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LABORATORY EVALUATIONS OF METHYLATED SOY OIL AND MONOTERPENES AS MOSQUITO LARVICIDES

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ABSTRACT. The larvicidal toxicities of methylated soy oil (MSO) and surfactant combinations were compared to 2 commercially available oil larvicides (Golden Bear Oil 1111® and Bonide®) in standard laboratory bioassays of 4th-stage larvae of *Culex pipiens* Linn. The dose lethal to 50% of the test organisms (LD_{50}) and the dose lethal to 95% of the test organisms (LD_{95}) values are presented as microliters (µl) per beaker (treatment surface area of 54 cm^2). The 2 surfactant–MSO mixtures differed significantly in their toxicity to *Cx. pipiens* larvae; 2% Pyroter CPI-40® in MSO was more toxic than 2% Pluronic L121® in MSO (LD_{50} = 3.8 µl per 54 cm^2 and 11.3 µl per 54 cm^2, respectively). The 2 most active larvicides were Golden Bear Oil (LD_{50} = 3.6 µl per 54 cm^2) and the 2% Pyroter–MSO mixture. These 2 were not significantly different from each other. Bonide (LD_{50} = 6.2 µl per 54 cm^2) and the Pluronic L121–MSO mixture (LD_{50} = 11.3 µl per 54 cm^2) were less toxic than Golden Bear Oil and the MSO–Pyroter mixture and they were significantly different from each other. Bioassays with 4th-stage larvae of *Anopheles stephensi* Liston showed that toxicity of the Pyroter–MSO formulations increased about 2-fold from 18°C to 24°C (LD_{50} = 20.5 µl per 54 cm^2 and 11.8 µl per 54 cm^2, respectively). The laboratory bioassays suggest that MSO mixed with surfactants are potential mosquito larvicides. We also evaluated the influence of the 2 surfactants on the toxicity of 3 monoterpenes. The larvicidal activity of citral and limonene increased with the addition of surfactants, but neither surfactant enhanced the toxicity of cineole. All 3 monoterpenes, with and without surfactants, were considered poor candidates as surface larvicides because of their high volatilities.

KEY WORDS larvicide, soy, monoterpene, *Culex,* surfactant

INTRODUCTION

Surface-active larvicides have been used to control mosquito larvae in rain barrels, floodplains, swamps, and marshes for more than a century in the United States (Ginsburg 1943, McMullen et al. 1977, Mulla 1994). Larvicidal oils have the added benefit of being toxic to pupae and eclosing adults, and they may repel ovipositing mosquitoes (Beehler and Mulla 1996). Petroleum-based oil larvicides, primarily containing mineral oil, are the most widely used, although a variety of plant essential oils (e.g., citrus peel and eucalyptus oils) and natural and synthetic water-insoluble compounds (e.g., egg and soy lecithins, turpentine, cineole, limonene, and commercial surfactants) have been evaluated as alternative larvicides (Sukumar et al. 1991, Corbet et al. 1995 and references therein, Floore et al. 1998).

An important characteristic of any surface-active larvicide is its ability to maintain a uniform surface layer in different aquatic habitats (Toms 1945, McMullen et al. 1977). Seed oils have been evaluated as spray adjuvants and as control agents for insect pests (Davidson et al. 1991, Hamilton 1993). However, pure vegetable oils are generally too viscous to be used as mosquito larvicides. Physical and chemical properties of plant oils, including viscosity, can be modified by alkali transesterification to form methyl and ethyl esters of fatty acids, as in the processing of soybean oil to "biodiesel" (De Filippis et al. 1995). The spreading pressures of lipophilic products can also be increased by the addition of surfactants (Corbet et al. 1995).

