation of 1.0% temephos (temephos G) was tested for its efficacy in mosquito larval control, residual effects and effects on non-target microorganisms. *Culex pipiens* complex in septic tanks in Yangsan, Republic of Korea were used for this study. Microorganisms in the same septic tanks were used for study of non-target organisms. After the application of temephos G at the rates of 2.0 and 5.0 mg/L to selected septic tanks, mortalities were recorded for 50 days. The effect of temephos G on non-target organisms was studied by evaluating its effects on the bacterial populations in the treated septic tanks. It was observed that using 1.0% temephos G at a rate of 2.0 mg/L resulted in 100% mortality against *Cx. pipiens* larvae by the eighth day after treatment. The residual activity of temephos G was observed to continue for a time; for example 29 days after treatment, average larval mortality rates of >68.0% were observed for both concentrations. At the 50th day after treatment, the mortality rates of the *Cx. pipiens* larvae were observed to decrease to below 42.7% for both concentrations. Application of temephos G did not have significant effects on the bacteria populations in the septic tanks. Since temephos did not seem to have any major effects on the microorganisms, it was concluded that it is a safe and effective larvicide to use in the septic tanks at a rate of 2.0–5.0 mg/L of 1.0% temephos G.

Key words: *Culex pipiens*, Korea, microorganisms, septic tank, temephos.

Introduction

Culex pipiens complex is recognized as a dominant pest mosquito in urban areas in the Republic of Korea (ROK). This mosquito group of the ROK mostly consists of *Cx. pipiens pallens* Coquillett (Sohn 2005) and *Cx. pipiens molestus* Forskal was reported in Seoul in the middle of the Korean peninsula (Shim *et al*. 1989). However, Sohn (1996) reported that *Cx. pipiens molestus* was the dominant species with 92.5% of *Cx. pipiens* complex in underground structures. *Culex pipiens molestus* has been reported almost everywhere in the country during all seasons in urban areas including Busan located in the southern area of the peninsula (Lee & Lee 1992). *Culex pipiens* complex breed in pools, sewerage systems within the sewer water, marshes and ponds.

Most mosquito control in the ROK had been to use adulticides, and effective and high-level control was not achieved (Lee *et al*. 1997). The use of larvicides augmented the use of adulticides to achieve better results. Early studies on the use of larvicides were conducted on stagnant pit latrines (Maxwell *et al*. 1990). *Culex quinquefasciatus* Say and

Aedes albopictus (Skuse) were reported as successful breeders in domestic septic tanks (Lam 1989). Chang (1993) reported that *Ae. albopictus* was breeding in over 38% of the septic tanks in Kuching, Sarawak, Indonesia, while the infestation rate of *Cx. pipiens* complex was an average of 11.6% of septic tanks in four urban residential areas in the ROK (Lee 2007).

Studies conducted later found that *Cx. pipiens* complex was successfully breeding in septic tanks and a posed possible disease transmission threat and a biting nuisance to humans in the ROK in 2002 (Lee 2006). Yangsan near Busan, an urban residential area, was chosen to represent areas that face increased mosquito populations during the hot summer months in the southeastern areas of the Korean peninsula. *Culex pipiens*, a common domestic mosquito, has been linked with transmission of West Nile Virus in Europe and the USA. *Culex pipiens* is also linked with the mechanical transmission of the virus that causes the Rift Valley Fever in the Middle East and Africa (Rozendaal 1997).

