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these chemicals. However, the females lost their sterility in successive egg layings except at higher concentrations where oviposition was totally retarded. The loss of sterility depended upon the degree of initial sterility in the females so that the higher the initial sterility the lesser was the loss.

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Induction of Sexual Sterility in Indian Housefly, *Musca domestica nebulo* Fabr. Musharraf A. Ansari (Zoology Department, Aligarh Muslim University, Aligarh, India.) Received April 13, 1973. *Botyu-Kagaku*, 38, 135, 1973.

20. イエバエにおける不妊化の誘発 Musharraf A. Ansari (Aligarh Muslim 大学 動物学教室) 48. 4. 13 受理

イエバエに apholate, tepa, metepa を食餌混合投与，局所塗布，ペトリ皿内面塗布しての飼育の三方法によってテストし不妊効果をしらべた．

食餌に0.03125% tepa を混合して飼育したときには，100%の不妊化がみられた．局所塗布では，apholate, tepa, metepa がそれぞれ0.25, 0.0625, 0.5%の溶液 0.0018m*l* で，100%の不妊化がみられた．

Sc50 (sterility concentration 50) から各不妊剤の効力を比較したところ tepa は apholate の4.1倍，metepa の14.3倍，hempa の73.3倍，hemel の133.3倍であることがわかった．

また，おのおのの不妊剤をペトリ皿内面に塗布してイエバエ成虫を入れ接触させただけでも不妊効果があり，この方法でも tepa が最も効力があった．

The successful eradication of *Cochliomyia hominivorax* from the island of Curacao (Baumhover, *et al.*, 1955), Florida and South Estern States (Lindquist, 1959 and Knipling, 1960) gave a great impetus to the use of sterile males for insect control. Increasing attention is being paid to chemical sterilization approach advocated by Knipling (1955, 1959 and 1962) and Lindquist (1961) and a number of chemicals have been already shown promise as sterilants against *M. d. domestica* (Labrecque, 1961 and Labrecque *et al.*, 1960, 1963) when administered in the food of adults.

Of the various ways the chemosterilants act, the most interesting is that shown by radiomimetic compounds which completely destroy the genetic material of reproductive unit without affecting much the vigour and mating requirements of

the insect species (Smith, 1963). Painter and Kilgore (1964) tested a number of compounds and concluded that apholate and thiotepa could cause permanent sterility in *M. d. domestica*. Sacca *et al.* (1964) also succeeded in inducing sterility in *M. d. domestica* when tepa and apholate were given in sugar solution to adult flies. Mathis and Schoof (1965) reported that flies could be sterilized when fed on a bait containing 0.5 percent apholate and 12.0 percent sugar while Painter and Kilgore (1965) observed permanent sterility when 1.0 percent 5-fluorootic acid was adminstered in the food of adults. Compounds of low toxicity such as the non alkylating agents were tested with great optimism (Chang *et al.*, 1964) and the efficiency of hempa was confirmed by Labrecque and his associates in 1966 who observed 100.0 percent sterility in both sexes of *M. d. domestica* when 0.25 percent of the chemical was administered in the diet of adults. Hafez *et al.* (1969) were also able to produce sterility in the oriental housefly, *M. d. vicina* when the same compound was given in the food of adults.

The above studies relate to the use of baits and do not revel the specific amount of chemosterilant that may be required to sterile a fly. Attempts have, therefore, been made to develop a fast and reliable bioassy method which would reveal even slight differences in sterilizing potency. Chang and Borkovec (1964) injected the sterilant solution directly into the tissues of the housefly and found that tepa, metepa and apholate induced sterility in male houseflies, an observation later confirmed by Gouck *et al.* (1963) and Ascher (1964). Similar results were obtained by Ansari and Khan (1971) who applied measured drop of acetone solutions of hempa to the dorsum of *M. d. nebulo*.

Sterility has also been produced by tarsal contact of the adult insects to the residual films of chemosterilants as shown by Weidhaas (1962) and Harris (1962). Meifert *et al.* (1963) exposed the adults of *M. d. domestica* to residues of tepa and metepa on glass surfaces and reported that houseflies could be sterilized by tarsal contact to residues of these chemosterilants. However, similar tests with apholate did not cause any degree of sterility in adults. Contrary to this, Pershad and Naidu (1966) were able to produce sterility in males of *M. d. domestica* by exposing the adults to residue of apholate for 12 hours in intermittent dosages, 2 hours per day for 6 consecutive days. Labrecque and others (1966) obtained 100.0 percent sterility when the males were exposed to 200 mg/sq. ft. residue of hempa. However, only 38.4 percent sterility could be observed when the females were treated. Similar results have been obtained by Hafez *et al.* (1969) in case of *M. d. vicina* with tepa and metepa.

