Chemosterilant Resistance in *Musca domestica nebulo* Fabr. Musharraf A. ANSARI* (Department of Zoology, Aligarh Muslim University, Aligarh, U.P., India.) Received July 13, 1973. *Botyu-Kagaku*, 38, 239, 1973.

32. イエバエ *Musca domestica nebulo* Fabr. における化学不妊剤抵抗性の発現 Musharraf A. ANSARI* (Aligarh Muslim 大学 動物学教室) 48. 7. 13 受理

イエバエ *M. domestica nebulo* を累代飼育し各世代の成虫の食餌中に化学不妊剤として apholate, tepa, metepa, hempa および hemel を加えた場合に抵抗性がどのように発現するかについて調べられた．31～34世代目の成虫について各不妊剤の効果を比較したところ，hemel に対して最も顕著な抵抗性の獲得が見られ第1世代の20倍の dose でようやく同等の不妊効果を示すにすぎないことがわかった．また hempa と hemel とを交互に各世代の成虫に与えて撰択を行なったところ単独の不妊剤を施用したときよりもずっと速く，かつ両方の不妊剤に対して抵抗性の発現することがわかった．

Introduction

The advent of chlorinated hydrocarbon insecticides led the scientists to believe that man had after all succeeded in his fight against noxious insect pests, but the joy was shortlived. Houseflies and other insects took up the challenge and developed protective mechanism whereby they could escape the toxic effects of not only these chemicals but also develop tolerance against organophosphorus and even the recently introduced carbamate insecticides. And it seems that chemosterilants may also have the same fate. Though different from insecticides in their mode of action they are also prone to this danger and there is always the possibility of their becoming ineffective against insect pests. In fact Hazard *et al.* (1964) have already found evidence that the yellow fever mosquito, *Aedes aegypti* can develop significant resistance to apholate. Resistance to metepa has also been reported in this species by Klassen and Matsumura (1966).

That the housefly, *Musca domestica domestica* can become resistant to metepa has been reported by Sacca *et al.* (1966). Absa and Hansens (1969) succeeded in producing resistance to apholate in a strain of *M. d. domestica* initially resistant to lindane, diazinon, dimethoate, dimetilan and some other insecticides. Morgan and his associates (1967), however, failed to find any tolerance to apholate in a laboratory colony of *M. d. domestica* subjected to continuous selection with this chemical and similar results were obtained by Meifert *et al.* (1967) when semiweekly baits of metepa were given to adult flies for two years under field conditions. Since the tolerance of the Indian forms of houseflies to these chemicals has not yet been investigated, it was considered desirable to find out if *Musca domestica nebulo* can develop tolerance to the commonly used chemosterilants, apholate, tepa, metepa, hempa and hemel.

Materials and Methods

The flies used during the present studies were drawn from the normal laboratory stock that is being maintained in the laboratory since 1961 and has shown wide frequencies of resistant genes by developing resistance to dieldrin, DDT and BHC (Rahman, 1963, Khan and Ansari, 1964, Khan and Dildar, 1965). Larvae were reared on cotton pads soaked in dilluted milk at a temperature of 28 ± 1°C and 60 to 70 percent relative humidity.

On emergence, the flies were sexed and kept in cages, 8×8″ in size constructed of wire frames and covered over by mosquito netting and meshed cloth. A petri dish containing treated sugar was placed in each cage. Another petri dish containing water soaked cotton was also added in each cage in order to provide sufficient moisture. On the fifth day of emergence dishes were removed and regular fly food was provided. On each day following oviposition a random sample of 100 eggs was collected from each dish. The eggs were placed on a piece of moist black cloth

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and their rate of hatching was determined. The percent sterility and net sterility were calculated by the formulae as described by Hair and Adkins (1964).

The percentage sterility obtained in tests was converted into probit and plotted against log-concentrations on a graph paper. Regression lines were drawn by calculating the maximum and minimum values of probit.

Results

The detailed results obtained with each chemosterilant are presented in Tables 1-6 which clearly indicate that the species is liable to develop resistance to apholate, tepa, metepa, hempa and hemel.

Selection with apholate: The adults were selected with sugar bait containing 0.03125 percent apholate. This produced a sterility of 90.4 percent. The remaining 9.6 percent larvae were reared to produce the next generation which was again subjected to selection pressure. In this way selection was continued up to 34th generation when the selected stock was compared with the normal laboratory strain.

