of carbon atoms in the substituted moiety.

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Effects of Hempa on the Sterility and Mortality of *Drosophila melanogaster* (Meign). A. N. Chattoraj and B. B. L. Srivastava* (Entomological Research Laboratory, Department of Zoology, University of Allahabad, Allahabad, 211002, India) Received Jul. 25, 1977. *Botyu-Kagaku*, 42, 151, 1977.

23. Hempa によるショウジョウバエの不妊化率と死亡率 A. N. Chattoraj and B. B. L. Srivastava (University of Allahabad) 52. 7. 25 受理

ショウジョウバエの成虫，幼虫の餌に hempa を混入してその効力を検べた．幼虫時に処理した雌成虫は，正常雄と交尾すると産卵は増加するが，同時にその卵の孵化率も低下した．同様に処理した雄と正常雌では，産卵は少ないが，孵化率は処理雌の時より高かった．従って幼虫時の処理は雄よりも雌に行なう方が効果的である．0.5% hempa の混入で 100% の不妊化率がみられた．

成虫の餌では 2%混入して 100% の不妊化率であった．しかも，この時は雄成虫に処理した方が効果的であった．これは幼虫の場合とは，効果が逆転しているという結果になった．

The autocidal method of insect control has attracted much attention of the entomologists during the past decade and a large number of chemicals liable to induce sexual sterility in dipterans have been evaluated for this purpose (La Brecque 1960 & 1961)[14,15]. Chang *et al* (1961)[4] found that the non alkylating agent hempa could produce sterility in *M. domestica*, Fye *et al* (1966)[7] obtained 100% sterility in house flies when hempa was given in the food of the adult at a concentr ation of 0.05%. Similar results have also been reported by La Brecque *et al* (1966) when hempa was applied to pupae and adults of house flies. Davis and Eddy (1966)[6] found that hempa was more effective when given in food than in residual treatment in *Fannia canicularis* (L.) and that it affected males more than the females. McFadden and Rubio (1966) reported that when they fed 8 day old Mexican fruit fly larvae with food containing 0.5% hempa no emergence of adult flies was noted. They also found that pupae dipped in 5.0% solution of hempa for 60 seconds resulted in 62% of adult emergence with 100% sterility and that when adult flies were fed for 3

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days with 3% hempa mixed in food or when fed on 0.5% aqueous sol. of hempa for one day it produced 100% sterility. White (1966)[18] reported success with 500 ppm thiotepa treatment of pupae of *Aedes aegypti*. Das (1967)[5] reported failure to sterilize pupae of *Culex pipiens fatigans* with apholate. Kaur and Steve(1969)[12]reported that both sexes of the face fly *Mucsa autumnalis* DeGeer were sterilized with 0.1% hempa mixed in food. They also reported that at 1.0% concentration level the longevity of males was badly affected, while 2.0% concentration caused quick female mortality. Hafez *et al*(1969)[8,9] reported varying degrees of larval mortality in *M. d. vicinia* when treated with hempa. Patterson, Weidhass, Ford and Longfern (1970)[16] reported success in suppression and elimination of *C. p. quinquifasciatus* population using tepa and thiotepa treated pupae in an island near coast of Florida. Kaur and Wentworth (1972)[13] reported that pupal dipping of *Musca autumnalis* in 4.5% soln. of hempa produced initial sterility of 68.5% and 62.6% in male and female respectively. Sharma, Patterson, Grover and La Brecque(1973)[17] achieved 99.7% sterility in male *C. p. fatigans* by dipping pupae for 5 hours in .5% solution of thiotepa and 100% sterility by exposing pupae at .7% conc. of thiotepa for the same period. Ansari (1973)[3] dipped 1-3 day old house fly pupae for a period varying between 30-240 seconds in the ethanolic solutions of apholate tepa, metepa hempa and hemel at 1%, 2% and 4% conc. and obtained 100% sterility with apholate, tepa and metepa. Hempa produced maximum sterility of 53.4% when 1 day old pupae were dipped for 240 minutes in a 4% solution.

Materials and Methods

The pure wild variety of *Drosophila melanogaster* (Meign) flies were obtained from the Cytogenetic Laboratory of B. H. U., Varanasi, India and cultured in the departmental laboratory at 27±3.

The sample of hempa was obtained from Dr. A. B. Borkovec of U. S. D. A., Beltsville, Md. U. S. A.

In order to obtain virgin female and unmated male flies, pupae were collected and kept separately in vials without any food. The emerging adults were sexed and starved for 6 hours and used for oral feeding or pairing tests.

Administration of Hempa

1. *Oral treatment*

For oral feeding hempa was mixed with artificial diet of larvae and with sugar solution for adult flies. The selected and counted number of eggs were placed on the mixed diet and allowed to feed till pupation. The adults emerging from above pupae were separated and sexed as stated above, while for oral feeding of adults, the newly emerged adult flies of both sexes were fed for 48 hours on hempa mixed in 10% sugar solution diet and then transferred to separate vials for pairing experiments.