Mosquitoes need water to breed and the septic tanks in residential areas provide suitable breeding sites while the humans and animals provide possible blood meals for the females. Control of the mosquito larvae in the septic tanks before they emerge to adults may be an effective way of controlling the mosquito population and minimizing risks of disease transmission to the human population (Curtis 1989). Temephos is an organophosphate pesticide that acts by inhibiting the activity of cholinesterase enzyme and is toxic to insects and certain other non-target invertebrates (Smith 1987; Brown *et al*. 1996). It is used only in public health applications (World Health Organization 1988) and controls the larvae of mosquitoes (Brown & Orloski 1966; Moore & Breeland 1967; Rettich 1979; Yap *et al*. 1982). It is also used to control the larvae of midge (Tabaru 1975; Yasuno *et al*. 1982). Temephos has also been applied in the control of moths and sandflies (Tomlin 2000) and is used extensively in West Africa to control black flies, the vector for onchocerciasis (Opong-Mensa 1984). Temephos has the advantage of having very low toxicity to mammals, birds and aquatic organisms, and of being less persistent in the environment (Opong-Mensa 1984). It has been used to control *Ae. aegypti* larvae in drinking water in Africa, as it neither accumulates in the treated water nor is it harmful to humans (Laws *et al*. 1967, 1968). The World Health Organization authorized the use of temephos in drinking water at a dose of 1.0 mg/L (World Health Organization 1973).

Sludge microorganisms are essential to degrade organic matter in septic tanks. However, little information is available on the effects of survival and propagation of the non-target microorganisms in septic tanks when temephos is used for mosquito control. In the present study, temephos as granules (temephos G) was tested for its effects on mortality in larval mosquitoes, survival of non-target microorganisms and its residual effect. The data obtained may be useful to determine the mortalities of *Cx. pipiens* complex and microorganisms when using temephos G in septic tanks for mosquito control.

Materials and methods

Field efficacy of temephos G

Field assessments were conducted from June to August 2006. Nine septic tanks were randomly selected in the Yangsan area for use in the tests. The tanks varied in size and depth, and were divided into two chambers. The second (outlet) chambers were used for the tests because mosquito larvae bred only in the second chambers Lee (2006). The volume of wastewater in the second chamber was 0.4–8.0 m^3. The nine septic tanks were randomly divided into one group of six and one group of three tanks. The group of six tanks was used as the treatment group while the group of three tanks was used as the control (non-treatment) group. The group of six septic tanks was then randomly divided in two groups of three septic tanks each. One group of three septic tanks was treated with the 1.0% temephos G (Abate 1SG; Pharmcle, Seoul, Korea) at a rate of 2.0 mg active ingredient (AI) per liter while the second group of three septic tanks was treated with the 5.0 mg AI/L rate of 1.0% temephos G. Each application rate was mixed with tap water in a hand compression sprayer and then applied. The treatment was applied once during the whole test period.

The tests were observed for the residual effects of the temephos G for 50 days using the domestic mosquitoes *Cx. pipiens* complex collected in the septic tanks. Before the application of the temephos, mosquito surveys were conducted in both treatment and control groups of septic tanks to confirm the presence of the *Cx. pipiens* complex larvae. Also, the sizes of the septic tanks and water depths were measured using a ruler to determine the water volume in the septic tanks.

Plastic larval cages were made and used inside the septic tanks to carry out the tests. The cages were 14 cm (length) × 14 cm (width) × 9 cm (height) with openings measuring 6 cm × 5 cm on the sides and 8 cm × 8 cm at the bottom. The top of the cage was covered with a tightly fitting lid to contain any emerging adults. The openings were covered with size 12 mesh netting which could allow water to pass in and out of the cage without allowing the larvae or pupae to escape. *Culex pipiens* complex larvae were collected from each septic tank for the test. Twenty-five 3rd stage larvae of *Cx. pipiens* complex were put in each plastic cage and placed in the septic tanks one day before treatment and at 1, 2, 3, 8, 15, 22, 29, 36, 43 and 50 days post-treatment. Mortality was recorded at the designated days as the number

Table 1 Analysis of dissolved oxygen, turbidity and pH of water in septic tanks treated with 1.0% temephos G at rates of 2.0 and 5.0 mg/L and control septic tanks

	Mean ± SD				
Group	DO (mg/L)	Turbidity (NTU)	pH	Temp (°C)	Salinity (%)
n	12	30	8	8	8
Control	2.48 ± 0.10b†	12.39 ± 1.17b	7.94 ± 0.19a	24.0 ± 0.8a	0.05 ± 0.03a
Treatment 2.0	2.45 ± 0.13b	12.86 ± 0.56b	7.56 ± 0.22a	23.5 ± 1.5a	0.06 ± 0.04a
Treatment 5.0	2.66 ± 0.03a	15.25 ± 1.13a	7.61 ± 0.13a	24.4 ± 1.1a	0.03 ± 0.02a

†Means in the same columns followed by the same letter are not significantly different ($P = 0.05$; Duncan's multiple range test).
DO, dissolved oxygen; *n*, number of tanks; NTU, nepthelometric turbidity unit.

of dead larvae per cage. At the time of testing, mean water temperature of three groups ranged from 23.5 to 24.4°C in each group, whereas pH and salinity ranged 7.56–7.94 and 0.03–0.06%, respectively (Table 1).

