Effect of insecticide .Resistance on Reproductive Potential in Malaria Mosquito

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ABSTRACT

The third instar larvae of Anopheles stephensi were used to study effect of sub-lethal concentrations of three organophosphorus insecticides on fecundity, egg hatchability and sex-ratio. Fenthion was most effective (LC50 — 0.0014 ppm.), followed by methyl-Parathion (0.0063 ppm.) and Malathion (0.145 ppm.). Treatment with Fenthior. and methyl-Parathion decreased egg production and egg hatchability, along with sex-ratio distortion towards males in the treated strains and their F.; progeny. However, Malathion treated strain also showed a decrease for the same, out their Ft progeny indicated an increase (statistically insignificant), as compared to "control". All the above strains regained normal condition in the F2 generation. It is interesting to note that toxic effect of above insecticides is highly variable on reproductive potential to An. stephensi.

Key Words: Anopheles. stephensi, Organophosphorus insecticides, fecundity, egg-hatchability, reproductive potential.

Introduction

Anopheles. stephensi is one of the potential carriers of malaria in Indian sub-continent. It belongs to the order `Diptera' of the group `Cellia'. The mosquitoes develop insecticide resistance mainly due to agricultural applications (World Health Organization[15]). Though, Anopheline mosquitoes like An.stephensi have developed resistance for various insecticides, yet in developing countries including India, malaria eradication programme is solely dependent upon chemical control especially by using Organophosphorus (OP) and Carbamate insecticides.

The OP-insecticides have been presently used as larvicides/adulticides for the control of insect-pests, mosquitoes including An.stephensi (Brown[12]). The effect of insecticides on the fecundity and fertility of insects have been reviewed by several workers including Knutson[2] Affifi and Knutson[3], Ouyer and Knutson[4], Knutson[5], Adkission and Wellso[6], Gilotra[7], Georhiou[8], Grosch[9], Ferrari and Georghiou[10], Verma[13], Weide Liu et.al[14]. Since the information is scanty regarding impact of OP-insecticides on reproductive potential of An. stephensi, experiments were designed for the present investigation.

Materials and Methods

An. stephensi was collected as gravid females at Bangalore (WG), from cattle sheds, huts and human dwellings using an aspirator and torch light. The WG-strain was reared in Laboratory, free from insecticides for ten generations. Three experimental strains (synthesized from WG-strain) treated with Fenthion, methyl-Parathion and Malathion have been designated as "FN", "mP" and "ML" strains, respectively. The untreated WG-strain was used as "Control". The LC_{50} values of above insecticides for the WG-strain were obtained from log dosage percent mortality linear regression relationship. If mortality of larvae in 'Control' exceeded 10%, corrected mortality was obtained by using the formula of Abbott[1].

Organophosphorus insecticides

Fenthion

O, O-dimethyl 0-(3-rnethy! 1-4-methylthiophenyl) Phosphorothiote.

Fenthion was obtained as an emulsifiable concentrate containing 82.50% w/w Fenthion, from the Health Department, Bangalore City Corporation (BCC), Bangalore, India.

Methyl-Parathion

O,O-dimethyl O-(4-nitrophenyl) Phosphorothioate.

It was obtained in the form of technical concentrate (80%), from Rallies India Ltd., Bangalore, India.

Malathion

O,O-dimethyl S-(1, 2-dicarbethoxy) ethyl phosphorodithioate.

Malathion was obtained as technical grade concentration from World Health Organization (WHO), Regional Office, New Delhi, India.

Batches of one hundred third instar larvae (in three replicates), were exposed to sub-lethal concentrations of Fenthion (0.0001 and 0.0005 ppm.), methyl-Parathion (0.001 and 0.005 ppm.) and Malathion (0.01 and 0.05 ppm.) for 24 hrs. The exposed larvae were washed with tap-water, and reared in a white enamel pan (12" x 8") containing tap water. The larvae were fed with yeast tablets (Cyano-Pharma). The pu0e were collected in wide-mouthed bottles and upon emergence released into 8" x 8" x 8" cages. After 5 days, mice was provided for blood-meal. About 20-25 gravid females were randomly selected from each cage and aspirated individually into plastic vials containing tap-water lined with a strip of filter paper for oviposition.