It seems that the species is capable of developing tolerance to apholate when selected in the laboratory (Table 1). The rate at which the strain acquired resistance was quite low. At Sc50 and Sc90 levels (Table 5) the strain after being

Table 1. Effects of alkylating compounds on the sterility of different generations of *M. d. nebulo.*

Chemosterilant	Generation	Percent net sterility with indicated percent apholate bait									
		0.00195	0.0039	0.0078	0.0156	0.03125	0.0625	0.125	0.25	0.5	1.0
Apholate	P	—	4.4	38.7	62.1	90.4	99.2	*	*	*	—
	F15	—	—	18.05	53.7	66.6	96.03	100.0	*	*	—
	F21	—	—	19.07	33.1	44.1	95.3	100.0	*	*	—
	F34	—	—	—	7.6	19.9	35.8	76.5	*	*	—
Tepa	P	16.1	69.05	87.3	96.8	100.0	*	*	—	—	—
	F15	5.7	28.9	43.9	51.7	99.2	*	*	—	—	—
	F22	—	17.9	29.06	43.4	87.1	100.0	*	—	—	—
	F33	—	6.01	15.1	28.2	54.5	93.8	100.0	*	—	—
Metepa	P	—	—	4.8	52.8	86.3	96.8	100.0	*	*	—
	F13	—	—	5.8	38.6	58.7	92.9	100.0	*	*	—
	F20	—	—	—	10.6	17.1	58.8	90.7	100.0	*	*
	F31	—	—	—	7.4	11.6	23.4	57.5	75.7	100.0	*

* The females did not oviposit.

Table 2. Effects of non alkylating compounds on the sterility of different generations of *M. d. nebulo.*

Chemosterilant	Generation	Percent net sterility with indicated percent apholate bait							
		0.03125	0.0625	0.125	0.25	0.5	1.0	2.0	4.0
Hempa	P	10.7	39.2	60.1	86.5	96.5	100.0	*	—
	F16	—	2.1	17.9	29.7	50.4	88.3	*	—
	F24	—	—	5.7	11.1	35.08	56.7	92.3	*
	F32	—	—	—	9.7	24.8	37.1	86.5	100.0
Hemel	P	—	13.04	37.7	62.6	78.3	91.4	100.0	*
	F15	—	—	12.3	24.7	32.2	43.9	98.4	100.0
	F21	—	—	—	4.7	12.9	22.02	75.03	100.0
	F33	—	—	—	—	—	9.7	29.9	49.4

* The females did not oviposit.

Table 3. Effects of hempa and hemel on the sterility of different generations of *M. d. nebulo.*

Chemosterilant	Generation	Percent net sterility with indicated percent chemosterilant bait							
		0.03125	0.0625	0.125	0.25	0.5	1.0	2.0	4.0
Hempa	P	10.7	39.2	60.1	86.5	96.4	100.0	*	—
Hemel	P	—	13.04	37.7	62.6	78.3	91.4	100.0	*
Hempa	F15	—	4.8	16.3	20.6	31.3	86.04	100.0	*
Hemel	F16	—	—	3.9	13.7	16.7	29.9	86.04	100.0
Hemel	F24	—	—	—	—	4.9	18.0	45.4	93.2
Hempa	F25	—	—	—	9.09	28.1	38.4	95.2	*
Hempa	F33	—	—	—	7.0	20.9	35.8	75.4	100.0
Hemel	F34	—	—	—	—	7.1	11.7	24.9	48.2

* The females did not oviposit.

Table 4. Effects of tepa and metepa on the sterility of different generations of *M. d. nebulo.*

Chemosterilant	Generation	Percent net sterility with indicated percent chemosterilant bait									
		0.00195	0.0039	0.0078	0.0156	0.03125	0.0625	0.125	0.25	0.5	1.0
Tepa	P	16.1	69.05	87.3	96.8	100.0	*	—	—	—	—
Metepa	P	—	—	4.8	52.8	86.3	96.8	100.0	*	—	—
Tepa	F14	—	47.02	56.7	84.8	98.4	*	—	—	—	—
Metepa	F15	—	—	—	19.7	57.2	87.6	100.0	*	—	—
Metepa	F23	—	—	—	11.01	21.7	42.6	82.5	100.0	*	—
Tepa	F24	—	14.9	28.01	41.9	82.2	100.0	*	—	—	—
Metepa	F33	—	—	—	—	7.2	16.7	54.1	86.6	100.0	*
Tepa	F34	—	10.6	23.04	38.7	76.05	100.0	*	—	—	—

* The females did not oviposit.

Table 5. Sc50 and Sc90 values of normal and resistant strains.

Chemosterilant	Strain	Sc50	Resistance ratio	Sc90	Resistance ratio
Apholate	Normal	0.01148	6.3	0.02884	13.2
	Resistant	0.070795		0.37154	
Tepa	Normal	0.0036308	8.3	0.0087096	10.8
	Resistant	0.025704		0.087096	
Metepa	Normal	0.017373	10.2	0.042658	13.0
	Resistant	0.17783		0.52481	
Hempa	Normal	0.091201	10.0	0.30903	10.6
	Resistant	0.93325		3.2359	
Hemel	Normal	0.23342	20.0	0.7224	24.1
	Resistant	4.6774		16.982	

selected for 34 generations developed 6.3 and 13.2 times tolerance respectively. This is significantly low as compared to the 26.0 fold resistance obtained by Absa and Hansens (1969) in the case of *M. d. domestica* selected with apholate for 35 generations.

