ARTICLES

TOXICITY OF CERTAIN OIL EMULSIONS TO MOSQUITO LARVAE AND PUPAE

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During 1940–42 the writer conducted special investigations in Oregon to increase the efficacy of oils against mosquito larvae and pupae through the use of emulsifying agents or spreaders, or both. The investigations demonstrated that certain emulsifiers greatly increased the efficacy of a given amount of oil against larvae and pupae. Results of field tests and suggestions for the use of certain formulations in mosquito control were published in 1943.[1] About this time the use of DDT for controlling mosquito larvae was developed. The performance of this and other available mosquito larvicides was so vastly superior that there seemed to be little need for a practical consideration of oils alone.

In recent years, however, strains of mosquitoes that have developed a marked resistance to DDT have appeared in certain parts of the United States. Experience with similar resistance in the house fly indicates that this problem may become more general and widespread. It may be necessary, therefore, to resort to oils for controlling mosquito larvae where the resistance problem is acute.

The control of mosquito pupae with DDT has always been unsatisfactory. The application of oil emulsions containing special emulsifying and spreading agents may therefore be advantageous for controlling mosquito pupae, because pupae are inherently quite resistant to insecticides such as the chlorinated hydrocarbons.

This paper presents data obtained with some of these emulsions in the field as well as in the laboratory. Since the efficacy of oils against larvae, and particularly against pupae, was increased, the information given here may stimulate other workers to investigate emulsifying agents as a means for improving the control of mosquito larvae and pupae.[2] Further studies along this line are now under way at the Bureau's Corvallis, Oregon, laboratory.

LABORATORY TESTS

In the laboratory tests enameled pans about 11 inches in diameter and 4½ inches deep with sloping sides were used. Into each pan was placed 3 liters of water. The water was about 2½ inches deep and had a surface area of 80 square inches. No. 2 fuel oil or diesel oil obtained locally was used. Emulsions of the oils were added to the water in desired amounts with a pipette. Various oil emulsifiers were used in comparison with coconut oil soap, which was the standard emulsifier for the New Jersey pyrethrum larvicide. A stock emulsion (without the addition of pyrethrum) was prepared by mixing 2 parts of oil and 1 part of water containing 6 percent of coconut oil soap, and was diluted with water as desired. The same preparation with pyrethrum added was also used in some tests. Although this pyrethrum emulsion had been used successfully in New Jersey and other states in

[1] E. F. Knipling, C. M. Gjullin, and W. W. Yates. A New Oil-Emulsion Mosquito Larvicide. U. S. Bur. Ent. and Plant Quar. E-587, issued April 1943.

[2] D. M. Jobbins, of the New Jersey Agricultural Experiment Station, encouraged the writer to publish some of the data obtained.

the East, its performance in Oregon was erratic and generally undependable.

Larvae and pupae of *Aedes vexans* Meigen and *A. sticticus* Meigen were used in most of the laboratory tests. The eggs were collected in soil and debris along the Columbia River, and the larvae and pupae were reared in the laboratory. In most cases the ratio of *A. vexans* to *A. sticticus* was approximately 3:1. The two species were about equally susceptible to insecticides. Some tests were also made with other species of mosquitoes.

Most of the emulsifiers selected for comparison with coconut oil soap were oil-soluble. Three percent of the emulsifier was added to the oil before it was diluted with water.

All applications were calculated on the basis of a given amount of oil per acre of water surface. Usually 25 fourth-instar larvae or 10 to 25 pupae were used in each test. Mortality records were taken after 24 and 48 hours, but results are given for the 24-hour period only as they are considered more valid. Moribund larvae were included in calculating mortality. A larva was considered moribund when it could not remain on the surface. Experience indicated that larvae in such condition following exposure to oils would soon die.

The results with some of these tests are presented in Tables 1, 2, and 3.

Table 1 shows that an emulsion made with Nopco 1216 [3] required less than half as much oil to kill *A. vexans* larvae as an emulsion made with coconut oil soap. Furthermore, a given amount of oil emulsified with the Nopco emulsifier proved equally as toxic as oil containing pyrethrum and emulsified with coconut oil soap. The emulsifier B-1956 [4] was only slightly less effective than Nopco 1216. Several other emulsifiers tested at about the same time showed only slight or no advantage over coconut oil soap.

Table 2 shows additional data obtained with Nopco 1216 and also the results with

TABLE 1.—Toxicity of fuel oil emulsified with coconut oil soap, Nopco 1216, and B-1956 to fourth-instar larvae of *Aedes vexans* and *A. sticticus*. Laboratory tests.