The percentage mortality was calculated as the number of dead larvae at 24 h after being introduced into the cage in the septic tanks divided by the total and multiplied by 100. The mortality data were collected 10 times during the 50 days with the cage contents being changed 24 h before the designated dates. Percent mortality values of the larvicide against *Cx. pipiens* complex larvae were subjected to a log $x + 1$ transformation. The data were then analyzed using Duncan's multiple range test and a Student's *t*-test at $P = 0.05$ by SPSS software v12 for Windows (SPSS, Chicago, IL, USA) to determine the differences among the groups. Each treatment and control was replicated three times. This method was similar to that used by Fortin *et al.* (1987), Steelman *et al.* (1967) and Beehler *et al.* (1991) except that in those three cases researchers carried water from the treatment sites to the laboratory for tests and analysis while in the present study the larvae were observed in the cages at the sites.

Effects of temephos granules on aerobic and anaerobic bacteria

The effects of temephos on sludge microorganisms (bacteria) were evaluated in terms of decrease or increase of aerobic and anaerobic bacteria in the control (untreated) and treated septic tanks using a total cell count method. The samples of septic water for bacteria counts were collected on days 0, 1, 3 and 6 after the introduction of 1.0% temephos G (Abate 1SG, Pharmcle) at a rate of 2.0 mg AI/L while the second group of three septic tanks was treated with the 5.0 mg AI/L rate of the temephos G. The treatment was applied only once during the whole test period. The water samples on day 0 were collected before treatment in all septic tanks in control and treated groups to confirm the presence and number of the bacteria.

The samples were collected using sterilized stainless steel ladles and placed aseptically in to Whirl-Pak (Nasco Co., Fort Atkinson, WI, USA) containers and then put into an icebox and transported to the laboratory for microorganism analysis tests. Each 1 mL of the samples was put into 99 mL of sterilized dilution peptone water. The diluted samples were plated into Petri dishes and standard plate count agar (Difco, Sparks, MD, USA) was added and maintained at about 43–45°C. Duplicate plates were made. Solidified Petri dishes were turned over and held in an incubator at 35 ± 1°C for 48 h. Colonies formed after incubation were counted as an aerobe total cell count.

Total cell count of the anaerobic bacteria was carried out using methods similar to those for aerobic bacteria but the incubation of plates was carried out in an anaerobic device, the GENbox Jar (BioMerieux Co., Marcy l'Etoile, France). Anaerogen (Oxoid Co., Basingstoke, UK), which removes oxygen from the air, was set in the jar and an anaerobic indicator (Oxoid), which confirms the removal of oxygen from the system, was added to the jar. The jar was hermetically sealed and the GENbox placed into an incubator at 35 ± 1°C for 48 h. Three replicates were made.

The physical and the chemical characteristics of the septic tanks were suspected as having possible effects on the tests. The analytical methods given here were based on those given in field methods for physiochemical factors of septic water (Brower & Zar 1977). During the period of testing, a reading for each of the water temperature, salinity, dissolved oxygen (DO), pH and turbidity were measured for each septic tank including those in the control group at 14:00 hours on the designated dates for the 50 days of the test. The salinity and temperature of water in the septic tanks were measured on the designated test dates using a portable temperature and salinity meter (TM-30D, Takemura Elec., Tokyo, Japan). The DO, pH and turbidity of the septic water were measured using a portable DO meter (DKK-Toa Corp., Tokyo, Japan), pH meter (TS-1, Suntex, Taipei, Taiwan) and a turbidimeter (LaMotte Co., Chestertown, MD, USA). These physiochemical properties of the septic tanks were

