

**Laboratory Experiments on the Effect of Insecticides against Blackfly Larvae (Diptera: Simuliidae) and Fishes.** Kikuo MATSUO and Tatsuo TAMURA (Department of Medical Zoology, Kyoto Prefectural University of Medicine, Kyoto.) Received September 8, 1970, *Botyu-Kagaku* 35, 125, 1970.

17. 実験室内における各種殺虫剤のブユ幼虫殺虫性と魚毒性. 松尾喜久男, 田村辰夫, (京都府立医科大学医動物学教室) 45. 9. 8 受理.

ブユ幼虫殺虫性が強く, かつ, ブユ幼虫発生水域に生息する魚類に対して毒性のより弱い殺虫剤を選択する目的で14種の殺虫剤について室内実験を行なった. 用いたブユはアオキツメトゲブユならびにヒメアシマダラブユ, 魚類はアユ (体長 8~9cm), ニジマス (体長 9~11cm) ならびにカワムツ (体長 7~8cm) である. 各種の実験結果を総合すると, 供試した殺虫剤のうち, 魚毒性を考慮して, ブユ幼虫殺虫効力のある殺虫剤は Baytex 水和剤と Sumithion 水和剤であった.

### Introduction

In the basin of Takano River which runs through the northeastern suburbs of Kyoto City, there were a large number of blackflies and the inhabitants, the sight-seeing visitors and fishermen were injured by the blood sucking of the blackflies. In 1955, Yoshida and Shogaki, carrying out a survey of the blackfly fauna of the vicinity, noted that *Simulium nacojapi* was the dominant species. Furthermore they tested preliminary control of the blackfly larvae with Lindane emulsion in a part of the river. Since 1958, the control of blackfly larvae with Lindane emulsion and DDT wettable powder had been carried out by the Kyoto City Health Center. However, in 1963, this control was discontinued because the fishes in the river sometimes died and the effectiveness of the insecticide to the blackfly larvae was reduced. As the necessity of using other insecticides instead of Lindane and DDT arose, the authors undertook laboratory experiments for the purpose of finding insecticides that would be more effective on blackfly larvae and would have lower toxicity to the fishes. After considering the results of these experiments, since 1964 blackfly control with Sumithion wettable powder had been carried out by the Kyoto City Health Center and the population of blackfly larvae in the river remarkably reduced. At present, injuries from the blackfly adult have disappeared and there has been no damage to the fishes.

This paper reports the results of the laboratory

experiments made from 1963 to 1968.

### Material and method

The insecticides tested were *pp'*-DDT, Lindane, Heptachlor, Dieldrin, Abate, Baytex, Butonate, DDVP, Diazinon, Dibrom, Dipterex, Malathion, Nankor and Sumithion, all of which given by the Sankyo K.K. Of these, Abate was a 5 per cent emulsion, a commercial sample, and all the others were made up in 10 per cent wettable powder by the authors in the laboratory.

*Test on blackfly larvae:* The larvae used in the tests were *Simulium aokii* and *S. venustum* collected from several breeding spots in a river which had had no application of any insecticides for the purpose of blackfly control. The trailing vegetations to which blackfly larvae had attached themselves were put into a plastic bucket together with river water and then were carried to the laboratory. The larvae were reared in the bucket in which the water was bubbled by compressed air under 20°C. air temperature for one hour. Then the vigorous mature larvae were used in the tests. Forty-mesh screen strainers, 5 cm in diameter, into which about 50 larvae were removed from the bucket, were dipped in the test suspension, bubbling by air, of 1ppm for 10minutes, 10ppm for one minute, 0.1ppm for 10 minutes and 1 ppm for one minute, respectively. After that the strainers were dipped in clean water bubbling by air for 10 minutes and the larvae were rinsed. The larvae in the strainers treated by this method were removed to a clean quarium (capacity

500 cc.) bubbling by air and reared for 24 hours. The control tests were carried out by the same process, but the larvae were dipped in clean water instead of the test suspension. Observations on the effect of the insecticides were made after 24 hour rearing. Larvae which moved slightly but were unable to attach themselves to things were considered as dead. The rate of mortality of the larvae exposed to the insecticides was corrected by Abbott's formula when the mortality of the control group was lower than 10 percent, otherwise it was discarded. All experiments were tested in water temperatures from 19°C to 21°C.