2. *Dipping treatment*

The normal selected pupae of 1, 3 and 5 day old pupae were immersed in a 5% aquous solution of hempa for period varying from 30 to 120 mins. The mortality in the pupal stage and sterility in the adults emerging out of the treated pupae were noted by subjecting them to different pairing tests.

Pairing Experiments

The adults emerging from all the experiments were subjected to pairing experiments to find out the sterility effect. Single pair reciprocal crosses were established between treated male and treated females and also between normal and treated males and females. The pairing experiments were started on third day of their emergence and each pair was kept separately in a vial containing food medium for egg laying. Five pairs of each type with three replications were observed for first ten days for fecundity and sterility. Control experiments were run simultaneously by mixing requisite amount of distilled water in larval food medium.

Net sterility and net mortality was calculated by the following formula based on Abbot (1925)[1] and Hair and Adkins (1964)

$$\text{Net sterility } \% = \frac{\%\text{ sterility in test} - \%\text{sterility in normals}}{100 - \%\text{ sterility in normals}} \times 100$$

for calculation of net mortality, the sterility in the above formula is substituted by mortality at

every step.

Results

A. *Larval feeding*

(1) *Sterility effects*

The Table 1 shows the result of larval feeding treatment on fecundity, hatching and sterility in the adults emerging out of the treated larvae. It is evident from the table that when treated (in larval stage) female was paired with normal male it increased the fecundity but lowered the hatching percentage. Similarly treated male mated with normal female recorded a fall in the fecundity but a rise in the hatching percentage. The maximum net sterility percent recorded was 21.3, when treated male and females were paired. In general, it has been noted that hempa was more effective on females than males, but did not decrease the fecundity of the females.

(2) *Mortality effect*

The Table 2 shows that the larval feeding of .1% and .5% hempa produces 94.6 and 100% mortality of larvae respecitvely. At .1% concentration the adults which emerge out of the treated larvae also died on 2nd or 3rd. day without laying any egg.

B. *Adult treatment*

The Table 3 shows that when adults are fed with hempa in solution it produces complete sterility of the flies. The maximum net sterility percentage recorded was 99.5 at 2.0%, when treated males and females were paired. It is clearly evident from the result that at all concentrations of treatment hempa was more effective on the adult males than females and that the sterility increased with the increase in the dosage of treatment from .5% to 2%.

C. *Pupal treatment*

(1) *Sterility effect*

Tables 4, 5 and 6 show sterility effect on the adults emerging out of the hempa treated pupae of 1, 3 and 5 days. Hempa produces maximum effect on the 3 day old pupae treated for 120 minutes at 5% conc.; maximum net sterility produced at this conc. was 46.5 when both the sexes emerging from treated pupae were mated. It appears that 1 and 5 day old pupae are less sensitive to the treatment with hempa as the maximum net sterility produced at the age of 1

Table 1. Rresults of larval feeding treatment with Hempa, Details of fecundity, hatching and sterility in the adults emerging out of the treated larvae.

Conc. of hempa in food (%)	Type of pairing experiment	Average No. of eggs laid per female	Average No. of eggs hatched per female	Hatching (%)	Sterility (%)	Net sterility (%)
Control	NM×NF	161.3	142.2	86.5	15.5	—
0.01	TM×NF	146.4	110.6	75.5	24.5	12.7
0.01	TF×NM	183.7	124.4	67.7	32.3	21.7
0.01	TM×TF	128.0	83.8	65.4	34.6	24.3

TM=Treated Male, TF=Treated Female NM=Normal Male NF=Normal Female

Table 2. Mortality effect of Hempa in larval feeding treatment

Conc. of hempa in food (%)	Average No. of eggs exposed on media	Average No. of larvae pupated	Average No. of adults emerged	Average No. of mortality in larvae & pupae	Mortality (%)	Net Mortality (%)
Control	50	38	36	14	28	—
0.01	50	38	34	16	32	5.5
*0.1	50	26	2	48	96	94.6
0.5	50	—	—	50	100	100

* Adults emerged at this conc. died on 2nd and 3rd day of emergence.

and 5 days are 26.3 and 24.3 respectively. In general, it has been noted that hempa has no effect on the fecundity of the females emerging from treated pupae.

(2) *Mortality effect*

The Table 7 shows that the maximum net mortality noted at 1, 3 and 5 day old pupae was 4.9, 21.4 and 17.2 respectively at 120 mins. The mortality was noted to increase with the period of treatment at every age group, the dosage remaining constant.

Table 3. Results of Adult feeding treatment with Hempa. Details fecundity, hatching and sterility in adults.

