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CHEMOSTERILIZATION OF LABORATORY-REARED AEDES AEGYPTI (L) BY SUBSTITUTED—PHOSPHINE OXIDES AND PHOSPHORUS AMIDES

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Abstract. Toxicity and sterilizing activity of alkylating phosphine oxides were greater than nonalkylating phosphorus amides. Among phosphine oxides the activity appeared to decrease with increasing size of the radicals. Sulphur-containing phosphorus amides were much more effective sterilants.

Male mosquitoes were found to be more susceptible to sterilization than females and when both sexes were treated the effect was cumulative. Second instar larvae of *A. aegypti* (PCSIR strain) appeared to be more susceptible to these chemosterilants in both homologous series reported here.

Chemosterilization studies of larvae of the yellow-fever mosquito, *Aedes aegypti* (L) have been undertaken by various workers. The susceptibility of larvae of this mosquito to Apholate, Tepa and Metepa has been amply documented. White tried Thiotepa on the larval stages of *Aedes aegypti*. Hafez *et al.*[2] investigated the effects of Tepa and Hempa on *Anopheles pharoensis*.. Recently Madhukar *et al.* [3,4] have worked on the second instar larvae and pupae of *Aedes aegypti* with aziridinyl compounds (phosphine oxides and phosphrous amides). Shams and Qureshi[5] reported the chemosterilization studies on third instar larvae of *Aedes aegypti* (PSCIR strain) with substituted phosphine oxides and methylmelamines. Phosphine oxides and some of the phosphorus amides reported here have already been screened against a different strain of *Aedes aegypti*.

The activity of the chemosterilant is known to vary under different experimental conditions, methods of applications, stages of development of insect, sex, concentrations and treatment times, besides the strain specificity. The present studies were undertaken to study the sterilization of laboratory-reared *A. aegypti* at second stage with alkylamino-phosphine oxides and phosphorus amides under laboratory conditions, The studies also include the data on induced mortality and sterility.

Materials and Methods

The candidate compounds in the first category are biaziridinyl phosphine oxides with different substituents on phosphorous with the general structure $R\text{-}PO(N\Delta)_2$, where $R=CH_3NH$, C_2H_5NH, C_3H_7NH, iso-C_3H_7NH, C_4H_9NH, $C_8H_{17}HN$. The compounds in the second category are nonalkylating bi and triamides of phosphoric acid related to Hempa (hexamethylphosphoric triamide). One of them (AI3-50918) is the amide of phosphorothioic acid. The full names of these compounds are as under:

AI3-50918. Hexamethylphosphorothioic triamide.
AI3-51007. N,N,N′,N′-tetramethyl-*p*-piperidine-phosphonic diamide.
AI3-51008. N,N,N′,N′-tetramethyl-*p*-morpholino-phosphonic diamide.
AI3-51128. N,N,N′,N′-tetramethylphosphoric-tiamide.
AI3-51136. Bis-(dimethylamino)-tetramethyl guanidinylphosphine oxide.
AI3-51163. Bis-(dimethylamino)-isopropylamino-phosphine oxide.
AI3-51164. 3-Bis (dimethylamino) phosphinyl carbazic acid, *t*-but-*ester*.
AI3-51295. Pentamethylphosphoric triamide.
AI3-61117. N,N,N′,N′-tetramethyl-*p*-(4-methyl-I-piperazinyl) phosphonic diamide.
AI3-61175. N,N,N′,N′-tetramethyl-*p*-I-pyrrolidinyl-phosphonic diamide.
AI3-61177. N,N,N′,N′-tetramethyl-*p*-(4-methyl-I-piperidino) phosphonic diamide.

The procedures used for the tests were a modification of that reported by Madhukar *et al.*[3,4] In each experiment 100 second stage larvae were exposed in 250 ml water containing various concentrations of chemosterilants at 83±5°F and 84±4% R.H. No food was provided for 24 hr, but on the second day of treatment, larvae were fed yeast and kept in the treated medium till pupation. The mortality of larvae was recorded daily. The pupae were removed, washed in water and placed in cages for emergence. The per cent control of reproduction reported in these experiments is on mixed sexes; sex specificity tests for some of the effective compounds were also undertaken by segregation of male and female pupae according to the method of Weidhaas.[6]

The males were fed on 1% glucose solution soaked in cotton, while females were brooded on rats on every third day for 15 days. The females were allowed to lay eggs on moist filter papers placed on the inner sides of plastic bowls containing water. A sample of 100 eggs taken at random from each replicate was observed for the hatchability and corrected per cent hatchability was calculated by the formula of Crystal.[7]

$$\text{Corrected \% hatch} = \frac{\text{Test \% hatch}}{\text{Control \% hatch}} \times 100$$

The per cent control of reproduction induced by each chemosterilant was calculated by the formula of Chemberlain.[8] Each experiment reported here was replicated twice.