Oil Concentrate (figures in percent)	Dilution	(gallons per acre) Emulsion	Oil	Number of tests	Percent mortality after 24 hours
Oil 66.7, water 31.3, and coconut oil soap 2.0	1:4	10	1.3	5	22
		15	2.0	5	66
		20	2.6	5	90
Same as above with pyrethrins 0.07 (New Jersey larvicide)	1:9	5	.33	6	34
		7.5	.5	10	56
		10	.67	11	68
		15	1.0	7	87
		20	1.3	1	100
Oil 97 and Nopco 1216 3	1:6.5	5	.67	11	57
		7.5	1.0	7	92
		10	1.3	8	99
		15	2.0	7	99
		20	2.6	4	100
Oil 97 and B-1956 3	1:6.5	10	1.3	3	92
		15	2.0	3	100
		20	2.6	3	100

[3] A sulfated sperm oil produced by the Nopco Chemical Company.

[4] A phthalic glyceryl alkyl resin produced by Rohm and Haas Company.

TABLE 2.—Toxicity of diesel oil emulsified with Nopco 1216 and Amine 230 X to fourth-instar larvae of different species of mosquitoes.

Species	Emulsified with Nopco 1216			Emulsified with Amine 230 X		
	Gallons of oil per acre	Number of tests	Mortality after 24 hours	Gallons of oil per acre	Number of tests	Mortality after 24 hours
Aedes vexans	0.5	4	52	0.5	4	34
A. sticticus	.75	5	85	.75	3	81
	1.0	5	91	1.0	5	94
	1.5	1	100	1.5	1	100
A. cinereus	0.5	3	99	0.5	3	82
	1.0	3	100	1.0	2	100
	1.5	2	100	1.5	1	100
A. hexadontus	1.0	2	100	...	..	..
	1.5	1	100	...	..	..
C. pipiens [1]	1.0	6	61	1.0	6	74
	1.5	6	79	1.5	6	97

[1] Diesel oil emulsified with coconut oil soap in one test at 2 gallons of oil per acre caused 68% mortality.

another promising emulsifier called Amine 230 X.[3] Data in this table show the effectiveness of the emulsifiers used with diesel oil against several species of mosquitoes. Amine 230 X in general proved equally as effective as Nopco 1216. Larvae of *A. cinereus* Meigen and *A. hexadontus* Dyar were found to be at least as susceptible as *A. vexans* to the emulsified oils. *Culex pipiens* L. was indicated to be more resistant than the *Aedes* species, although a rather high mortality was obtained with relatively small amounts of emulsified oil.

Table 3 shows the relative effectiveness of fuel oil to *A. vexans* and *A. sticticus* pupae when emulsified with Nopco 1216 and coconut oil soap. Data are also given for certain quantities of oil containing pyrethrins applied in the form of the New Jersey larvicide. The fuel oil alone emulsified with Nopco 1216 was far more effective than the coconut oil soap emulsions with or without the pyrethrins. With Nopco 1216 only one-sixth to one-eighth the amount of fuel oil was required to produce a given mortality as when coconut oil soap was used. Oil alone emulsified with Nopco 1216 was two to three times as effective as the oil containing pyrethrum emulsified with coconut oil soap.

Tests were also run to compare the efficacy against pupae of oils emulsified with Nopco 1216 and Amine 230 X. These tests were made with the diesel oil employed by the Portland (Oregon) Mosquito Abatement District. Table 3 shows that the diesel oil was at least as effective as fuel oil, and that the two emulsifiers were about equal in performance.

SEMI-FIELD TESTS

Further tests under controlled conditions were run in natural mosquito-breeding waters. Galvanized-iron cylinders 11 inches in diameter and 20 inches long, inside of which screens were soldered 4 inches from the bottom, were set up in the water by forcing them into the bottom soil.

Laboratory tests (data not given) utilizing waters from various sources had shown that various contaminants, such as soil, hay infusion, etc., greatly influenced the effectiveness of oils, particularly the New Jersey pyrethrum larvicide. It seemed im-

[3] An 18-carbon-chain complex amine produced by the Carbide and Carbon Chemical Corporation.

TABLE 3.—Susceptibility of *Aedes vexans* and *A. sticticus* pupae to various types of oil emulsions.