*Test on fishes:* The fishes used in the tests were three species, *Salmo irideus* (rainbow trout, Nijimasu in Japanese), *Plecoglossus altivelis* (a kind of fresh-water trout, Ayu in Japanese) and *Sacco temminchi* (a kind of fresh-water fish, Kawamutsu in Japanese). Of these, the Ayu were young fish (body length 8-9 cm.) bred in Lake Biwa and then transferred to Takano River, the Nijimasu were young fish (body length 9-11 cm.) commercially cultured in an artificial pool in the city, and the Kawamutsu were wild fish (body length 7-8 cm.) living in rivers in the city. Fishes carried to the laboratory from the fields

were held in an aquarium at the laboratory for 24 hours prior to testing. Then the same size fish of each species were placed in 20 liter test suspensions or emulsions for 15, 30 and 60 minutes, respectively. After being exposed to the insecticides, the fish were removed to clean water for 2-3 minutes for rinsing. The fish treated by this method were then removed to a clean aquarium. The control tests were carried out by the same process but the fish were placed in clean water instead of the insecticidal test solutions. Observations of the toxicity to the fish were made during the exposure and at 1, 24 and 48 hours after removal to the clean aquarium. At the time of each observation, the number of fish that showed abnormal behavior and the number of dead ones were counted. If any of the fish in the control group died, the data from that series of tests was eliminated. All experiments were conducted in water at temperatures from 9.0°C to 13.5°C bubbling by compressed air.

#### Results and discussion

*The blackfly larvae:* The results are summarized in Table 1. In the test of 1 ppm. for 10 minute exposure, Diazinon was the most effective among

Table 1. Effect of insecticides (10% wettable powder) on the blackfly larvae in water temperatures from 19°C to 21°C in the laboratory

Insecticides	Dosage of insecticides and period of exposure								
	1 ppm for 10 minutes		10 ppm for 1 minute		0.1 ppm for 10 minutes		1 ppm for 1 minute		Control Mortality %
	No. of larvae tested	Morta- lity* %	No. of larvae tested	Morta- lity* %	No. of larvae tested	Morta- lity* %	No. of larvae tested	Morta- lity* %	
Baytex	380	85.5	192	93.6	198	16.7	191	26.5	1.7
Butonate	117	4.2	101	12.1	106	3.8	102	1.1	0.9
DDT	106	2.9	129	2.2	122	0.7	107	1.1	0.9
DDVP	147	70.3	125	92.5	131	0.8	137	2.1	3.8
Diazinon	140	92.6	150	93.1	150	15.3	130	0.8	1.5
Dibrom	106	18.9	116	11.1	119	0.8	116	1.7	0
Dipterex	109	1.8	112	1.8	74	0	93	0	0
Dieldrin	118	28.7	116	17.3	122	10.0	116	7.1	7.1
Heptachlor	49	34.7	59	25.4	57	5.3	56	5.4	0
Lindane	120	65.0	135	65.2	110	10.0	105	19.0	0
Malathion	327	34.5	320	26.7	330	6.4	333	2.8	4.8
Nankor	125	2.4	123	3.2	119	1.7	115	0.9	0
Sumithion	387	86.4	206	74.1	204	9.4	206	6.3	2.1

\* Rate of mortality corrected by Abbott's formula.

the 13 insecticides tested. Sumithion, Baytex, DDVP and Lindane were slightly less effective (mortality: 65.0~86.4%), while Heptachlor, Malathion, Dieldrin, Dibrom, Butonate, DDT, Nankor and Dipterex were much less effective. In the test of 10 ppm for one minute exposure, Baytex, Diazinon and DDVP were remarkably effective (mortality: more than 90%), Lindane and Sumithion were slightly less effective (74.1% and 65.2%, respectively) and the others were much less effective. In both tests of 0.1ppm. for 10 minute exposure and 1 ppm for one minute exposure, all the insecticides were much less

effective and Baytex with the highest mortality was only 26.5%. So that, on the level of total 1 ppm concentration, none of the insecticides could be considered as blackfly larvicides. On the level of total 10 ppm concentration, Baytex, DDVP, Diazinon, Sumithion and Lindane were effective as blackfly larvicide if the toxicity to fishes is not considered. Comparing the results with those obtained by Jamnback (1962), Baytex, Diazinon, Lindane and Sumithion were similarly effective and Nankor and Malathion were similarly less effective. But the differences were that DDT and Dibrom were reported by him as being

Table 2. Toxicity of insecticides (10% wettable powder) to *Plecoglossus altivelis* in water temperatures from 16°C to 18°C in the laboratory.