The number of eggs laid by each female was counted under the dissection microscope to obtain egg production ratio. The egg hatchability (No. of larvae/No. of eggs x 1(l0) was calculated for each generation. The larvae of each female was reared as a single family. Precautions were taken to avoid selective mortality as a source of bias in collection of data for sex-ratio distortion. If mortality of larvae exceeded 10%, the entire family was discarded. The pupae upon emergence were screened and the number of freshly emerged males and females were scored for each female, at each generation. The ratio of male:female and number of female off-springs, produced by each female was also calculated for each generation.

Results

The data on the egg production ratio, percent egg hatchability and sex ratio distortion towards males in An. stephensi, for three generations (treated, F1, and F2), using two sub-lethal concentrations of each insecticide (Fenthion, methyl-Parathion and Malathion) have been given in Tables 1, 2 and 3. The LC50 values for above insecticides for WG strain have been represented in Figure 1.

TABLE 1

The effect of Fenthion on reproductive potential of 'An.stephensi

TABLE 1

The effect of Fenthion on reproductive potential of *An. stephensi*

Sample	Egg Production				Egg Hatchability					Sex Ratio distortion				
	Mosquitoes No. × replicates	Total eggs	Eggs/ Female	Ratio	Total Larvae	Larvae/ Female	Ratio	% hatch	Ratio	No. of Males	No. of Females	M:F	Females/ female	Ratio
Fenthion (FN)														
0.0001ppm	•25×3	4572	60.96	0.72	3484	46.45	0.60	76.20	0.84	1660	1824	0.91:1	24.32	0.67
0.0005ppm	20×3	3210	53.50	0.63	2095	34.92	0.45	65.26	0.72	946	1149	0.82:1	19.15	0.52
CONTROL-1	25×3	6393	85.24	1.00	5805	77.40	1.00	90.80	1.00	3076	2729	1.13:1	36.39	1.00
F1 of FN Strain														
0.0001ppm	15×3	2618	58.18	0.6e	1823	40.51	0.49	69.93	0.74	839	984	0.85:1	21.87	0.54
0.0005ppm	•20×3	2527	42.12	0.48	1398	23.30	0.28	55.32	0.59	601	797	0.75:1	13.28	0.33
CONTROL-2	25×3	6591	87.88	1.00	6198	82.64	1.00	94.04	1.00	3157	3041	1.04:1	40.55	1.00
F2 of FN Strain														
0.0001ppm	•20×3	5298	88.30	1.03*	4626	77.10	0.99	87.32	0.99	2371	2255	*1.05:1	37.58	1.01*
0.0005ppm	20×3	5720	76.26	0.89	4749	63.32	0.82	83.02	0.82	2418	2331	*1.04:1	31.08	0.83
CONTROL-3	25×3	6446	85.95	1.00	5814	77.52	1.00	90.29	1.00	3018	2796	1.08:1*	37.28	1.00

* Statistically insignificant

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TABLE 2

The effect of methyl-Parathion on reproductive potential of *An. Stephensi*

Sample	EGG Production				EGG Hatchbility					Sex Ratio Distortion				
	Mosquitoes No. × replicates	Total eggs	Eggs/ Female	Ratio	Total Larvae	Larvae/ Female	Ratio	% hatch	Ratio	No. of Males	No. of Females	M:F	Females/ female	Ratio
m-Parathion (mP-strain)														
0.001ppm	20× 3	3894	64.90	0.73	2886	48.10	0.57	74.11	0.78	1355	1531	0.89:1	25.50	0.65
0.005ppm	20× 3	3313	55.22	0.62	2065	34.42	0.41	62.33	0.66	931	1134	0.82:1	18.90	0.48
CONTROL-4	25× 3	6627	88.36	1.00	6281	83.75	1.00	94.78	1.00	3332	2949	1.13:1	39.32	1.00
F_1 of mP-strain														
0.001	25× 3	4659	62.12	0.72	3348	44.64	0.55	71.86	0.76	1608	1740	0.92:1	23.20	0.61
0.005	20× 3	2984	49.73	0.58	1760	29.33	0.36	58.98	0.63	810	950	0.81:1	15.83	0.41
CONTROL-5	25× 3	6472	86.29	1.00	6097	81.29	1.00	94.21	1.00	3231	2866	1.13:1	38.21	1.00
F_2 of mP-strain														
0.001	25× 3	6471	86.28	0.96	5803	77.37	0.92	89.68	0.96	3019	2784	*1.08:1	37.12	0.92
0.005	25× 3	5863	78.17	0.87	4721	62.95	0.75	80.52	0.86	2408	2313	*1.04:1	30.84	0.77
CONTROL-6	25×3	6744	89.92	1.00	6284	83.79	1.00	93.18	1.00	3268	3016	1.08:1	40.21	1.00