Oil concentrate (figures in percent)	Gallons of oil applied per acre [1]	Number of tests	Total number of pupae	Percent mortality after 24 hours
Fuel oil 66.7, water 31.3, and coconut oil soap 2.0	1.0	4	100	2
	1.3	2	30	3
	2.0	10	200	44
	2.6	5	70	46
	3.3	4	60	70
	4.0	2	40	100
Same as above with pyrethrins 0.07 (New Jersey larvicide)	.67	2	20	35
	1.0	10	180	66
	1.3	1	10	80
	1.67	1	10	60
Fuel oil 97 and Nopco 1216 3	.3	5	90	51
	.5	9	200	88
	.65	10	130	100
	1.3	8	90	100
Diesel oil 97 and Nopco 1216 3	.25	4	100	81
	.5	2	50	100
	1.0	2	50	100
Diesel oil 97 and Amine 230 X 3	.25	5	125	77
	.5	2	50	100
	1.0	2	50	100

[1] The dilution rates were the same as given in Table 1.

portant, therefore, to conduct laboratory-type tests, yet in the same water and under conditions as they exist in the field. The method proved satisfactory. Larvae from the breeding area were collected with dippers and placed in the water inside the cylinders. One hundred fourth-instar larvae (practically all *A. vexans*) were used in each test.

Results with the New Jersey larvicide and the oil emulsion made with Nopco 1216 are given in Table 4. The data show that under simulated natural conditions fuel oil alone emulsified with Nopco 1216 was far more effective than fuel oil containing pyrethrum and emulsified with coconut oil soap.

The tests in the cylinders fully confirmed the findings in the laboratory. Actually the results with the Nopco oil

TABLE 4.—Comparative effectiveness of the New Jersey larvicide and the oil emulsion containing Nopco 1216 against fourth-instar larvae of *Aedes vexans* in natural breeding areas. Semi-field tests; emulsions prepared with water from the breeding area.

Rate of application (gallons per acre)		Number of tests	Percent mortality after 24 hours
Emulsion	Oil		
New Jersey Larvicide diluted 1:9			
15	1.0	1	24
25	1.7	2	74
35	2.3	2	82
Fuel oil (96%) plus Nopco 1216 (4%) diluted 1:6.5			
10	1.3	1	99
15	2.0	2	100
25	3.3	1	100

emulsion were more favorable in the semi-field tests than in the laboratory.

FIELD TESTS

Several field tests with diesel or fuel oil emulsified with 4 percent of Nopco 1216 were made under a wide range of conditions in the summer of 1941. The emulsions were prepared by diluting the mixture with water from the breeding area. The sprays were applied with a knapsack sprayer.

C. M. Gjullin and W. W. Yates of the Portland laboratory conducted additional tests during 1942, the results of which have been reported in publication E-587.

The field tests were conducted against a number of species, both larvae and pupae, and in various types of breeding areas, including heavily polluted waters. Excellent control was obtained in all tests when the total amount of oil applied amounted to 5 gallons per acre.

DISCUSSION

These investigations definitely established that the emulsifier can have a marked influence on the efficacy of oil used in emulsions against mosquito larvae and pupae, and particularly against pupae. With Nopco 1216 and Amine 230 X as emulsifiers a given quantity of oil was more toxic to pupae than to larvae.

The addition of emulsifiers such as Nopco 1216 greatly increased the spreading properties of the oil. In the opinion of the writer the reason for increased larvicidal action of the oil containing the emulsifiers was due to its lowered surface tension, which permitted penetration into the tracheal tubes of both larvae and pupae more readily. It appeared that the effects on the larvae were largely physical—that an oil film spread over the spines and walls of the siphon tubes and made it impossible for them to remain suspended at the surface. Evenutally such larvae died by drowning, although the oil alone in sufficient quantities will kill larvae.

That kills were due in part at least to drowning was shown in special tests in which dissolved oxygen was removed from tap water (by boiling or by addition of sodium sulfite) before the oil larvicide was applied. Larvae in treated water containing less than 1 p.p.m. of dissolved oxygen were killed within 2 hours, whereas the same amount of oil in tap water containing about 9 to 10 p.p.m. of oxygen did not kill the larvae until they had been held for 24 hours.

SUMMARY

Tests conducted at Portland, Oregon, during 1940–42 showed that fuel oil or diesel oil emulsions made with the oil-soluble emulsifiers Nopco 1216, Amine 230 X, and B-1956 increased the efficacy of the oils in killing larvae of several species of mosquitoes. A given quantity of oil emulsified with these materials and applied to the water surface was about twice as effective as the same amount of oil emulsified with coconut oil soap. In the laboratory oil emulsified with the oil-soluble emulsifiers was comparable in effectiveness with oil containing pyrethrum and emulsified with coconut oil soap, as used in the New Jersey larvicide. In semi-field tests the oil alone plus the emulsifier Nopco 1216 was two or three times as toxic as the New Jersey larvicide.

Nopco 1216 and Amine 230 X increased the efficacy of fuel or diesel oil against pupae of *Aedes vexans* Meigen to a much greater extent than against larvae. A given quantity of oil applied with these emulsifiers was six to eight times as toxic as when applied with coconut oil soap and about three times as toxic as the coconut oil soap emulsion containing pyrethrum.

Field tests against various species of larvae and pupae confirmed the laboratory results.