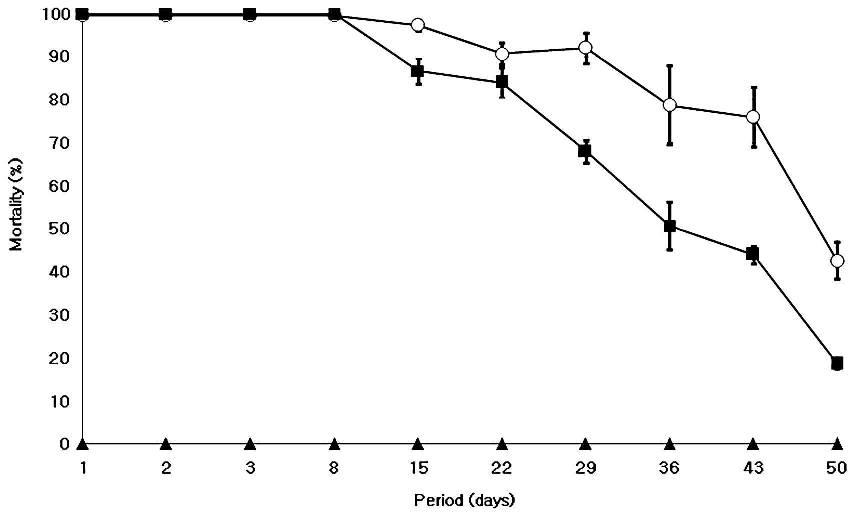

Figure 1 Average percent mortality (± SD) of *Culex pipiens* complex in septic tanks by treated with 1.0% temephos granules; 3 replicates. 2 ppm; 5 ppm; Control.

analyzed and compared using a Duncan's multiple range test to find out if they influenced the activity of temephos G or its residual effects.

Results

In both 2.0-mg AI/L and 5.0-mg AI/L applications, 1.0% temephos G was observed to provide both initial and residual activities in the control of *Cx. pipiens* complex larvae in the septic tanks. The results of the analysis of the physiochemical factors of the water in the septic tanks are shown Table 1. The amount of salinity, pH and water temperature did not differ significantly between control and treatment septic tanks except for the DO and turbidity in the 5.0-mg/L treatment septic tanks.

The results for daily mortality of *Cx. pipiens* complex in the septic tanks are shown in Figure 1. Treatment of water in the septic tanks using 1.0% temephos G at 2.0- and 5.0-mg/L rates resulted in 100% larval mortality for up to 8 days after treatment. The larval mortality on the 15th day after treatment was still high at 97.3% for 2.0 mg/L and 86.7% for the 5.0-mg/L treatments. Although the mortality levels dropped over time, there was continuous indication of the residual killing effect of the temephos granules even at 50 days. At day 50 after treatment, the mortality shows a sharp decrease (42.7% for 2.0 mg/L, 18.7% for 5.0 mg/L), indicating either a need for repeat treatment or the dilution effect of increase volumes of sewage water in the system. There was no significant difference between the mortality rates of the two temephos concentrations in each test period until the end of the study. The results suggest the dosage for successful control of *Cx. pipiens* complex larvae in septic tanks from dwellings. Generally, the application rates of both 2.0 mg AI/L and 5.0 mg AI/L of 1.0% temephos G showed high mortalities (>80%) for at least 22 days, compared with control tanks.

Effects of temephos granules on aerobic and anaerobic bacteria

The means of the data on the total bacteria cell count were compared with each other group and the results are shown in Table 2. The results show no significant difference between the population of the bacteria in the control septic tanks and two treatment groups, which indicates that 1.0% temephos granules did not significantly affect the bacteria population in the waters of the septic tanks. In some of the test sites, the population of bacteria seemed to increase at the higher concentration (5.0 mg/L) of temephos, a further proof that temephos did not have negative effects on the population size of the bacteria in the sewage water.