Insecticides	Dosage (ppm)	Rate of mortality after 0, 1 and 48 hours following 15, 30 and 60 minute exposure											
		15 minutes				30 minutes				60 minutes			
		No. of fish tested	0 hr	1 hr	48hrs	No. of fish tested	0 hr	1 hr	48hrs	No. of fish tested	0 hr	1 hr	48hrs
Baytex	10.0	10	0	0	0	10	0	0	0	10	0	30	10
	5.0	10	0	0	0	10	0	0	0	10	(90) 0	0	0
DDT	60.0	10	0	0	0	10	0	0	0	10	0	0	0
	*60.0	10	0	0	0	10	0	0	20	10	0	10	30
DDVP	5.0	10	0	0	0	10	0	0	0	10	60 (40)	30	10
Diazinon	10.0	10	0 (100)	0	0	10	0 (100)	10	0	10	70 (30)	30	—
	5.0	10	0	0	0	10	0 (40)	0	0	10	0 (100)	20	0
Dipterex	500.0	10	0	0	0	10	0	0	0	10	0	0	0
Dieldrin	20.0	10	0	0	20	10	0 (100)	100	—	10	70 (30)	30	—
Heptachlor	50.0	10	0	0	0	10	0	0	0	10	0	0	10
Lindane	0.5	10	0 (100)	40	0	10	0 (100)	40	0	10	20 (80)	30	0
	0.1	10	0	0	0	10	0	0	0	10	0 (50)	0	0
Malathion	15.0	10	0	0	0	10	0	0	20	10	0 (80)	20	10
	10.0	10	0	0	0	10	0	0	0	10	0	0	0
Sumithion	10.0	10	0 (70)	20	0	10	40 (60)	30	10	—	—	—	—
	5.0	10	0	0	0	10	0	0	0	10	0 (50)	10	0

In table 2-4, figures in parenthesis indicate the percentage of the number of the fish acting abnormally among fish tested.

\* Test with smaller fish (body length: 5-7cm.).

effective. This is probably due to differences in testing methods. He used ethanol as solvent for his formula and exposed for 20 minutes in 3.3ppm against a mixture of *Simulium venustum*, *S. verecundum* and *S. tuberosum*, while we used wettable powder and exposed for 10 minutes in 1.0 ppm and 1 minute in 10 ppm against a mixture of *S. aokii* and *S. venustum*. Besides the tests shown in Table 1, the test was carried out on *S. nacojapi* collected from the river where blackfly control with DDT had been carried out. The results indicated that DDT was ineffective

(mortality: 2.1% in 1 ppm for 10 minute exposure and 4.7% in 10 ppm for 1 minute exposure.).

*The fishes:* The results on *Plecoglossus altivelis*, the Ayu in Japanese, are summarized in Table 2. Of 10 insecticides in the formula of wettable powder tested, Lindane was the most toxic. In the tests of 15 minute exposure in the 0.5 ppm all fish showed abnormal behavior during the exposure and 40% of the fish tested died within one hour following the exposure. Dipterex was the least toxic and all fish were normal in the 60 minute exposure in the 500 ppm. The decreasing

Table 3. Toxicity of insecticides (10% wettable powder) to *Salmo irideus* in the laboratory.

Insecticides	Dosage (ppm)	Water temperature (°C)	Rate of mortality after 0, 1 and 48 hours following 15, 30 and 60 minute exposure.													
			15 minutes				30 minutes				60 minutes					
			No. of fish tested	0hr	1hr	48hrs	No. of fish tested	0hr	1hr	48hrs	No. of fish tested	0hr	1hr	48hrs		
Abate	*1.0	20.0	10	0	30	70	—	—	—	—	—	—	—	—	—	—
	*0.6	20.0	20	(50)	0	(70)	0	0	0	0	0	0	0	0	0	0
Baytex	10.0	12.0	—	—	—	—	10	100	—	—	—	—	—	—	—	—
	5.0	20.0	20	0	0	0	20	0	30	10	10	70	10	0	0	0
	3.0	20.0	—	—	—	—	—	—	—	—	—	40	0	0	0	0
	*0.4	20.0	—	—	—	—	—	—	—	—	—	10	0	0	0	0
	*0.3	20.0	—	—	—	—	—	—	—	—	—	20	(40)	0	0	0
DDT	40.0	12.0	5	0	0	40	5	0	0	80	5	0	20	60	0	0
	40.0	9.0	5	0	0	0	5	0	0	0	5	0	0	0	0	0
	20.0	12.0	15	0	0	0	15	0	0	0	15	0	0	0	0	0
DDVP	10.0	9.5	10	0	0	0	10	0	0	0	10	0	100	—	—	—
Diazinon	15.0	12.5	5	0	20	40	5	0	20	40	5	20	20	40	0	0
	15.0	9.5	5	(100)	0	0	5	(100)	0	0	5	(80)	0	0	0	0
	5.0	20.0	10	(60)	0	0	10	(100)	0	30	0	(100)	—	—	—	—
Dipterex	1000.0	12.0	5	0	0	0	5	0	0	0	5	0	0	0	0	0
Heptachlor	10.0	13.5	5	0	0	0	5	0	40	0	5	80	0	0	0	0
	10.0	9.0	5	0	0	0	5	0	0	0	5	20	0	0	0	0
Lindane	0.5	12.0	5	0	0	0	5	0	20	0	5	0	40	0	0	0
Sumithion	15.0	10.0	5	0	0	0	5	0	0	0	—	—	—	—	—	—
	10.0	20.0	—	—	—	—	—	—	—	—	10	100	—	—	—	—
	5.0	20.0	10	0	0	0	10	0	0	0	20	0	0	15	0	0
	3.0	20.0	—	—	—	—	—	—	—	—	40	(10)	0	0	0	0
	*2.0	20.0	—	—	—	—	—	—	—	—	10	100	—	—	—	—
	*1.0	20.0	—	—	—	—	—	—	—	—	20	0	0	0	0	0