We compared the larvicidal activity of methylated soy oil (MSO) mixed with 2 nonionic surfactants to 2 commercially available petroleum-derived larvicides, Golden Bear Oil 1111® (Golden Bear Speciality Products, Oildale, CA) and Bonide® (Bonide Products, Inc., Yorkville, NY), using *Culex pipiens* L. larvae as the target organisms. The influence of temperature on the toxicity of 1 surfactant–MSO mixture was evaluated with *Anopheles stephensi* Liston. In addition, we determined the impact of the 2 surfactants on the toxicity of 3 lipophilic monoterpenes considered to have pest control potential (Jacobson 1990).

MATERIALS AND METHODS

Dosage–mortality tests were conducted on early 4th-stage *Cx. pipiens* and *An. stephensi* from colonies maintained by the Medical Entomology Program at the Illinois Natural History Survey. The mosquitoes were reared under a long-day photoperiod (18 h light and 6 h dark) at 24°C (±4°C).

A modified World Health Organization (WHO) technique was used to determine susceptibility of mosquito larvae to the various treatments (WHO 1985). For each bioassay, 20 4th-stage larvae were transferred to 600-ml glass beakers containing 200 ml of distilled water. The surface area of each beaker at the point of treatment was 54 cm^2. Chemicals were applied either with glass microcapillary pipettes or micropipetters. For each treatment, an initial test was conducted at 5, 10, 20, 50, and 100 µl to determine the toxicity range. In subsequent tests,

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Table 1. Toxicity of oil larvicides (microliters per beaker) to 4th-stage *Culex pipiens* larvae.[1]

Treatment	LD_{50} (μl/54 cm²) (95% CI)	LD_{95} (μl/54 cm²) (95% CI)	Probit regression line
Golden Bear Oil 1111	3.6 3.4–3.8	7.8 7.2–8.5	4.9 log(x) − 2.7; r^2 = 0.78
2% CPI-40 + MSO	3.8 3.5–4.0	9.3 8.4–10.5	4.2 log(x) − 2.4; r^2 = 0.71
Bonide	6.2 5.9–6.5	13.4 12.2–15.2	4.9 log(x) − 3.9; r^2 = 0.70
2% PL-121 + MSO	11.3 10.4–12.3	35.6 29.7–45.3	3.3 log(x) − 3.5; r^2 = 0.74

[1] Median lethal dose (LD_{50}) and 95% lethal dose (LD_{95}) values are given in μl dosage per 600-ml beaker with 200 ml of water and a treatment surface area of 54 cm². CI is the confidence interval, which is asymmetric because of logarithmic transformation. MSO, methylated soy oil.

8–10 replicates were conducted over narrower dosage ranges. Untreated controls were included in each bioassay. Response to the treatments was recorded at 24 and 48 h. Larvae were prodded with a wooden applicator stick and those that did not move away from the stimulus were recorded as dead. This method combines both dead and moribund larvae.

The treatments included 2 petroleum-derived oil larvicides, Bonide (Bonide Products, Inc.) and Golden Bear Oil 1111 (Golden Bear Specialty Products), and 2 mixtures of MSO (SoyClean®, Interchem Inc., Kansas City, MO) with nonionic surfactants. The surfactants were Pluronic L121® (BASF Co. Performance Chemicals, Parsipanny, NJ) and Pyroter CPI-40® (Ajinomoto USA, Inc., Teaneck, NJ). Preliminary tests showed that toxicity to mosquito larvae did not vary between 1 and 5% of each surfactant in MSO; therefore, all subsequent comparisons were conducted with 2% surfactant in MSO. The toxicity of pure surfactant was determined by treating *Cx. pipiens* larvae (20 per beaker) with 100 μl of each surfactant (8 replicates). The breakdown of the intact surface layer was determined by the presence of separated oil beads on the surface of the water.

Pluronic L121 is a block copolymer of propylene oxide and ethylene oxide and Pyroter CPI-40 is a protein-based surfactant (monopyroglutamic and monoisostearic diesters of polyol ethylene-40-hydrogenated castor oil) (McCutcheon 1995). The Pluronic surfactant is practically insoluble in water, but Pyroter CPI-40 is readily soluble in both water and MSO. Methylated soy oil is insoluble in water.