Selection with tepa : The adults were exposed to 0.0156 percent tepa and the larvae obtained were bred to produce the next generation. In this way the flies were selected up to 18th generation when this concentration become ineffective. Hence they were selected with baits containing 0.03125 percent tepa till the 33rd generation. Sc50 and Sc90 values were calculated and when compared with the corresponding values for the normal strain showed that *M. d. nebulo* is liable to develop a significant degree of tolerance to tepa (Table 1 and 5). The selected population exhibited 8.3 and 10.8 times tolerance in 33 generations at Sc50 and Sc90 levels respectively. Though no significant differences in sterility levels were observed during earlier generations the percent net sterility decreased gradually after the tenth generation.

Selection with metepa : Selection with metepa was made at an Sc level of approximately 86.3 percent for 31 generations. The initial Sc50 and Sc90 values of 0.017 and 0.04 obtained for the normal strain when compared with the corresponding values for the selected stock suggest that the species acquired 10.2 and 13.0 times tolerance to metepa. This substantiate the earlier work of Sacca *et al.* (1966) who detected metepa resistance in a colony of *M. d. domestica* when selected for 22 generations.

Selection with hempa : In tests with hempa the flies were selected at 96.4 percent net sterility levels and the larvae were reared to produce the F_1 generation. After twenty two generations they were selected with 1.0 percent hempa up to the 32 generation when the selected stock was compared to the normal susceptible one. It was found that the species could develop resistance to hempa under laboratory conditions. The selected strain when compared with the normal strain showed 10.0 and 10.6 times tolerance at Sc50 and Sc90 levels respectively (Tables 2 and 5), an observation contradictory to that of Sacca *et al.* (1966) who could not find any tolerance to hempa in *M. d. domestica* in 20 generations of laboratory selection.

Selection with hemel: Selection with hemel was made at sterility level of 91.4 percent. The remaining 8.6 percent larvae were reared to produce the next generation. The process of selection was continued up to 33rd generation when the species was found to have developed a tolerance of 20.0 and 24.1 times at Sc50 and Sc90 levels respectively (Tables 2 and 5).

Discussion

The studies clearly show that *M. d. nebulo* is capable of developing resistance to both the alkylating and the non alkylating compounds. In early stages of selection no significant tolerance is developed but as soon selection has been made for 15 generations, marked differences in the tolerance of the selected and the normal

Table 6. Sc50 and Sc90 values of normal and resistant strains.

Chemosterilant	Strain	Sc50	Resistance ratio	Sc90	Resistance ratio
Hempa	Normal	0.091201	—	0.30903	—
Hemel	Normal	0.23442	—	0.7224	—
Hempa	Resistant	1.2023	13.3	5.0119	16.6
Hemel	Resistant	5.1286	25.5	—	—
Tepa	Normal	0.0036308	—	0.0087096	—
Metepa	Normal	0.017378	—	0.042658	—
Tepa	Resistant	0.02884	9.3	0.13490	15.5
Metepa	Resistant	0.095499	9.0	0.47863	10.0

strain are noticeable. However, the rate of development of resistance to non-alkylating agents is much faster than that for the alkylating agents. A 10.0 and 20.0 times tolerance was achieved with hempa and hemel as against 6.3, 8.3 and 10.2 times tolerance developed with apholate, tepa and metepa respectively.

When selected with hempa and hemel in alternate generations the strain acquired tolerance to both the chemicals and such tolerance was higher than the one acquired when the strain was selected with a single chemical. At an Sc50 levels the strain developed 13.3 and 25.5 times tolerance to hempa and hemel respectively as against 10.0 and 20.0 times when selection was made with either of these chemicals (Tables 3 and 6). It seems that similar structure and mode of action of the two compounds selected resistant individuals for each other with the result that increased tolerance was acquired to both the chemosterilants. Yet another strain was selected with tepa and metepa for 34 generations and the Sc50 and Sc90 values derived from regression lines were compared with the corresponding values of the normal laboratory strain. Here also the flies developed resistance to both the compounds (Tables 4 and 6). It is interesting that the ratio of resistance to tepa increased from 10.8 to 15.5 while the resistance ratio for metepa decreased from 13.0 to 10.0 at Sc90 levels. Though no definite explaination can be offered it is possible that tepa does not select the metepa resistant individuals in contrast to metepa which may select individuals tolerant to tepa.