Most of the above studies relate to *M. d. domestica* and very little is known concerning the sterilization of *M. d. nebulo* by these methods. The present studies were, therefore, made to observe the degree of sterility induced by apholate, tepa, metepa, hempa and hemel in this species by using different methods of treatment.

Materials and Methods

The flies used during the present tests were obtained from the normal laboratory stock maintained at a temperature of 28±1°C and 60 to 70 percent relative humidity. On emergence the adults were sexed and about 100 males and femlaes were segregated in cloth cages measuring 8×8″ in size. They were fed on sugar treated with the desired concentration of the chemosterilant for four days when the dishes containing treated sugar and water were removed and regular fly food was given to the flies. Random samples of 100 eggs were collected daily and placed on moist black cloth to determine the hatch rate. Observations were recorded for twenty days and the percenty sterility and net sterility was calculated after the manner described by Hair and Adkins (1964).

In topical treatments measured drops of the desired solutions were applied on the dorsum of each fly. The size of the drop applied was 0.0018 cc throughout the experiments. After treatments the flies were kept in cages and regular fly food was supplied to them.

Yet another experiment was performed by spraying 10 cc solution of apholate, tepa, metepa, hempa or hemel in acetone on petri dishes, 4″ in diameter. The dishes thus treated were

rotated on the surface till the solvent evaporated. In this way an even film of the chemosterilant was obtained. Newly emerged adults were slightly anaesthetized with carbon dioxide and released in between the two petri dishes for a desired period of time. The flies were exposed to such films for 15 to 240 minutes and freshly treated dishes were used for each test. After treatments the adults were allowed to escape in cloth cages and were fed on regular fly food. Oviposition and fertility of eggs was observed by collecting random samples of 100 eggs each on black moist cloth and determining the percent sterility and net sterility.

The percentage sterility obtained in tests was converted into probit and plotted against log-concentrations on graph papers. Regression lines were drawn by calculating the maximum and minimum values of probits as described by Finney (1952).

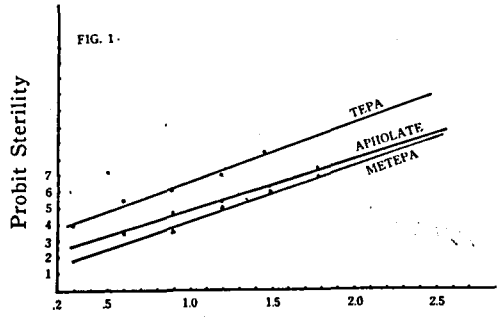

Fig. 1. Susceptibility of *M. d. nebulo* to apholate, tepa and metepa administered in the food of adults.

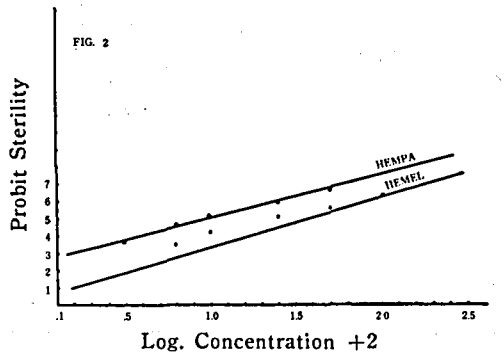

Fig. 2. Susceptibility of *M. d. nebulo* to hempa and hemel administered in the food of adults.

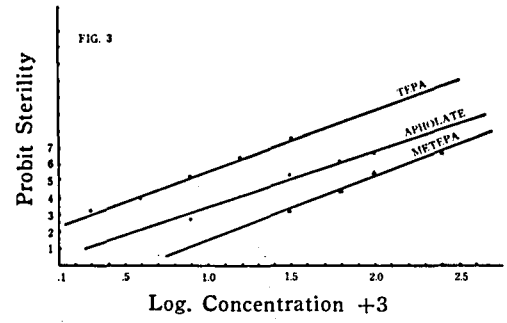

Fig. 3. Susceptibility of *M. d. nebulo* to apholate, tepa and metepa when acetone solutions of these chemicals were applied topically to the dorsum of individual flies.