The influence of temperature on larval mortality was examined by simultaneously testing a series of dosages of 2% Pyroter CPI-40 in MSO at 3 temperatures, 18°C, 24°C, and 30°C (±2°C) with *An. stephensi* larvae. Because spreading pressure increases with temperature for most oils (McMullen et al. 1977), the hypothesis was that toxicity to mosquito larvae would also increase. The test beakers were placed in incubators (Dual Program Illuminated Incubator 818, Precision Scientific, Winchester, VA) that had 18 h light and 6 h dark photoperiods. Mortality was recorded at 24 and 48 h as previously described. Four replicates were conducted of each dosage.

The influence of Pyroter CPI-40 and Pluronic L121 on the toxicity of 3 monoterpenes, cineole, citral, and limonene (Sigma Chemical Co., St. Louis, MO), was evaluated with 4th-stage *Cx. pipiens* larvae. Formulations of each monoterpene with 2% surfactant were bioassayed as previously described at 20°C (±3°C) with 4 replicates of each dosage.

The data from the bioassays were subjected to probit analyses (Norusis 1995) and the dose lethal to 50% of the test organisms (LD_{50}) and the dose lethal to 95% of the test organisms (LD_{95}), their 95% confidence intervals, and the slopes of the probit regression lines were recorded. Within a bioassay series, LC_{50} and LC_{95} values of different treatments were considered significantly different if their 95% confidence intervals did not overlap.

RESULTS

MSO formulations compared to petroleum-derived larvicides

Both the surfactants and the pure MSO were essentially nontoxic to *Cx. pipiens* larvae at the dosage of 100 μl after 24 h (less than 5% mortality in 8 replicates of each). Larval mortality in controls was consistently less than 5% in all bioassays.

Methylated soy oil did not form a uniform layer on the water surface over the temperature ranges used in our study (18–30°C). However, the addition of surfactants, either 2% Pyroter CPI-40 or Pluronic L121, to MSO resulted in a rapid spreading, surface-active larvicide (Table 1). The toxicity of the surfactant–MSO formulations to *Cx. pipiens* larvae differed by about 3-fold depending upon the surfactant; 2% Pyroter CPI-40 in MSO had an LD_{50} = 3.8 μl per 54 cm² and 2% Pluronic L121 in MSO had an LD_{50} = 11.3 μl per 54 cm² (Table 1). The 2% Pyroter CPI-40 formulation in MSO was as active as Golden Bear Oil 1111 based on the overlap

Table 2. Toxicity of 2% Pyroter CPI-40 in methylated soy oil (MSO) at 24 h and 48 h (μl/beaker) to 4th-stage larvae of *Anopheles stephensi* at 3 temperatures.[1]

Temperature	24 h	48 h
18°C		
LC_{50}	20.5	15.8
95% CI	15.8–52.0	12.7–22.7
LC_{95}	56.7	38.4
95% CI	31.3–1,440.0	25.3–182.7
Probit equation	3.7 log(x) − 4.9	4.3 log(x) − 5.1
r^2	0.81	0.61
24°C		
LC_{50}	11.8	9.2
95% CI	10.1–13.8	7.3–11.1
LC_{95}	37.0	19.7
95% CI	27.3–65.0	15.6–29.9
Probit equation	3.3 log(x) − 3.5	5.0 log(x) − 4.8
r^2	0.86	0.92
30°C		
LC_{50}	9.9	6.3
95% CI	10.1–11.2	5.3–7.2
LC_{95}	24.1	14.5
95% CI	19.8–33.2	12.1–19.2
Probit equation	4.2 log(x) − 4.2	4.6 log(x) − 3.7
r^2	0.92	0.90

[1] Median lethal dose (LD_{50}) and 95% lethal dose (LD_{95}) values are given in μl dosage per 600-ml beaker with 200 ml of water and a treatment surface area of 54 cm². CI is the confidence interval, which is asymmetric because of logarithmic transformation.

of the 95% confidence intervals for both LD_{50} and LD_{95} values. Both Golden Bear Oil and the 2% Pyroter–MSO formulation were more potent larvicides than either Bonide or the 2% Pluronic L121 formulation in MSO. The 2% Pluronic L121 formulation in MSO was the least active larvicide of the 4 treatments.