* Statistically insignificant

TABLE 3

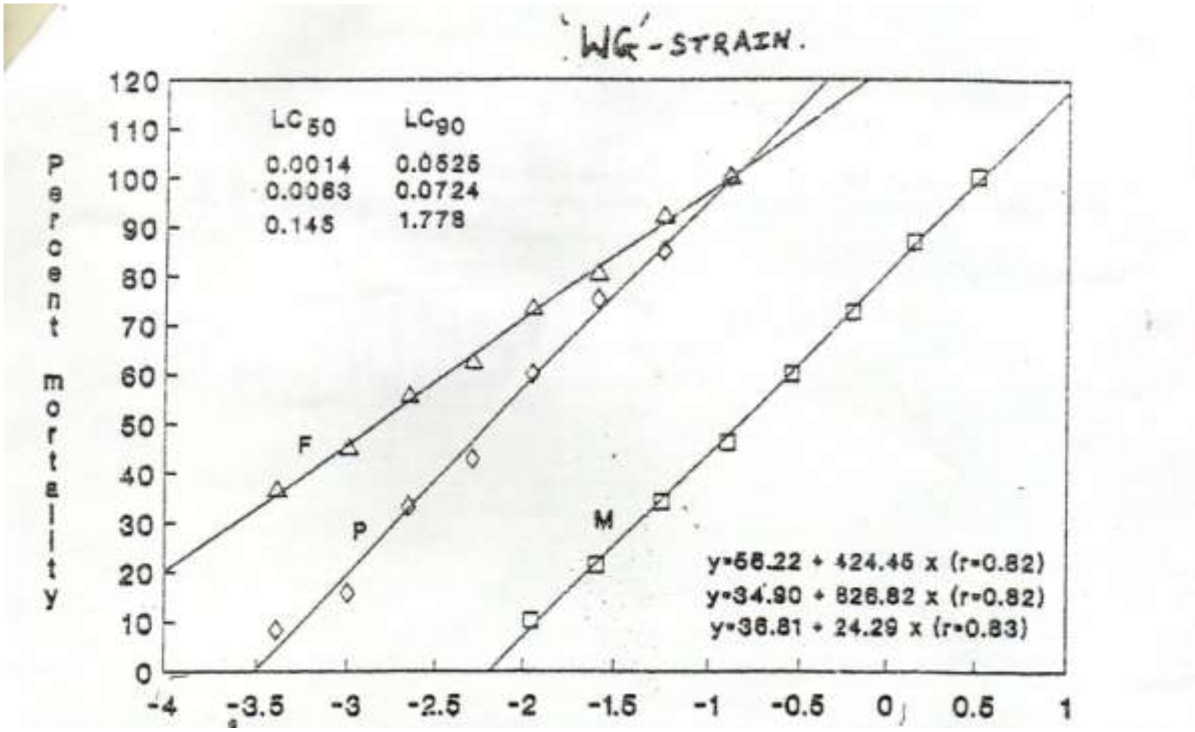
The Effect of Malathion on reproductive potential of *An. stephensi*

Sample	EGG Production				EGG Hatchability					Sex Ratio Distortion				
	Mosquitoes No. × replicates	Total eggs	Eggs/ Female	Ratio	Total Larvae	Larvae/ Female	Ratio	% hatch	Ratio	No. of Males	No. of Females	M:F	Females/ female	Ratio
Malathion (ML-strain)														
0.01ppm	23× 3	4278	62.00	0.76	3389	49.12	0.66	79.22	0.88	1627	1762	0.92:1	25.54	0.72
0.05ppm	20× 3	3494	58.23	0.71	2436	40.60	0.55	69.72	0.77	1194	1242	0.96:1	20.70	0.58
CONTROL-7	25× 3	6152	82.03	1.00	5564	74.19	1.00	90.44	1.00	2893	2671	1.08:1	35.61	1.00
F_1 of ML-strain														
0.01 ppm	20× 3	4984	83.07	1.03*	4758	79.30	1.07*	95.47	1.04*	2474	2284	*1.08:1	38.07	1.05*
0.05 ppm	20× 3	4872	81.20	1.01*	4589	76.48	1.03*	94.19	1.03*	2432	2157	*1.13:1	35.95	0.99
CONTROL-8	25× 3	6023	80.31	1.00	5554	74.05	1.00	92.21	1.00	2833	2721	1.04:1	36.28	1.00
F_2 of ML-strain														
0.01 ppm	25× 3	5859	78.12	0.92	5245	69.93	0.88	89.52	0.96	2675	2570	*1.04:1	34.27	0.90
0.05 ppm	25× 3	6051	80.68	0.95	5209	69.45	0.87	86.08	0.87	2656	2553	*1.04:1	34.04	0.90
CONTROL-9	25× 3	6345	84.60	1.00	5928	79.04	1.00	93.43	1.00	3083	2845	1.08:1	37.93	1.00

