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Toxicity of Blackfly Larvicidal Formulations to Some Aquatic Insects in the Laboratory[1]

Zohair H. Mohsen and Mir S. Mulla

Department of Entomology, University of California, Riverside, CA 92521

Currently, larviciding is the method of choice for the control of Simulium vectors of Onchocerciasis. Many chemical substances have been tested for effectiveness as simuliid control agents (CHANCE 1970, JAMNBACK 1973, LACEY & MULLA 1977a, McKAGUE et al. 1978, MUIRHEAD-THOMSON 1977, 1978a, b, and others). DDT was one of the earliest insecticides used successfully to control blackfly larvae (COLLINS & JAMNBACK 1958). But with the discovery of its long-term persistence and accumulation in the environment and the evidence that some blackfly species developed resistance to DDT (ASAHINA et al. 1966, SUZUKI 1963), its use was phased out in 1965 in New York and in 1970 in Canada (JAMNBACK 1973). To find substitute larvicides, many new organophosphorous insecticides, pyrethroids, and insect growth regulators have been tested as candidate blackfly larvicides.

There is concern that repeated applications of larvicides may adversely affect the nontarget fauna associated with blackfly larval populations in streams and rivers. For implementing a practical and safe Simulium vector control program, it is essential that we gather information on the effects of larvicides on some important nontarget biota. To date several reports have been published on the susceptibility of aquatic nontarget insects to blackfly larvicides in the laboratory. GAUFIN et al. (1961, 1965) developed bioassay procedures to determine the short- and long-term toxicity of commonly used insecticides such as DDT and others against a range of nontarget aquatic invertebrates. MUIRHEAD-THOMSON (1978a) evaluated the lethal and behavioral impact of pesticides on a range of stream macroinvertebrates under continuous trough-flow and simulated stream conditions. Mortality was assessed after 1-h exposure period at different concentrations followed by a 24-h holding period in a continuous flow of clear water. Among the species of macroinvertebrates studied, the naiads of Baetis were the most susceptible organisms to temephos.

To find more selective blackfly larvicides with minimum potential for harmful effects on nontarget organisms, the present

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studies were initiated. Reported here are the results of the evaluation of 6 insecticides and formulations against Simulium larvae and also data are presented on the impact of these insecticides on selected nontarget insects under laboratory conditions.

MATERIALS AND METHODS

In laboratory evaluation of larvicides against Simulium larvae, the flushing bioassay system described by LACEY & MULLA (1977b) was used. For nontarget bioassays the modified jar system described by the same authors (1977b) was employed. Two blackflies, S. argus Williston and S. virgatum Coquillett and the most abundant aquatic nontarget insects, Baetis parvus Dodds (Ephemeroptera:Baetidae) and Hydropsyche californica Banks (Trichoptera:Hydropsychidae) were selected as nontargets and used in these studies. The 6 larvicides tested were: FMC-45497, 10% EC (NRDC-160), a pyrethroid [(-) cyano-3-phenoxybenzyl (±)cis-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane carboxylate] obtained from FMC; decamethrin, 2.5% EC (K-Othrin, NRDC 161) a pyrethroid [(-)cyano-2-phenoxybenzyl (+)cis-3(2,2-dibromoethenyl) -2,2-dimethylcyclopropanecarboxylate] provided by Procida (Roussel Uclaf); temephos, 50% EC (Abate) an organophosphorus compound provided by American Cyanamid; chlorpyrifos-methyl, 10% encapsulated (Reldan, 2 formulations, UK 57/C and UK 57/D, 'C' has a lower level of cross linkage in the capsule wall than 'D') provided by Dow Chemical Co. and OMS-1356 20% EC (GH 74), a DDT-related compound [1,1-bis-(4-ethoxyphenyl)-2-nitropropane] provided by WHO.