All 4 of the treatments formed an oil layer covering the water surface for at least 24 h at dosages greater than 4 μl per beaker (54 cm²). However, at low dosages, such as 1–2 μl per beaker, only the surfactant–MSO formulations maintained an intact surface layer for at least 24 h at 20°C. Bonide was the most odiferous treatment and the loss of volatile components probably explains the absence of a surface layer after 8 h at dosages less than 4 μl per 54 cm².

Influence of temperature on toxicity

The toxicity of the 2% Pyroter–MSO formulation to *An. stephensi* larvae increased with increasing temperature from 18°C to 30°C (Table 2). The greatest change in LD_{50} and LD_{95} values was between 18°C and 24°C. The Pyroter–MSO formulation was about twice as active at 24°C and 30°C than at 18°C based on the LD_{50} values. The 95% confidence intervals of the LD_{50} and LD_{95} values at 18°C did not overlap with those at 24°C or 30°C at either 24 or 48 h. However, the 95% confidence intervals of the LD_{50} and LD_{95} values at 24°C and 30°C did overlap at 24 h, indicating no difference in toxicity between these 2 temperatures. Based on the LD_{50} and LD_{95} values, the 2% Pyroter CPI-40 formulation in MSO is less toxic to *An. stephensi* larvae (Table 2) than to *Cx. pipiens* larvae (Table 1).

Influence of surfactant on toxicity of monoterpenes

Limonene was the most active monoterpene, without the addition of a surfactant, to *Cx. pipiens*

Table 3. Influence of surfactants on the toxicity of 3 monoterpenes to 4th-stage *Culex pipiens* larvae.[1]

Treatment	LD_{50} (μl/54 cm²) (95% CI)	LD_{95} (μl/54 cm²) (95% CI)	Probit regression line
Cineole	33.3 27.9–40.4	82.7 63.8–123.1	4.2 log(x) − 6.3; r^2 = 0.96
Cineole + 2% CPI-40	24.1 19.0–38.5	71.6 46.1–200.5	3.5 log(x) − 4.8; r^2 = 0.92
Cineole + 2% PL-121	23.1 20.2–28.1	45.1 36.6–64.1	5.6 log(x) − 7.7; r^2 = 0.90
Citral	51.9 38.9–76.5	481.6 236.6–1,984.9	1.7 log(x) − 2.9; r^2 = 0.76
Citral + 2% CPI-40	3.5 2.6–4.2	6.5 5.2–14.9	6.2 log(x) − 3.4; r^2 = 0.61
Citral + 2% PL-121	3.8 3.3–4.5	7.8 6.2–12.6	5.4 log(x) − 3.1; r^2 = 0.80
Limonene	17.7 14.1–24.0	66.7 41.7–179.5	2.9 log(x) − 3.6; r^2 = 0.76
Limonene + 2% CPI-40	8.5 7.4–9.7	17.4 14.2–24.7	5.3 log(x) − 4.9; r^2 = 0.97
Limonene + 2% PL-121	9.5 8.2–11.1	23.5 18.4–35.9	4.2 log(x) − 4.1; r^2 = 0.95

[1] Median lethal dose (LD_{50}) and 95% lethal dose (LD_{95}) values are given in μl dosage per 600-ml beaker with 200 ml of water and a treatment surface area of 54 cm². CI is the confidence interval, which is asymmetric because of logarithmic transformation.

larvae, followed by cineole and citral, in that order (Table 3). The relative toxicity of the monoterpenes changed after 2% of either surfactant, Pyroter CPI-40 or Pluronic L121, was mixed with them. Citral with surfactant was more toxic than limonene with surfactant, which in turn was more toxic than cineole with surfactant. The addition of either surfactant dramatically increased the toxicity of citral and limonene, but did not enhance the toxicity of cineole, an oxygenated monoterpene (Table 3). The LD_{50} values for citral with surfactants were about 10 times less than the LD_{50} value of citral alone. For limonene, the surfactants increased larvicidal activity about 2-fold.