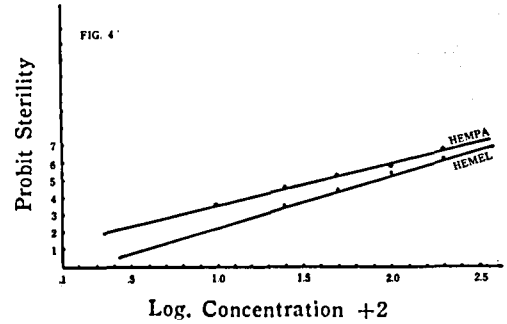

Fig. 4. Susceptibility of *M. d. nebulo* to hempa and hemel when acetone solutions of these chemicals were applied topically to the dorsum of individual flies.

Results

The results obtained with different methods are presented in Tables 1 to 7. It is evident from Tables 1 and 2 that all the compounds tested can induce sterility in *M. d. nebulo* when administered in the food of adults. In general the degree of sterility developed was dependent on the concentration of the chemosterilant applied ; 0.03125 percent tepa caused 100.0 percent sterility as against 90.4, 80.3 and 10.7 percent net sterility obtained with the same concentration of apholate, metepa and hempa respectively. Hemel did not induce any degree of sterility at this concentration. On comparing the two groups of sterilants it seems that aziridine compounds

Table 1. Viability of eggs obtained from adults fed on diet treated with aziridine compounds.

Chemosterilant	Concentration (%)	Total no. of eggs observed	Viable eggs (%)	Percent sterility	Percent net sterility
Apholate	0.0039	3600	74.6	25.4	4.4
	0.0078	4000	47.8	52.2	38.7
	0.0156	3200	29.5	70.5	62.1
	0.03125	2000	7.4	92.6	90.4
	0.0625	2800	0.5	99.5	99.2
	0.125	**	—	—	—
Tepa	0.00195	3600	65.9	34.1	16.1
	0.0039	2600	24.1	75.9	69.05
	0.0078	2700	9.8	90.2	87.3
	0.0156	2800	2.4	97.6	96.8
	0.03125	1800	0.0	100.0	100.0
	0.0625	**	—	—	—
Metepa	0.0078	2800	74.3	25.7	4.8
	0.0156	3200	36.8	63.2	52.8
	0.03125	2700	10.6	89.4	86.3
	0.0625	2400	2.4	97.6	96.8
	0.125	2100	0.0	100.0	100.0
	0.25	**	—	—	—

Percent sterility of control flies was 21.9
** The females did not oviposit.

Table 2. Viability of eggs obtained from adult fed on diet treated with non alkylating agents.

Chemosterilant	Concentration (%)	Total no. of eggs observed	Viable eggs (%)	Percent sterility	Percent net sterility
Hempa	0.03125	2200	69.7	30.3	10.7
	0.0625	2000	47.4	52.6	39.2
	0.125	1600	31.06	68.94	60.1
	0.25	1600	20.4	89.6	86.5
	0.5	1600	2.7	97.3	96.4
	1.0	2200	0.0	100.0	100.0
	2.0	**	—	—	—
Hemel	0.0625	3000	67.9	32.1	13.04
	0.125	4300	48.6	51.4	37.7
	0.25	3400	29.1	70.9	62.6
	0.5	4500	16.8	83.2	78.3
	1.0	3100	6.6	93.4	91.4
	2.0	3200	0.0	100.0	100.0
	3.0	2800	0.0	100.0	100.0
	4.0	**	—	—	—

Percent sterility of control flies was 21.9
** The flies did not oviposit.

are more promising than non-alkylating agents. The results obtained when compared with those of other workers show that *M. d. nebulo* is more sensitive to chemosterilants than *M. d. domestica*. Murvosh *et al.* (1964) obtained 96.2, 100.0 and 98.8 percent sterility with 0.25, 0.2 and 0.1 percent of apholate, metepa and tepa respectively while 99.2, 100.0 and 96.8 percent net sterility was observed in case of *M. d. nebulo* with 0.0625, 0.03125 and 0.0625 percent of apholate, tepa and

metepa, respectively. However, in the case of hempa the results were similar to those of Labrecque *et al.* (1966) who obtained 100.0 percent sterility in *M. d. domestica* when the adults were fed on sugar treated with 1.0 percent hempa. Of the five chemosterilants tested, tepa proved to be the most effective one.

Sterility could also be induced when apholate,

Table 3. Viability of eggs obtained from adults treated topically with aziridine compounds.