Considering all the results obtained with apholate, tepa, metepa, hempa and hemel it is safe to conclude that though the Indian housefly, *M. d. nebulo* can develop tolerance to chemosterilants under laboratory conditions, the degree of resistance developed is not very high. Chemosterilants, may, therefore, be safely employed for the control of this form of housefly. Morever such a resistance phenomenon will not jeopardise the idea of releasing sterile males in the field for reducing population of flies as there would be no chance of selection.

Summary

Development of resistance to chemosterilants in *Musca domestica nebulo* was studied by incorporating the candidate chemosterilant in the food of the adults. The larvae obtained were reared on cotton pads soaked in diluted milk to produce the next generation. When the selected stocks were compared with the normal laboratory stock it was found that the species is liable to develop tolerance to apholate, tepa, metepa, hempa and hemel. However, the degree of the tolerance acquired greatly varied with each chemosterilant and in 34, 33, 31, 32 and 33 generations of laboratory selection the species developed 6.3 8.3, 10.2, 10.0 and 20.0 times tolerance to apholate, tepa, metepa, hempa and hemel respectively. The rate of development of resistance to hemel was faster as compared to the degree of tolerance detected for apholate, tepa, metepa and hempa. Selection was also made with different chemosterilants in successive generations of rearing. It was found that alternate selection with hempa and hemel induced resistance to both the chemicals and the rate of development of such resistance was much faster than when the flies were selected with a single chemical. The strain selected with tepa and metepa in alternate generations of laboratory rearing also acquired tolerance to both the chemicals.

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ワタアカミムシの性誘引物質
—前構造の検討と新構造の提出—

Clarification of the Chemical Status of the Pink Bollworm Sex Pheromone.
H. E. Hummel, Lyle K. Gaston, H. H. Shorey, R. S. Kaae, Kevin J. Byrne and Robert M. Silverstein. *Science*, 181, 873 (1973).

"Propylure" 10-*n*-propyl-*trans*-5, 9-tridecadienyl acetate および deet, (*N, N*-diethyl-*m*-toluamide) は，それぞれワタアカミムシ (pink bollworm moth, *Pectinophora gossypiella* Saunders) の性誘引物質およびその協力物質であると 1966年に Jones らにより報告され，また翌年に Eiter らにより，その化合物を合成した結果まったく活性を持たなかったという反論もあった．さらにその活性のなかった理由を *cis* 異性体によるマスキングにより説明した報文もあった．そこでこれらの問題の収捨をはかるため新たな研究をおこなった．

それぞれ異なった起源の3組のメス虫体より得られた抽出物の g.l.c 分析をおこなったが，いずれの場合も上記二つの物質はまったく検出できなかった．またこれら二つの化合物の室内および野外試験もあわせおこなったが，それらは単独でもまた組合わされた場合でもまったくか，あるいはわずかしか活性を示さなかった．

"Hexalure" *cis*-7-hexadecenyl acetate は，合成化合物のスクリーニングの結果発見されたワタアカミムシの合成誘引物質であり，その虫体中の存在の有無も問題となっていた．しかし，g.l.c 分析の結果この化合物も虫体中より検出できなかった．

FID およびオス成虫を検知器として用い，5種の極性の異なるカラムを使った g.l.c 分析により1雌成虫あたり 5 ng に相当する性誘引物質のピークが確認された．

この物質をオゾン酸化し還元的に分解をおこないその g.l.c 分析をおこなうと生成物として 1-pentanal, 1, 4-butanedial, 7-acetoxy heptanal の3者を与えた．この結果ワタアカミムシの性誘引物質は，7, 11-hexadecadienyl acetate であると決定された．また精製物の g.l.c 分析をおこなうと，部分的にかさなり合った2ピークとして現われ，*n*-hexadecyl acetate に対する相対保持時間 (4% DEGS, 175°) はそれぞれ1.40, 1.46であった．それら2つのピークを別々に分取し IR 分析をおこなうと前者は合成 *cis*-7, *trans*-11-hexadecadienyl acetate, 後者は *cis*-7, *cis*-11-hexadecadienyl acetate の IR チャートと完全に一致した．またそれぞれの天然物，合成物を用いた coinjection 方式による g.l.c 分析をおこなうと単一ピークとして検出された．以上の結果より，ワタアカミムシの性誘引物質は，7, 11-hexadecadienyl acetate の *cis, cis*;*cis, trans* 異性体の混合物であると同定された．なお "gossyplure" と名づけられたこの性誘引物質は野外試験において両異性体が同時に存在する時のみ活性を有し，おのおの単独の場合は，活性がなかった．これらの異性体の混合比についてのくわしいことは現在検討中である．　(山岡亮平)