Various dilutions of each formulation were prepared in distilled water 15 min prior to treatment. All target and nontarget organism treatments were arranged in randomized block design. Water temperature was maintained at 20±1°C. Penultimate and ultimate larvae of blackflies (with histoblast apparent to well developed) were collected from creeks and rivers in southern California by brushing rocks with a 1-in. artist soft plastic brush into 1-L polystyrene containers filled with water. Larvae were obtained from a creek in Thousand Palms Canyon, 100 km east of Riverside, CA; White Water River, 65 km east of Riverside, CA; and a creek in Temescal Canyon near Corona, CA. After sufficient larvae (1000-2000) were collected, water was decanted and the larval containers were transported to the laboratory in an ice chest. In the laboratory, larvae were transferred into aerated 1-L pyrex beakers immediately after arrival. Thirty-five to 40 larvae were introduced into each flushing bioassay unit within 2-3 h with a dropper having a large opening. Food suspension (3 parts ground Purina lab chow + 1 part Brewer's yeast) was added at the rate of 35 mg/unit. After an acclimation period of 4-6 h, dead larvae resulting from handling were removed through the lower funnel stem of the flushing bioassay units. Reaction of larvae, in treated and check units, was observed at 5, 15, 30, 45 and 60 min after treatment and the number of detached or dead Simulium larvae was recorded. After 60 min of exposure period, all units were flushed with clean water at the rate of 1 L/min

for 5 min and 35 mg of food suspension was added after flushing. Mortality was assessed 24 h later excluding pupated larvae. Moribund larvae which did not respond to a needle were considered dead. Each treatment and the control were replicated 4 times. Mortalities were corrected by Abbott's formula (ABBOTT 1925). The LC_{50}, LC_{90}, slope and correlation coefficients were determined.

In assessing the toxic effects on nontarget organisms, 2-L glass beaker units were used. Beakers were provided with a layer of small gravel (2-6 mm diameter) at the bottom to simulate natural conditions. Larvae of H. californica and naiads of B. parvus were collected from White Water River and Thousand Palms Canyon. The number of late instar B. parvus and H. californica used in bioassays was 25 and 15/replicate, respectively. Larvae of H. californica were transferred into the 2-L beaker by lightweight spring steel forceps, but B. parvus naiads were transferred in the same manner as Simulium larvae. Seventy mg of food suspension (the same mixture used for Simulium larvae) were added to each beaker after transferring the nontargets into the beakers, prior to treatment and after removal of the treated water after 1 h of exposure. Prior to treatment, B. parvus and H. californica were acclimated in the 2-L beakers for 4-6 h. After exposure of 1 h, the water was decanted from all the treatment and control units and clean water was then added to the beakers. Escape of test organisms during water change was prevented by pouring the water through a 1-mm mesh net. After use and between experiments all glassware was cleaned with hot Cascade solution by soaking them for 24 h then rinsing with clean tap water.

To avoid repeated field trips to collect Simulium larvae, it became necessary to study the survival of field-collected larvae under laboratory conditions. In the laboratory, field-collected larvae were held in 1-L aerated pyrex beakers for varying periods of time. Water in these beakers was changed once a day and 70 mg of food suspension was added after each water change. Change of water was conducted by decanting the used water and adding fresh water. Survival of field-collected S. argus was assessed by holding 35-40 penultimate and ultimate larvae in flushing bioassay units (LACEY & MULLA 1977b). Water in these units was changed and 35 mg of the same food mixture as stated before was added every day. Survival among test larvae was computed after 1, 3, 7 and 14 days by counting larvae which died on daily basis excluding pupated ones.

RESULTS AND DISCUSSION

Toxicity of blackfly larvicides to Simulium and nontarget species

The toxicity of FMC-45497, K-Othrin, Abate, Reldan UK 57/C, Reldan UK 57/D, and OMS-1356 formulations against the targets S. argus and S. virgatum and nontargets B. parvus and H. californica are presented in Table 1. The two closely related pyrethroids

FMC-45497 and K-Othrin (decamethrin) showed the highest toxicity to all the target and nontarget species. It is important to note that both nontarget species were more susceptible to these pyrethroids than the target species S. virgatum.