\* Test with 5% emulsion.

order of toxicity to the fish was Lindane, DDVP, Diazinon, Sumithion, Dieldrin, Baytex, Malathion, Heptachlor, DDT and Dipterex.

The results on *Salmo irideus*, the Nijimasu in Japanese, are shown in Table 3. The tests were carried out on 8 wettable powder insecticides and three emulsion ones. In the wettable powder test, Lindane was again found to be the most toxic. All fishes showed abnormal behavior in the 15 minute exposure in 0.5 ppm. Dipterex was the least toxic and all fish were normal in the 60 minute exposure in 1000 ppm. Heptachlor that showed comparatively low toxicity to the Ayu was more toxic to the Nijimasu. The decreasing order of toxicity to the fish was Lindane, Diazinon, Baytex, Sumithion, DDVP, Heptachlor, DDT and Dipterex. Diazinon and Heptachlor were tested in the same concentration of each in the two temperature zones, 9.0°C~9.5°C and 12.0°C~13.5°C. The results indicated that both insecticides were more toxic in the higher temperature zone. In the emulsion test on Abate, Baytex and Sumithion, Sumithion was the least toxic and all fish were normal during the 60 minute exposure in the 1 ppm. The order of toxic level was Baytex, Abate and Sumithion. Comparing the toxicity of the wettable powder with that of the emulsion, on Baytex and Sumithion, the emulsion was five to ten times more toxic than the wettable powder in both

cases.

The results on *Sacco temmincki*, the Kawamutsu in Japanese, are summarized in Table 4. In the test, 60 minute exposure was carried out on wettable powder and emulsion. In the emulsion test, 2 ppm was lethal on Abate or Baytex while that of Sumithion was nonlethal. From the results, the order of toxicity was Baytex, Abate and Sumithion and the emulsion was more toxic than the wettable powder in the case of Baytex and Sumithion.

In blackfly control, larvicides are regularly applied to a river living the larvae. Fishes in the river are exposed to the larvicides several times during the blackfly season. In the following test, the Ayu and the Kawamutsu were exposed to insecticides for 60 minutes every day. The tests were carried out with the 10% wettable powder of Baytex, Diazinon and Sumithion, which are effective to the larvae and have comparatively low toxicity to the fishes. After twenty fish of each species had been exposed to 1 ppm of each insecticide for 60 minutes, the fish were removed into clean water and re-exposed the next day. The exposure was carried out twenty times during twenty days at 18°C to 21°C water temperature. The control tests were carried out by the same process except that the fish were placed in clean water instead of insecticidal

Table 4. Toxicity of insecticides (10% wettable powder) to *Sacco temmincki* in the tests of 60 minute exposure.