Results

Tables 1 and 2 show the data on the toxicity and sterilizing effects of the candidate compounds. In general phosphine oxides were more toxic to larvae than phosphorus amides, but no obvious structure–toxicity relationship was evident within each group. The mortality of the adutls after treatment with phosphine oxide was also noted to be very high.

The sterilizing activity of the phosphine oxides was quite high at 2.5–5 p.p.m. concentrations (Table 1). These compounds were obviously more active than the phosphorous amides (Table 2). Among the phosphine oxides, ethyl (2), propyl (3) and isopropyl (4) compounds indicated higher sterilizing activity than methyl (1), butyl (5) and octyl (6) compounds and beyond 2.5 p.p.m. the larvae did not pupate with these three compounds (2, 3 and 4, Table 1). None of the larvae survived beyond 30 days.

The activity of phosphine oxides appeared to decrease with increasing size of the alkyl radicals.

Among the phosphorus amides the sulphur-containing compound (AI3-50918) at 10 p.p.m. induced 65.88% control of reproduction (Table 2). It was toxic beyond this dose. The effects of compounds (3, 5 and 10, Table 2) at 50 p.p.m. indicated higher sterilizing activity than other amides tested at 100–250 p.p.m. Table 3 shows the results of sex specificity of five selected compounds from both series described in this paper. Males were found to be more susceptible to sterilization than females, and as expected the accumulated effects on the control of reproduction was higher when both sexes were treated.

Nonalkylating phosphorus amides (4 and 5, Table3) also prove less effective than alkylating phosphine oxides (1–3) when tested in separate sexes.

Discussion

Toxicity and sterility induced by alkylating biaziridinyl phosphine oxides were much more than nonalkylating phosphorus amides. This is due to the fact that structurally phosphine oxides are close to Tepa while phosphorus amides are close to nonalkylating Hempa which is a relatively unreactive, thermally stable compound as compared to Tepa (a highly reactive, thermally unstable, alkylating agent). Chang and Borkovec[9] reported the activity of Hempa in male houseflies much lower than that of well-known chemosterilant Tepa. Fye *et al.*[10] observed the activity of nonalkylating phosphoramides much lower than that of similar aziridinyl derivatives when rested against houseflies. Hempa has already been tried by the present authors against third instar larvae of *A. aegypti* (PCSIR strain) and was found to be less effective as compared to alkoxy phosphine oxide. The sterilizing activity of the ethyl (2), propyl (3), and isopropyl (4) phosphine oxides indicated higher sterilizing activity as compared to the methyl compound (1, Table 1). Madhukar *et al.*[3] reported that the activity of these compounds decreased with increasing size of the radical, when tested against second stage larvae of a different strain of *A. aegypti*. They reported the highest control of reproduction with the methyl compound. Borkovec *et al.*[11] also reported the methylamino derivative of biaziridinyl phosphine oxide as the most potent male housefly sterilant with an activity higher than Tepa. The present authors also observed a decrease in activity of these chemosterilants with increasing size of the alkyl radical except for the methyl (1) compound. This difference of susceptibility may be due to partial solubility of this compound in the solvent used in the present test which could cause a difference in the rate of uptake of this compound by PCSIR strain.

The mortality caused by these compounds was much higher than reported by Madhukar *et al.*[3] This indicates that PCSIR-strain of *A. aegypti* was more susceptible to phosphine oxides as compared with the strain reported by them.[3]

Among the phosphorus amides (Table 2), toxicity of the thio-compound (1) was the highest among the series. This observations is in line with the report of Madhukar *et al.*[4] Terry and Borkovec[12] reported the activity of hexamethylthiophosphoric triamide as effective as Hempa among the 50 phosphoramides investigated for thier activity against housefly.