Table 1. Toxicity of fast-acting blackfly larvicides to Simulium larvae and two nontarget aquatic insects exposed for 1 h and after 24 h holding period under laboratory conditions.[a/]

Chemical	Organism	24 h lethal concentration LC_{50} (ppm)	LC_{90} (ppm)	Slope	r
FMC-45497	S. virgatum	0.0016	0.0053	2.5	0.93
	B. parvus	0.0011	0.0027	3.5	0.98
	H. californica	0.0007	0.0010	8.6	0.99
K-Othrin (decamethrin)	S. virgatum	0.0009	0.0045	1.8	0.91
	B. parvus	0.0004	0.0008	3.8	0.95
	H. californica	0.0004	0.0009	3.1	0.95
Abate (temephos)	S. argus	0.020	0.038	4.6	0.99
	S. virgatum	0.0082	0.020	3.3	0.98
	B. parvus	0.0097	0.018	5.0	0.99
	H. californica	1.3	4.0	2.7	0.99
Reldan UK 57/C (chlorpyrifos-methyl)	S. virgatum	0.035	0.091	3.1	0.94
	B. parvus	0.0009	0.0016	4.8	0.91
	H. californica	0.30	0.44	8.2	0.99
Reldan UK 57/D	S. argus	0.0065	0.015	3.5	0.95
	H. californica	0.21	0.25	18.3	0.99
OMS-1356	S. virgatum	0.0028	0.0040	8.8	1.00
	B. parvus	0.27	0.62	3.5	0.98
	H. californica	0.051	0.094	4.9	0.99

[a/] Values obtained using formulations, not pure insecticide standards.

Within the range of lethal concentrations (LC_{90}+), about 40% of S. virgatum larvae detached and subsequently died at the end of the 1 h exposure period with FMC-45497 and K-Othrin (Fig. 1a, b). Immediately after the pyrethroids were introduced, S. virgatum larvae stopped feeding as observed by the termination of movement of the cephalic fans. Rapid response was also evident in H. californica larvae; they left their cases 1-5 min posttreatment and this behavior was followed by nervous abnormal wriggling of body. Small proportion of larvae did recover and returned to their cases or they built new ones.

Recently, some photostable pyrethroids have been found which show exceptionally high level of activity against insects. In

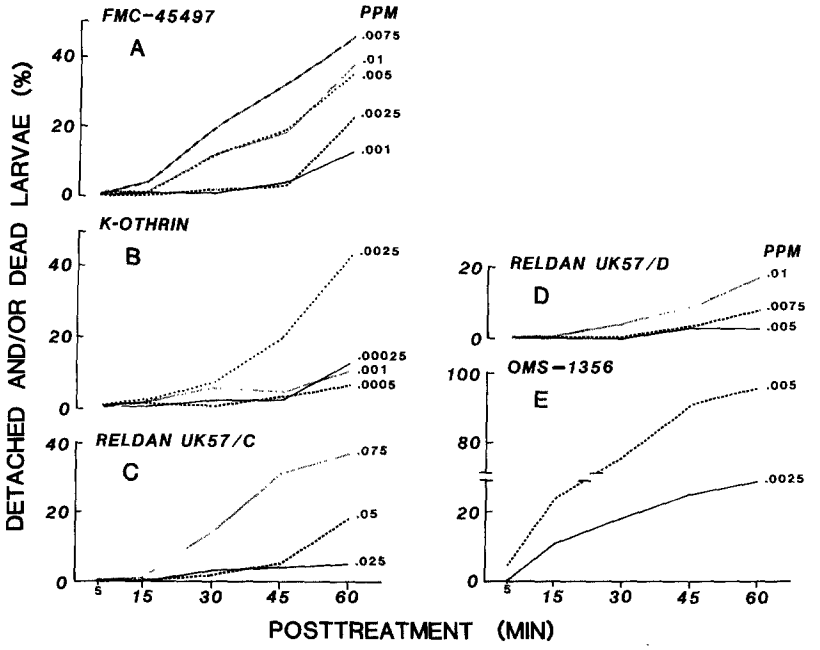

Fig. 1. Reaction of Simulium larvae to fast-acting larvicides at various concentrations a.i. (ppm) under laboratory conditions. There was no detachment in the check units.

