Genetic Study of Malathion Resistance in Anopheles Stephensi: A Malaria Mosquito

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Abstract

The genetic mechanism of malathion resistance in Anopheles stepensi was investigated. A diagnostic dosage of 3.125 ppm malathion was used for instar larvae of various genetic crosses including presumptive homozygous resistant. susceptible, F_1 hybrids, backcrosses and F_2 generation. The data on resistance susceptibility and time-mortality relationship clearly snow that malathion resistance was incompletely dominant and autosomal in Anaphelesstephensi.

Key Words: Anopheles stephensi, Genetic crosses, Malathion resistance.

Introduction

The extensive ore of insecticides or control of insect pests has resulted in the development or multiple resistance to various chemicals among mosquito vectors. The inherited ability to detoxify insecticides require a greater understanding of genetic mechanism of insecticide resistance Inn effective research on vector control.

Anopheles stephensi is an important malaria vector in the Indian sub-continent. The insecticide-resistance in An. stephensi has been reported (Shidrawi, 1990), The genetic basis of insecticideresistance has been studied in a few species of mosquitoes (RathorandToquir 1981, Rowland 1985, Malcolm 1990, Hemingway 1992). However, very little information is available on genetics of malathion resistance in mosquitoes.

The present investigationdeals with the resistance susceptibility and time motality relationship to determine the genetic basis of malathion resistance in Anopheles Stephensi under laboratory condition.

Material and Methods

An stephensiListon was collected as gravid females from some cattle shed in Bangalore (WG), South India. The WG strain wasa reared and maintainedinour laboratory as a temperature of 25+/- 1^{0}C; relative humidity of 75+/-5% and photoperiod of 15h. The adults were fed on 10% sucrose. Females were provided with blood meal of mice. Enamel bowls containing tap water were lined with a strip of filter paper and placed inside the cages for oviposition. The larvae were provided with synthetic yeast Malathion(O, O-dimethyly S-(1,2-ddicarbethosy) ethyl phosphorodithoiate) was obtained as technical grade concentration from World Health Organization. Regional Office. New Delhi. India. It is employed in malaria zones for eradicationof Anopheles Mosquitoes (Buchel 1982)

A discriminating dosage of 3.125ppm malathion (World Health Organisation, 1980) was used to separate the resistant and susceptible stocks. The larvae derived from iso female of WG strain were treated with the above dosage. The larvae which survived from this treatment were maintained as separate stocks. This process was repeated for 12 crore generations, in order to establish a homozygous resistant stock for malathion (MR strain). The untreated proportion of the above WG strain was used to establish the corresponding homozygous susceptible stock for malathion (MS Strain). The genetic croses were made between 15 males and 15 females of MR strain with that of corresponding ms strain. A part of F1 individuals were libered to get F2 generation and the remaining mosquitoes were backcrossed to parental type. The resistance/sysceptibility tests were carried out for third instar larvae by using diagnostic dosage of 3.125 ppm malathion as recommended for anopheles' larvae (World Health Organization 1980). Larval mortality (susceptible) and survivability (resistant) were scored after 24 h exposure period to the above dosage for all genetic crosses. The number of males and females were also scored individually for resistance susceptibility. The chi-square (X^2) values were calculated. The cumulative time-mortality for 24h. (at a regular interval of 4h) were recorded for third instar larvae for various types of genetic crosses. Lethal time (LT_{10}) values which killed 50% of larvae were determined by time mortality relationship (World Health Organization 1970)

Results and Discussion

In the crosses 3 and 4 (Table 1), the F_1 hybrids shoed 65.42 and 66.07% resistance and 34.50 and 33.93%, susceptibility for malathion. The time mortality relationship for the above crosses showed LT50 values of 15:12 and 14:48 h:min (Table 2)

The F1 hybrids (heterozygote's) were backcrosses with the presumptive homozygous of both sexes. The backcrosses 5,6,7 and 8 revealed 1:1 ratio of resistance: susceptibility (Table 1). The time-mortality relationship for the above crosses showed LT_{50} values of 10:48, 10:24, 10:54 and 10:42h.min(Table 2). The crosses 9 and 10 of F2 generation showed 67:47 and 66.61%

resistance and 32.53 and 33.59% susceptibility. The above crosses showed LT_{50}values of 15:36 and 15:25 h.min(Table2).