*Statistically insignificant.

Discussion

Among various OP-insecticides used for present study, Fenthion is effectively used against strains resistant to chlorohydrocarbons; methyl-Parathion is biologically active due to presence of 'Nitro' group and Malathion is employed for eradication of Anopheline mosquitoes (Buche1111). in mosquitoes, the use of malathion usually induces a type of resistance restricted to Malathion only, due to detoxication by a carboxyesteras enzyme whereas the other OP-insecticides induce a general OP-resistance, due to phosphatase detoxication (Brown[12]).



Log (Concentration of Insecticide-ppm)

Fig. 1. Dosage mortality regression lines for third instar Larva of Anopheles stephensi Liston to Fenthion (F). methyl-Parathion (P) and Malathion (M).

The present investigation revealed that FN-strain ofAn.stephensi showed decreased egg production by 28% and 37%, followed by 27% and 38% decrease in mP-strain, along with 24% and29% decrease in ML-strain for low and high dosages, respectively, against control. The F_1 progeny of FN-strain showed; remarkable depression of egg production by 34% and 52%, followed by 28% and 42% decrease in mP-strain. However, F_1 of =IN/11,' strain showed increase by 3% and 1% for low and high dosages. The F_2 showed a comparative lower reduction Late in egg production with 11%, 13% and 5% decrease for FN', ‘mP’ and 'Ml’ strains respectively, thereby indicating a tendency of reversion to normal condition as compared to "Control" (Weide Liu, et. al.[14]).

The egg hatchability of FN-strain in F1 progeny showed a drastic decrease by 51% and 72%, followed by 45% and 64% decrease for the mP-strain, whereas ML-strain showed an increase by 7% and 3%, at low and high dosages, against control.

The present study supports the investigation of Knutson2, that D. melanogaster produced 7.60% more eggs when treated with a sub-lethal dosage of dieldrin. Affifi and Knutson[3] reported that houseflies treated with dieldrin produced 62.9% and 9.3% more eggs in the F_1 and F_2 respectively against the control. However, Gilotra7 found no pertinent differences in either fecundity or fertility of Anopheles albimanus to dieldrin. Weide Liu et. al.,[14] showed that egg production was reduced by d-allethrin by 69% and 45% in low and high dosages respectively compared to the 'Control ; d-phenothrin increased egg production by 33% at low dose, but decreased by 30% at high dose relative to the 'Control', Ft showed 75% and 58% decrease for low and high doses, whereas F2 showed reversion to normal egg production.

The sex-ratio distortion (males:females) of FN-strain in the F_1 generation was found to be 0.82:1 and 0.72:1 followed by 0.81:1 and 0.72:1 for mP-strain at low and high dosages, respectively. However, mL-strain showed 1.03:1 and 1.07:1, against the control (1:1). The possibility of lower male-proportion,. as a consequence..of lower egg productionthat hatchability. or greater susceptibility towards insecticides than females, requires further elucidation of investigation regarding impact of insecticides on physiological mode of resistance, to confirm the reproductive potential of males in An. slephensi.

Conclusion

The significance of insecticide resistance on reproductive potential depends upon the inherent toxicity, selection pressure (dosage) and behaviour of mosquitoes towards the insecticidal application against the field populations. The occurrence of generally decreased fecundity and egg hatchability along with sex-ratio distortion towards mares following treatment with Fenthion, methyl Parathion and Malathion- does not appear to have been reported previously in An. stephensi.

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