this group K-Othrin is one of the most active insecticides evaluated against oarious species of mosquito larvae (MULLA et al. 1978a). K-Othrin presents a minimum risk to man, has low toxicity to birds, and it has low level of risk to honeybees exposed in mosquito control operations. It, however, possesses a high level of toxicity to fish, but still with relatively good margin of safety for mosquito larvicidal use (MULLA et al. 1978b). Since both pyrethroids tested were highly toxic to B. parvus and H. californica, their use as Simulium larvicides may have potential adverse effects on aquatic nontarget insects and other biota.

Abate (temephos), on the other hand, was a more selective Simulium larvicide than the 2 pyrethroids tested (Table 1). B. parvus showed similar susceptibility as did S. virgatum; Baetis spp. are known to be quite susceptible to various blackfly larvicides (MUIRHEAD-THOMSON 1978a, b). H. californica was not as susceptible as Baetis or Simulium species to Abate in this study. At the LC_{90} S. virgatum and S. argus were x200 and x100 more susceptible than H. californica, respectively. The safety of Abate to nontargets has been reported by other workers (SWABEY et al. 1976, MUIRHEAD-THOMSON 1978a, b). SWABEY et al. (1967) found no harmful effects on nontarget organisms (fish, crayfish and stonefly nymphs), when Abate was used at 0.05 ppm as blackfly larvicide. At 0.1 ppm, Abate was safe to fish, shrimp, amphipods and microorganisms, i.e., rotifers, Euglena and Coleps (Ciliata) (VON WINDEGUTH & PATTERSON 1966). Other laboratory studies have

shown that Abate is also safe for backswimmers, Notonecta undulata Say at mosquito larvicidal dose (FALES et al. 1968). Although Abate poses some potential hazards to Baetis species, it nevertheless has a good margin of safety to most other nontarget aquatic biota.

Compared to pyrethroids, Abate did not induce severe rapid response in Simulium larvae. No apparent detachment was evident in Simulium larvae during the 1 h exposure period. LACEY & MULLA (1977b), under similar laboratory conditions, found that Simulium larvae were not irritated and were not flushed prematurely from the bioassay units when Abate EC2 was removed by flushing and exchange of water. MUIRHEAD-THOMSON (1978b), under simulated stream conditions, also noticed no drifting activity of late instar Simulium larvae when exposed to sublethal or practical lethal concentrations of Abate for 30 min. However, using early instar larvae, detachment was negligible even at 0.2 ppm (effective concentration), but increased to 17% at 0.5 ppm and 27% at 1.0 ppm (higher than practical concentrations).

Studies on the evaluation of blackfly larvicides to date have shown that Abate is one of the most promising and relatively safe compounds (CHANCE 1970, JAMNBACK 1973, SWABEY et al. 1967) and is used effectively in Onchocerciasis control programs in west Africa (WALSH et al. 1979).

In the case of chlorpyrifos-methyl, both formulations, UK 57/C and UK 57/D, were toxic against Simulium larvae. For B. parvus UK 57/C was more toxic than to S. virgatum (Table 1); the other formulation was not tested against mayflies, and it is assumed to be equally toxic. Baetis naiads were also found susceptible to chlorpyrifos-methyl by MUIRHEAD-THOMSON (1978b); he observed that 74% of B. rhodani to be washed when exposed to 0.1 ppm for 30 min with 24 h overall mortality of 100%.