Chemosterilant	Concentration* (%)	Total no. of eggs observed	Viable eggs (%)	Percent sterility	Percent net sterility
Apholate	0.0078	3600	77.1	22.9	1.2
	0.0156	3800	62.1	37.9	20.4
	0.03125	4100	24.09	75.9	69.1
	0.0625	3500	7.7	92.3	90.1
	0.125	2000	2.5	97.5	96.6
	0.25	1600	0.0	100.0	100.0
	0.5	**	—	—	—
Tepa	0.00195	3600	74.6	25.4	5.7
	0.0039	3600	64.5	35.5	17.4
	0.0078	3600	28.4	71.6	63.6
	0.0156	1400	4.1	95.1	93.7
	0.03125	300	0.0	100.0	100.0
	0.0625	**	—	—	—
	0.125	**	—	—	—
Metepa	0.03125	3600	74.2	25.8	4.9
	0.0625	4200	51.6	48.4	32.6
	0.125	3600	22.6	77.4	70.9
	0.25	1300	3.9	96.1	94.8
	0.5	700	0.0	100.0	100.0
	1.0	**	—	—	—
	Control	4200	78.1	21.9	—

* A drop of 0.0018cc was applied to each fly.
** The female did not oviposit.

Table 4. Viability of eggs obtained from adults treated topically with non alkylating agents.

Chemosterilant	Concentration* (%)	Total no. of eggs observed	Viable eggs (%)	Percent sterility	Percent net sterility
Hempa	0.125	4200	72.1	27.9	7.6
	0.25	4100	51.2	48.8	34.3
	0.5	3500	30.8	69.2	60.4
	1.0	3100	14.7	85.3	81.07
	2.0	1800	2.6	97.4	96.5
	4.0	**	—	—	—
Hemel	0.25	4200	73.1	26.9	6.01
	0.5	4500	56.6	43.4	28.01
	1.0	3600	22.2	77.8	71.4
	2.0	3500	9.1	90.9	88.2
	4.0	1700	0.0	100.0	100.0
	Control	4200	78.1	21.9	—

* Adrop of 0.0018cc was applied to each fly.
** The females did not oviposit.

tepa, metepa, hempa and hemel were applied topically to both sexes (Tables 3-4). The aziridine compounds caused sterility at very low concentrations in comparison to non-alkylating agents. A 100.0 percent net sterility was achieved when the adults were treated with 0.25, 0.0625 and 0.5 percent of apholate, tepa and metepa respectively and 96.5 to 100.0 percent net sterility when 2.0 and 4.0 percent of hempa and hemel were used. Oviposition was completely inhibited at higher concentrations except in the case of hemel where concentrations above 4.0 percent could not be applied as it was not possible to obtain acetone solution of any higher concentration. The concentrations used during the present study did not cause any mortality after 24 hours of treatments as has been observed in the case of *M. d. domestica* (Gouck *et al.*, 1963).

The relative potency of the chemicals calculated from Sc50 values and presented in Table 5b,

Table 5(a). Sc50 and Sc90 values for adults fed on diet treated with chemosterilants.

Chemosterilant	Sc50	Sc90
Apholate	0.01148	0.02884
Tepa	0.0036308	0.0087096
Metepa	0.017378	0.042658
Hempa	0.091201	0.30903
Hemel	0.23342	0.7224

Table 5(b). Sc50 and Sc90 values for adults treated topically with desired concentrations of chemosterilants.

Chemosterilant	Sc50	Sc90
Apholate	0.028840	0.070795
Tepa	0.0067608	0.015488
Metepa	0.079433	0.18197
Hempa	0.43652	1.5849
Hemel	0.89125	2.2387

clearly indicates that tepa was 4.1 times as effective as apholate, 14.3 times as metepa, 73.3 times as hempa and 133.3 times as effective as hemel in sterilizing the adult flies. The present findings are not far from those of Chang and Borkovec (1964) who reported that tepa was 4.0 times as effective as apholate and 12.5 times as effective as metepa in producing sterility in the males of *M. d. domestica.*

The rate of potency widened considerably when compared at higher effective dose level. Tepa was found to be 5.3 times as effective as apholate, 14.6, 100.0 and 168.2 times as effective as metepa, hempa and hemel respectively at Sc90 level. Since the structure of apholate differs with that of tepa it could be assumed that the active molecule may be the aziridine ring while the other portion functions only as a carrier but by possessing 6 aziridine rings apholate was less effective than tepa with only 3 aziridine rings. Borkovec (1962) believes that though the presence of aziridine rings is an important factor in the effectiveness of any chemosterilant, the number of such rings can not be entirely responsible for the decrease or increase in sterilizing activity. Later Chang and Borkovec (1964) reported that on a weight basis, the aziridine group constitutes 73.0 percent tepa but only 65.0 percent of apholate.