TABLE 1 : Genetic study of malathion resistance in *An. stephensi* #

Genetic crosses	No. of larvae tested	Resistant				Susceptible				X^2
		Female	Male	Total	%	Female	Male	Total	%	
PARENTAL										
1. $MR_m \times MR_f$	1059	515	544	1059	100	-	-	-	-	-
2. $ms_m \times ms_f$	1162	-	-	-	-	569	593	1162	100	-
F_1 HYBRID										
3. $MR_m \times ms_f$	587	183	196	384	65.42	101	102	203	34.58	-
4. $ms_m \times MR_f$	513	199	206	405	66.07	102	106	208	33.93	-
BACK CROSSES										
5. $ms_f \times F_{1m}$ $(ms_f \times MR_m)$	1058	262	282	544	51.42	253	261	514	48.58	0.85*
6. $ms_f \times F_{1m}$$(MR_f \times ms_m)$	1047	264	272	536	51.19	251	260	511	48.81	0.60*
7. F_{1f} $(ms_f \times MR_m) \times ms_m$	1075	266	286	552	51.35	258	265	523	48.65	0.78*
8. F_{1f} $(MR_f \times ms_m) \times ms_m$	1069	268	280	548	51.26	255	266	521	48.75	0.73*
F_2 GENERATION										
9. F_2 $(MR_m \times ms_f)$	1196	394	413	807	67.47	188	201	389	32.53	-
10. F_2 $(ms_m \times MR_f)$	1174	386	396	782	66.61	192	200	392	33.39	-

\# Third instar larvae exposed to 3.125 ppm malathion for 24 h. * Statistically insignificant. Subscripts m and f correspond to male and female respectively.

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TABLE 2 : Lethal time (LT_{50}) values for malathion resistance in *An. stephensi* #

Genetic crosses	Cumulative per cent mortality (in h)							LT_{50}
	0 - 4	4 - 8	8 - 12	12 - 16	16 - 20	20 - 24	24 - 28	H : Min
PARENTAL								
1. $MR_m \times MR_f$	-	-	-	-	04	33	71	25 : 48
2. $ms_m \times ms_f$	31	73	100	-	-	-	-	05 : 54
F_1 HYBRIDS								
3. $MR_m \times ms_f$	-	-	24	53	85	100	-	15 : 12
4. $ms_m \times MR_f$	-	-	28	55	82	100	-	14 : 48
BACK CROSSES								
5. $ms_f \times F_{1m}$ $(ms_f \times MR_m)$	15	34	53	76	100	-	-	10 : 48
6. $ms_f \times F_{1m}$ $(MR_f \times ms_m)$	13	35	51	78	100	-	-	10 : 24
7. F_{1f} $(ms_f \times MR_m) \times ms_m$	11	33	54	74	100	-	-	10 : 54
8. F_{1f} $(MR_f \times ms_m) \times ms_m$	12	35	52	75	100	-	-	10 : 42
F_2 GENERATION								
9. F_2 $(MR_m \times ms_f)$	-	21	34	59	70	83	100	15 : 36
10. F_2 $(ms_m \times MR_f)$	-	23	39	58	74	85	100	15 : 25

\# Third instar larvae exposed to 3.125 ppm malathion. Subscripts m and f correspond to male and female respectively.

The malathion resistance is incompletely dominant and autosomal in An.stephensi. similar studies on the genetic basis of malathion resistance have been reported in different populations of An.stephensi. rather and Toquir (1981) reported that 5% malathion shoed 100% morality in 6h and 1h for resistant and susceptible strains respectively whereas the F_1 hybrids survived for 4h, indicating incomplete dominance. Hemingway (1983) reported that malathion resistant strain is 37 times more resistant than the susceptible strain at LT50 levels, whereas F_1 heterozygotes are 6 times more resistant than susceptible strains which indicate that the gene for malathion resistance was partially dominant in An.stephensi.

The genetic basis of malathion resistant was found to be partially dominant in An.arabiensis (Lines et al.1990) and Cx.quinquefasciatus (Shetty 1987) and semi dominant in An.culicifacies (Hearth et al.1987). hence the genetic basis of insecticide resistance among the different species of mosquitoes shoed inconsistent pattern of inheritance.

The effect of 'resistance' genes in the absence of insecticides is important for inhibiting evolution of resistance (Muggleton 1982). The availability of 'susceptible' alleles, selection pressure and degree of integration of resistant genotypes may affect the rate of adaptation to an insecticide free environment. Inaddition, insecticide resistance in mosquitoes in also associated with various parameters including gene amplification of detoxifying carboxylesterase (Mouches et al.1990) and sex ratio distortion towards male (Gangadhar Rao & Shetty 1992).

Conclusion

The present study on genetic basis of malathion resistance in An.stephensi indicates the simple Mendelian pattern of inheritance for various genetic crosses. However, the genetics of insecticide resistance also depends on variables such as the number of genes involved. Genetic variance, intensity of selection pressure. Environmental effects. Population size, etc. which are interrelated and such complex factors require future genetic research.

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