Insecticides	Dosage (ppm)	Water temperature (C°)	No. of fish tested	Rate of mortality after 0, 1 and 48 hours following the exposure.		
				0 hour	1 hour	48 hours
Abate	*3.0	20	10	100	—	—
	*2.0	20	10	0(20)	0	10
	*1.0	20	15	0	0	0
	10.0	20	30	40(30)	3	0
Baytex	5.0	20	30	0(10)	0	10
	3.0	20	25	0	0	0
	*2.0	20	5	0(80)	0	20
	*1.0	20	15	0	0	0
Sumithion	10.0	20	10	50(30)	10	0
	5.0	20	33	0	0	0
	*4.0	20	5	40(60)	0	0
	*3.0	20	15	0	0	0

\* Test with 5% emulsion.

solutions. In the test on Diazinon, fourteen of the Ayu tested died during the test (three died in the control group) and four of the Kawamutsu tested died (no deaths in the control group). In the Baytex test, three of the Ayu tested died on the eleventh day of the test and on the same day four died in the control group, while all of the Kawamutsu were alive during the test. In the Sumithion test, five of the Ayu treated and four of the control fish died, while all of the Kawamutsu were alive. From the results, it is suggested that Baytex and Sumithion are more suitable than Diazinon for the regular control of the blackfly larvae from the point of toxicity to fishes in the river.

#### Summary

The laboratory experiments were carried out for the purpose of finding out which insecticides would be more effective on blackfly larvae in a river and would have lower toxicity to the fishes. Fourteen insecticides were tested and the larvae used were *Simulium aokii* and *S. venustum* and the fishes used were *Plecoglossus altivelis*, *Salmo irideus* and *Sacco temmincki* (respectively the Ayu, the Nijimasu and the Kawamutsu in Japanese). From the results, it is suggested that of the insecticides tested under laboratory condition the wettable powder of Baytex and Sumithion are more suitable for regular control of the blackfly larvae living in a river.

**Acknowledgment** The writers are indebted to

Dr. K. Uemoto of the Epidemic Prevent Center, Kyoto City and Dr. A. Yamaguchi of the Sakyo Health Center, Kyoto City, for furnishing materials of blackfly larvae and fishes from the fields.

#### References

- 1) Davis, A. N., Gahan, J. B., Fluno, J. A. and Anthony, D. W.: *Mosquito News*, 17, 261 (1957).
- 2) Ferguson, D. E., Gardner, D. T. and Lindley, A. L.: *Mosquito News*, 26, 80 (1966).
- 3) Frempong-Boadu, J.: *Mosquito News*, 26, 562 (1966).
- 4) Jamnback, H.: *Mosquito News*, 22, 384 (1962).
- 5) Lea, A. O. and Dalmat, H. T.: *Jour. Econ. Ent.*, 47, 135 (1954).
- 6) Lewallen, L. L. and Wilder, W. H.: *Mosquito News*, 22, 369 (1962).
- 7) Matsuo, K. and Tamura, T.: *Scientific Pest Control*, 29, 21 (1964).
- 8) Muirhead-Thomson, R. C.: *Am. J. Trop. Med. & Hyg.*, 6, 920 (1957).
- 9) Nagahana, M., Matsuo, K., Tamura, T. and Uemoto, K.: *Sani. Injur. Insects*, 8, 1 (1964).
- 10) Shogaki, Y. and Yoshida, Y.: *Jap. Jour. Appl. Ent. Zool.*, 2, 157 (1958).
- 11) Suzuki, T., Ito, Y. and Harada, S.: *Jour. Exp. Med.*, 33, 41 (1963).
- 12) Swabey, Y. H., Schenk, C. F. and Parker, G. L.: *Mosquito News*, 27, 149 (1967).
- 13) Yoshida, Y. and Nakanishi, Y.: *Jap. Jour. Sani. Zool.*, 9, 46 (1958).

---

**An Apparatus for Bioassaying the Pheromones of Moths.** Shozo TAKAHASHI and Chikayoshi KITAMURA (Pesticide Research Institute, College of Agriculture, Kyoto University, Kyoto, Japan) Received October 7, 1970, *Botyu-Kagaku* 35, 130, 1970.

18. ガルの性フェロモンの生物検定装置について。高橋正三, 北村実彬 (京都大学 農学部 農薬研究施設) 45. 10. 7. 受理。

ヤガ類の性フェロモンの定量的生物検定について, Shorey らは新しい装置を考案した。我々は, この装置が他の種類のガの性フェロモンの定量的生物検定にも使用できるかを試し, 改良を加えた。それを用いて, カイコ, エリサン, スジマダラメイガについて, それぞれ性フェロモンに対する BR<sub>50</sub> を求めた。

#### Introduction

An quantitative bioassay apparatus with completely closed air flow system was used for the

sex pheromone of the noctuid moths by Shorey *et al.*<sup>1)</sup> Availability of the apparatus in the bioassay of the other species of moth was tested for the silkworm moth, the eri-silkworm moth and the