In the last set of experiments the flies were exposed by tarsal contact method and the results obtained (Tables 6-7) show that a deposit of 0.42mg/sq. cm. of apholate, tepa and metepa totally retarded oviposition at all exposure periods which varied from 15 to 240 minutes. Inhibition of oviposition was also observed in tests with hempa at 0.84 mg/sq. cm., but hemel failed to inhibit oviposition even at 1.68 mg/sq. cm. The degree of sterility was directly proportional to the concentration tested and the exposure period. A 0.0065 mg/sq. cm. apholate caused 50.7 percent net sterility at exposure period of 0.25 hours as against 69.4, 75.4, 93.2 and 100.0 percent net sterility obtained with deposits of 0.013, 0.026, 0.05 and 0.105 mg/sq. cm. Similarly 0.026 mg/sq. cm. residue of apholate produced 75.4, 87.2, 96.9 and 100.0 percent net sterility at exposure periods of 0.25, 0.5, 1.0 and 2.0 hours respectively while no eggs were laid by females at an exposure period of 4.0 hours. The same pattern was noticed with tepa, metepa, hempa and hemel. Tepa was most promising and hemel the least in producing sterility in all tests performed with these chemicals.

While Meifert *et al.* (1963) reported that apholate was ineffective in causing sterility in *M. d. domestica* by contact exposure and Pershad

Table 6. Percent sterility of houseflies when exposed to residues of aziridine compounds on treated petri dishes.

Chemosterilant	Concentration residue (mg/sq. cm.)	Percent net sterility after exposure of indicated hours				
		0.25	0.5	1.0	2.0	4.0
Apholate	0.0065	50.7	51.1	72.6	72.3	91.3
	0.013	69.4	79.1	96.1	100.0	100.0
	0.026	75.4	87.2	96.6	100.0	**
	0.052	93.2	95.7	100.0	**	**
	0.105	99.7	100.0	**	**	**
	0.21	100.0	**	**	**	**
	0.42	**	**	**	**	**
Tepa	0.0065	64.8	69.05	87.5	96.6	100.0
	0.013	94.7	98.3	100.0	100.0	100.0
	0.026	99.8	100.0	**	**	**
	0.052	100.0	100.0	**	**	**
	0.105	**	**	**	**	**
	0.21	**	**	**	**	**
	0.42	**	**	**	**	**
Metepa	0.0065	5.6	19.8	19.4	26.9	76.8
	0.013	33.6	58.1	68.1	88.2	96.5
	0.026	51.1	90.02	93.6	100.0	100.0
	0.052	85.4	87.7	98.2	100.0	**
	0.105	94.3	93.4	**	**	**
	0.21	100.0	**	**	**	**
	0.42	**	**	**	**	**

Percent sterility of control flies was 21.9
** The f males did not oviposit.

Table 7. Percent net sterility of houseflies when exposed to residues of non-alkylating agents on treated petri dishes.

Chemosterilant	Concentration residue (mg/sq. cm.)	Percent net sterility after exposure indicated hours				
		0.25	0.5	1.0	2.0	4.0
Hempa	0.105	38.6	35.3	52.6	56.2	78.1
	0.21	44.2	57.6	69.05	87.9	93.3
	0.42	88.4	94.3	100.0	100.0	**
	0.84	**	**	**	**	**
	1.68	**	**	**	**	**
Hemel	0.21	5.6	23.01	26.08	32.9	46.8
	0.42	23.2	36.4	44.6	60.1	77.1
	0.84	31.8	40.1	53.3	73.01	91.4
	1.68	49.3	51.6	72.6	93.5	100.0

Percent sterility of control flies was 21.9.
** The females did not oviposit.

and Naidu (1966) found that considerably longer exposure period was required for causing complete sterility in the males of this species, the present author observed that apholate was capable of producing sterility in *M. d. nebulo* at deposits as low as 0.105 mg/sq. cm. and at exposure periods varying from 0.25 to 0.5 hours. Concentrations of apholate, tepa, metepa and hempa above 1.0 mg/sq. cm. caused over 80 percent mortality in the flies. This is in partial agreement with the observations of Labrecque *et al.* (1966) who observed high mortality at deposits

of hempa above 200 mg/ft.