H. californica larvae, at the LC_{90} level of chlorpyrifos-methyl, was x5 more tolerant to Reldan UK 57/C and x16 more tolerant to Reldan UK 57/D than the Simulium larvae (Table 1). The LC_{90} for UK 57/C and UK 57/D formulations against H. californica was 0.44 and 0.25 ppm, respectively. The difference in the level of cross linkage in both encapsulated formulations of Reldan had no apparent effect on their toxicity against H. californica.

The magnitude of detachment of late instar of S. virgatum larvae due to exposure to chlorpyrifos-methyl (UK 57/C) at LC_{90}+ was 38% (Fig. 1c). In late instar larvae of S. argus, Reldan UK 57/D produced 18% detachment at LC_{90}+ (Fig. 1d).

In a study on the drift behavior of other species of Simulium, MUIRHEAD-THOMSON (1978b) found that at 0.1 ppm and 30 min exposure, only 6% of late instar larvae drifted at the end of 60 min following exposure to chlorpyrifos-methyl. However, detach-

ment among these larvae increased to 76% at the end of 24 h.

Signs of immediate reaction in Simulium larvae to both chlorpyrifos-methyl formulations were less pronounced than in the pyrethroids; larvae stopped feeding 15 min after introducing chlorpyrifos-methyl, whereas they stopped feeding immediately in the case of the pyrethroids.

OMS-1356 (a DDT-related compound) showed the greatest selectivity against Simulium (Table 1). S. virgatum larvae were more susceptible than both B. parvus and H. californica. At the LC_{90}, B. parvus was x16 and H. californica was x23 more tolerant than S. virgatum (Table 1). This compound was the only one tested here that was not toxic to Baetis naiads at blackfly larvicidal concentrations.

Reaction of S. virgatum at the LC_{90}+ to OMS-1356 was substantial; over 90% of larvae detached and died after 1 h of exposure (Fig. 1e). Other organochlorines, such as DDT, have also been found to be extremely effective in controlling blackfly larvae, but because of their persistence and biomagnification in aquatic ecosystems these organochlorines have not been considered good choices as blackfly larvicides. If the field toxicity hazards of OMS-1356 against nontargets are minimal, then this compound offers a good potential for the control of Simulium vectors of Onchocerciasis.

Among the 6 chemicals and formulations studied against Simulium and nontarget organisms, the pyrethroids were nonselective and will likely cause detrimental effects in at least some organisms in the aquatic ecosystems. Abate, on the other hand, showed much lower toxicity to H. californica but was toxic to both Simulium larvae and Baetis naiads. The DDT-related OMS-1356 showed excellent selectivity against Simulium larvae, being more toxic to these than the selected nontarget insects. However, further laboratory and yield studies are needed to fully assess the impact of these Simulium larvicides on various components of the aquatic fauna.

Survival of Simulium larvae in the laboratory

The rate of survival of S. argus larvae under laboratory conditions in the flushing bioassay units where water was changed by flushing once a day and temperature was maintained at 20±1°C, was quite high. In this system the survival of larvae was in the range of 79-99% during a period of 14 days. The daily change of water in these units helped remove the larval toxic by-products and pollutants resulting from daily addition of 35 mg of food. By using the same flushing bioassay system and regimen using one week holding period, higher mortality (12%) was noted by LACEY & MULLA (1977b) with a mixed population of S. vittatum Zetterstedt (57%) and S. argus (43%), but when water was flushed and food was added twice a day, mortality dropped to 4%. However, mortalities in both cases were not significantly different.

The flushing bioassay system developed by LACEY & MULLA (1977b) and employed here proved very useful in maintaining high survival rate among Simulium larvae designed for bioassay studies.

It is apparent that larvae of Simulium species could be maintained under suitable laboratory conditions with minimum mortality provided that pollutants and other larval by-product toxic materials are removed daily. The holding regimen employed here provided adequate numbers of healthy larvae for bioassay experiments and reduced the need for more frequent field larval-collecting trips. Under conditions where Simulium laboratory colonies are lacking and their breeding sites are far away from the laboratory, a similar holding procedure should be considered to minimize the number of costly field trips.

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