The sterilization capacity of this compound was greater than Hempa when results were compared with an earlier publication by the present authors.[5] Seawright *et al.*[13] compared the absorption rate of PO and PS compounds (Tepa and Thiotepa) and found that pupae of *C.P. quinquefasciatus* absorbed PS compound 4–10 times more than PO compound Tepa and any given concentration of Thiotepa induced a much higher degree of sterility than the same concentration of Tepa. Lofgren *et al.*[14] also compared a homologous series of PS and PO compounds and reached the same conclusion. The other phosphorus amides (2–11) had less pronounced differences of activity between them. Because of the uncertain relationship between the concentration of the agent and its absorption by the organism (Madhukar *et al.*[4]) only qualitative effects of the structural variables can be gleaned from the present data.

When the results of toxicity induced are compared with Hempa it seems evident that Hempa is comparatively less toxic than some of the phosphorus amides reported here.

When the results of three amides (AI3–51295, AI3–51007 and AI3–50918) which were also included by Madhukar *et al.*[4] in their experiments were compared with the data reported here, it become evident that PCSIR–strain was more susceptible to these compounds. The data on sex specificity with 5 selected compounds showed that male were more susceptible to sterilization than females, and the

TABLE 1. MORTALITY AND REPRODUCTION OF *Aedes aegypti* TREATED WITH PHOSPHINE OXIDES FROM THE SECOND LARVAL INSTAR UNTIL PUPATION IN VARIOUS CONCENTRATIONS OF THE CHEMOSTERILANTS IN AQUEOUS SOLUTIONS.

No.	Compound A 13 No.	Concn p.p.m.	Mortality %	Average No. of eggs/♀	Hatch %	Control of reproduction %
1	51254	5.0	38	23.30	88.41	66.79
2	50787	2.5	4	13.79	13.48	97.00
3	51253	2.5	13	6.62	—	100.00
4	51256	2.5	4	3.00	—	100.00
5	51028	5.0	15	10.60	32.62	94.10
6	50788	10.0	49	14.90	44.30	92.52
7	Control	—	2	66.57	98.15	—

TABLE 2. TOXIC AND STERILIZING EFFECTS OF PHOSPHORUS AMIDES IN *Aedes aegypti* SECOND INSTAR LARVAE OF MIXED SEXES REARED IN TREATED WATER UNTIL PUPATION.

No.	Compound A 13 No.	Concn p.p.m.	Mortality %	Average No. of eggs/♀	Hatch %	Control of reproduction %
1	50918	25	100			
		10	49	43.57	75.41	65.88
2	51007	150	63	17.53	39.38	92.96
		100	57	29.38	64.23	80.40
3	51008	100	100			
		50	16	40.44	38.57	83.79
4	51128	250	71	24.71	55.48	84.62
		100	41	68.80	78.05	44.21
5	51136	100	100			
		50	53	62.80	91.19	40.58
6	51163	150	31	51.76	43.79	75.41
		100	27	58.50	85.48	48.06
7	51164	250	63	43.50	75.87	63.12
		100	31	54.25	85.14	52.02
8	51295	250	66	27.22	53.45	74.62
		100	31	50.90	70.53	62.73
9	61117	250	73	35.76	64.80	75.84
		100	30	48.00	72.91	63.64
10	61175	100	93			
		50	62	38.81	72.09	73.57
11	61177	250	64	34.80	48.70	82.31
		100	39	52.00	67.96	63.29
12	Control	—	13	105.88	90.93	—

TABLE 3. SEX SPECIFICITY OF SOME OF THE MOST EFFECTIVE PHOSPHINE OXIDES AND PHOSPHORUS AMIDES IN *Aedes aegypti* BY LARVAL TREATMENT UNTIL PUPATION.

Compound A 13 No.	Concn p.p.m.	Sex treated	Average eggs/♀	Hatch %	Control of reproduction %
50787	2.5	M	33.67	15.34	91.65
		F	17.96	78.72	77.11
		Both	13.79	13.48	97.00
51028	5.0	M	22.60	27.66	90.97
		F	25.50	66.66	72.54
		Both	10.60	32.62	94.10
50788	10.0	M	29.77	53.54	90.42
		F	21.83	70.08	81.54
		Both	14.90	44.30	92.52
		Neither	66.57	98.15	
51008	50.0	M	42.57	43.88	78.85
		F	34.33	80.83	68.57
		Both	40.44	38.57	83.79
61175	50.0	M	63.75	77.47	51.55
		F	72.22	46.57	36.79
		Both	38.81	72.09	73.57
		Neither	105.88	90.93	

M, Male; F, Female.

accumulated effects on mixed sexes resulted in greater control of reproduction (Table 3). This effect is in agreement with previously reported findings of other workers.[3,14,15,16]

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