A comparison of topical and feeding methods (Tables 5a and 5b) indicates that flies are more susceptible when the chemicals were incorporated in the food than when applied topically on the dorsum of individual flies except in the case of tepa which is more or less equally effective when tested by both these methods. It seems reasonable to conclude that adding the chemical in the food of the flies is perhaps the best and most convenient method which can be used for large scale control operations.

Summary

The potentialities of apholate, tepa, metepa, hempa and hemel as chemosterilants were determined against the adults of *M. d. nebulo* by different methods of treatment. The results obtained showed that tepa was found to be the most promising and caused complete sterility at a concentration as low as 0.03125 percent when administered in the food of adults for four days. Hemel was the least effective in producing sterility. The efficiency of these chemicals was further tested by applying measured drops of acetone solutions of the desired concentration of a chemosterilant on the dorsum of each fly. It was found that tepa was 4.1 times as effective as apholate, 14.3 times as metepa, 73.3 times as hempa and 133.3 times as effective as hemel. Sterility could also be induced when the adults were exposed to residual films of apholate, tepa, metepa, hempa and hemel at various concentrations and periods of time. The degree of sterility was directly proportional to the concentration tested and the exposure period. Tepa again showed the greatest promise and hemel was the least effective in producing sterility.

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Resistance Spectrum of Alkylating and Non-Alkylating Compounds in *Musca domestica nebulo* Fabr. Musharraf A. Ansari (Department of Zoology, Aligarh Muslim University, Aligarh, India.) Received April 13, 1973. *Botyu-Kagaku*, 38, 143, 1973.

21. イエバエのアルキル化および非アルキル化不妊剤に対する抵抗性スペクトラム

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イエバエの apholate 抵抗株, tepa 抵抗株, metepa 抵抗株, hempa 抵抗株, hemel 抵抗株について, 交差抵抗性の発現について研究を行なった. Apholate 抵抗株は, hemel に対する許容量が, 実験室で飼育している非抵抗株の 2.6 倍もあるが, tepa, metepa および hempa には感受性であった. Tepa 抵抗株は metepa, hempa および hemel に対し許容量の増加を示し, metepa 抵抗株は tepa には3.6倍, hempa には3.4倍, hemel には6.5倍の抵抗性を示した. Hempa 抵抗株は tepa, metepa および hemel にそれぞれ 3.3, 2.5, 9.1 倍の許容量の増加がみられた. Hemel 抵抗株は tepa, metepa および hempa にそれぞれ 4.6, 2.4, 8.7 倍の許容量の増加があることがわかった.

The development of tolerance to chemosterilants other than the one to which a strain has been selected, has raised a number of problems for the control personnel. A species resistant to an chemosterilant may be expected to show cross tolerance to other chemicals having similar structure and manner of detoxification in the insect body but a serious threat is posed when it becomes resistant to a chemical having an entirely different structure. Such cross tolerance have already been reported by Absa and Hansens (1969) in *M. d. domestica* who found that houseflies resistant to apholate were not only tolerant to this chemical but also showed increased tolerance to metepa. Similar results were obtained by Patterson and his associates (1967) in the yellow fever mosquito, *Aedes aegypti* that had been selected with apholate for 30 generations developed cross resistance to tepa and 3 to 4 fold increase in tolerance to metepa.

No effort has, however, been made to investigate the cross tolerance to chemosterilants in Indian forms of housefly, *Musca domestica nebulo*. Hence, tests were performed to observe if strains resistant to apholate, tepa, metepa, hempa and hemel developed any tolerance other than the one to which a strain has been selected.

Materials and Methods

During the present studies five strains of *M. d. nebulo* namely the AR strain, resistant to apholate, the TR strain, resistant to tepa, the MR strain, resistant to metepa, the HR strain, resistant to hempa or the PR strain resistant to hemel were tested for their susceptibility to other compounds by incorporating the candidate chemosterilant in the food of freshly emerged adults for four days and determining the hatch rate of the eggs in random samples of 100 eggs each. They were initially developed by selecting the adults at an Sc level of 90.0 percent or above with each of the chemicals in successive generations of laboratory rearing at a temperature of 28±1°C and 60 to 70 percent relative humidity and the larvae were reared on cotton pads soaked in diluted milk.

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