Discussion

The results show that the larvicidal effect of temephos at 1.0% persisted for up to 50 days with at least 22 days of effective larval control. The persistence of temephos G in an aquatic environment is affected by the rate of degradation due to hydrolysis, sunlight and microbial effects, evaporation, dissolution, dispersion and adsorption to suspended particles, sediments and other surfaces (Forgash 1976; Opong-Mensa 1984). It was important therefore to understand the environmental factors under which temephos is to be used. Factors such as fluctuations and disruption of sewage water levels may affect the surface cover by the larvicide, hence influencing the results of treatment (Lam 1989; Cetin *et al*. 2006). In this study, the continuous addition of sewage water from flushing of toilets in the residential buildings could have had an effect on the temephos although this did not show significant effects on the larvicidal effects or the physiochemical characteristics of the water in the septic tanks. The characters of physiochemical factors did not differ significantly among septic tanks except

Table 2 Average number of aerobes and anaerobes in the water of septic tanks treated with 1.0% temephos G

Concentration (mg/L)	Type	Day after treatment (cfu/mL)				
		0	1	3	6	Mean
Control	Aerobes	4.2×10^5a[†]	2.3×10^6a	1.5×10^6a	1.2×10^6a	1.4×10^6a
	Anaerobes	3.8×10^4a	4.1×10^4a	1.8×10^4a	2.8×10^4a	3.1×10^4a
2.0	Aerobes	1.1×10^5a	9.0×10^4a	9.1×10^4a	8.6×10^5a	2.9×10^5a
	Anaerobes	8.1×10^4a	1.7×10^5a	1.1×10^5a	2.3×10^5a	1.5×10^5a
5.0	Aerobes	1.5×10^5a	4.6×10^5a	5.0×10^5a	1.6×10^6a	6.8×10^5a
	Anaerobes	1.7×10^5a	2.2×10^6a	7.7×10^5a	7.3×10^5a	9.6×10^5a

[†]Means in the same rows followed by the same letter are not significantly different ($P = 0.05$; Duncan's multiple range test).
cfu, colony-forming unit.

for the DO and turbidity in the 5.0-mg/L treatment septic tanks. These could have been caused by other environmental factors and not the temephos. The volume of sewage water inflow into each septic tank was different and this could influence those physical and chemical characters of the water in the tanks. It might be the reason why the larval mortalities of *Cx. pipiens* seemed to be lower at higher concentration (5.0 mg/L) of temephos. There was no evidence that the DO and turbidity affects the abundance or the control of mosquito larvae in the septic tanks.

For long-lasting residual effects and activity by the larvicide, a stable water level is necessary and overflowing should be discouraged as it seems to carry the larvicide away (Curtis 1989). Temephos could work well if included in an integrated mosquito control program as mosquitoes must have water to breed (Rozendaal 1997). Temephos is an active larvicide against larvae of mosquitoes and other aquatic insects but has very low toxicity on fish, mammals and humans (Rozendaal 1997). These results agree with the supposition that temephos has low toxicity to non-target organisms. It was also shown to be effective at low dosage, making temephos appropriate in many situations such as in West Africa where it is effectively used for the control of black fly larvae (Rozendaal 1997) in water used for domestic purpose. At the studies on the residual toxicity of four insecticides, including temephos, to *Aedes triseriatus* (Say) in scrap tires, Beehler *et al*. (1991) demonstrated that temephos provided 14 months of 100% mortality for larval control with a drop to 21% mortality after 21 months. Using studies on relative toxicity by selected larvicides to bacteria populations, Steelman *et al*. (1967) demonstrated that temephos caused the least death to bacteria compared to Dursban, phoshorothioate, naled and fenthion. The results of this study agree with Steelman *et al*.'s (1967) findings, hence supporting the use of 1.0% temephos G in septic tanks to control *Cx. pipiens* complex. At some of the test sites, the population of bacteria seemed to increase at the higher concentration (5.0 mg/L) of temephos, as Steelman *et al*. (1967) suggested this could be as a result of the daily addition of new sewage and bathroom sludge water. The results of this study confirmed that it is safe to use 1.0% temephos G as a larvicide in sewage systems including septic tanks without compromising the decomposition function of the bacteria.

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