Cineole and citral did not form a uniform layer on the surface of water in the test beakers over the dosage range of 5–100 μl, except when surfactants were added to them. Limonene without surfactant formed a surface layer, but it rapidly broke up within 5–10 min. All of the monoterpene treatments with and without surfactants were rapidly lost from the water surface because of their high volatilities. In contrast, the MSO treatments maintained intact surface layers for more than 16 h at dosages greater than 5 μl.

DISCUSSION

In our laboratory tests, MSO formulations with 2% surfactant, either Pyroter CPI-40 or Pluronic L121, were effective oil larvicides for *Cx. pipiens* or *An. stephensi*. The 2% Pyroter formulation of MSO was as effective as Golden Bear Oil and significantly more toxic than either Bonide or the Pluronic–MSO formulation with *Cx. pipiens* larvae. With *An. stephensi* larvae, the Pyroter–MSO formulation increased in toxicity as temperature increased with the LD_{50} values at 24°C and 30°C about 2 times lower than that at 18°C. This is probably due to an increase in spreading pressure with increased temperature.

The petroleum oil products, especially Bonide, tended to have a rapid kill and steeper slopes to their probit regression lines. In contrast, the MSO formulations exhibited a slower rate of mortality. A comparison of LD_{50} values of the 2% Pyroter CPI-40 formulation in MSO at 24 and 48 h over 3 temperatures showed the 48-h values were one-third to one-fourth smaller than the 24-h values with *An. stephensi* larvae. Observations of the surface layers through time suggest that MSO–surfactant formulations make a surface film at lower dosages that persist for longer periods of time than the petroleum-based products. The effective field rates for oil larvicides are sometimes lower than predicted based upon laboratory bioassays, possibly because the oils tend to adhere to emerging vegetation (the edge of the water), which is where larvae and pupae are frequently found (Levy et al. 1982). Methylated soy oil formulations should be evaluated under field conditions and include their impact on nontarget plants and animals.

Essential oils with monoterpenes, such as eucalyptus oil and lemon peel oil, have been suggested as potential mosquito larvicides (Mwaiko and Savaeli 1994, Corbet et al. 1995). Limonene is considered a broad-spectrum insecticide that can be used as a mosquito larvicide or as a mosquito repellent (Hwang et al. 1985, Kassir et al. 1989, Mohsen et al. 1989). None of the monoterpenes in our bioassays were as effective as the petroleum-based products or the MSO–surfactant formulations; however, citral and limonene showed significant increases in toxicity after the addition of either 2% Pyroter CPI-40 or Pluronic L121. Cineole toxicity was not enhanced by either surfactant. Corbet et al. (1995) reported that undiluted cineole was toxic to 4th-stage larvae of *Cx. pipiens*; however, mortality was extremely low at relatively high dosages (1 μl/15 cm^2). Larvae contacting the monoterpenes, with or without the surfactants, exhibited an almost immediate behavioral response, probably due to the rapid penetration of these compounds through the lipophilic cuticle. In general, larvicidal activity of these products in neat or concentrated formulation would be for very short periods of time because of their volatility (Mwaiko and Savaeli 1994).

The 2 surfactants used to spread the MSO and the monoterpenes (citral and limonene) on the surface of water have different chemical and physical properties (McCutcheon 1995). Surfactants used in mosquito larvicides are typically insoluble in water (Corbet et al. 1995). The partial solubility of the Pyroter CPI-40 surfactant in both water and MSO may allow oil larvicides to be enhanced with the addition of either lipophilic or hydrophilic adjuvants.

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