Conc. of Hempa in foods (%)	Type of pairing experiment	Average No. of eggs laid per female	Average No. of eggs hatched per female	Hatching (%)	Sterility (%)	Net sterility (%)
Control	NM×NF	99.4	89.1	89.6	10.4	—
0.5	TM×NF	103.5	26.5	25.6	74.4	71.4
0.5	TF×NM	97.6	74.5	76.3	23.7	14.8
0.5	TM×TF	92.6	22.5	24.2	75.8	72.9
1.0	TM×NF	91.6	3.9	4.2	95.8	95.3
1.0	TF×NM	86.0	54.2	63.0	37.0	29.6
1.0	TM×TF	92.2	4.0	4.2	95.8	95.3
2.0	TM×NF	81.0	0.7	0.8	99.2	99.1
2.0	TF×NM	62.0	35.4	67.0	43.0	36.3
2.0	TM×TF	90.0	0.4	0.4	99.6	99.5

Concentration treatment = Significant at 1% level
Critical difference = 9.33

Pairing experiment = Significant at 1% level
Critical difference 9.33

Table 4. Results of one day old pupal dipping treatment with 5% aqueous solution of Hempa. Details of fecundity, hatching and sterility in the aduelts emerging out of the treated pupae.

Dipping periods (mts)	Type of pairing experiment	Average No. of eggs laid per female	Average No. of eggs hatched per female	Hatching (%)	Sterility (%)	Net sterility (%)
Control	NM×NF	108.8	97.6	89.7	10.3	—
30	TM×NF	113.6	100.8	88.7	11.3	1.1
30	TF×NM	105.2	94.2	89.5	10.5	0.2
30	TN×TF	83.4	73.5	88.1	11.9	1.7
60	TN×NF	89.6	69.0	77.0	23.0	14.1
60	TF×NM	73.6	56.7	77.0	23.0	14.1
60	TM×TF	82.4	63.4	77.0	23.0	14.1
120	TM×NF	98.8	67.9	63.7	31.3	23.4
120	TF×NM	68.8	49.4	71.8	28.2	19.9
120	TM×TF	72.3	47.8	66.1	33.9	26.3

Dipping treatment = Significant at 1% level
Critical difference = 5.198

Pairing experiment = Insignificant.

Discussion

Larval feeding

Larval feeding of hempa produces only partial sterility in adult flies. Females were found to be more affected than males. Similar result was also recorded by Kaur and Wentworth(1972)[13]in the larval treatment of the face fly *Musca autumnalis*. In the present study, hempa was found to be highly toxic to larvae and it produced 100% mortality at .5% conc. Whereas, Hafez *et al.* (1969b)[9] reported similar mortality in *Musca domestica vicinia* at a concentration of 50mg. of hempa in 50g. of larval food, McFadden and

Table 5. Results of three days old pupal dipping treatment with 5% aqueous solution of Hempa. Details of fecundity, hatching and sterility in the adults emerging out of the treated pupae.

Dipping periods (mts)	Type of pairing experiment	Average No. of eggs laid per femals	Average No. of eggs hatched per female	Hatching (%)	Sterility (%)	Net sterility (%)
Control	NM×NF	127.6	113.4	88.8	11.2	—
30	TM×NF	125.6	88.7	70.6	29.4	20.4
30	TF×NM	131.8	101.5	77.0	23.0	13.2
30	TM×TF	123.4	87.2	70.6	29.4	20.4
60	TM×NF	116.6	72.6	62.2	37.8	29.9
60	TF×NM	111.4	81.3	72.9	27.1	17.9
60	TM×TF	97.2	58.1	59.7	40.3	32.7
120	TM×NF	133.2	65.7	49.3	50.7	44.4
120	TF×NM	107.2	62.5	58.3	41.7	34.3
120	TM×TF	112.6	53.5	47.5	52.5	46.5

Dipping treatment = Significant at 1% level
Crrtical difference = 7.36
Pairing experiment = Significant at 1% level
Critical difference=7.36

Table 6. Results of five day old pupal dipping treatment with 5% aqueous solution of Hempa. Details of fecundity, hatching and sterility in the adults emerging out of treated pupae.

Dipping periods (mts)	Type of pairing experiment	Average No. of eggs laid per female	Average No. of eggs hatched per female	Hatching (%)	Sterility (%)	Net sterility (%)
Control	NM×NF	102.8	91.7	89.2	10.8	—
30	TM×NF	129.4	114.4	88.4	11.6	0.8
30	TF×NM	126.2	112.5	89.1	10.9	0.1
30	TM×TF	117.2	103.8	88.5	11.5	0.7
60	TM×NF	85.8	71.8	83.6	16.4	6.2
60	TF×NM	86.8	74.8	86.1	13.9	3.4
60	TM×TF	97.2	82.9	85.2	14.8	4.4
120	TM×NF	108.0	75.2	69.6	30.4	21.9
120	TF×NM	77.0	63.0	81.8	18.2	8.2
120	TM×TF	85.5	57.4	67.1	32.9	24.7

Dipping treatment = Significant at 5% level
Control difference = 10.81
Pairing experiment = Insignificant.

Table 7. Mortality effect of 5% aquous solution of Hempa in pupal dipping treatment.

Age of pupa days	Dipping period	Average No. pupae dipped	Average No. of adults emerged	Average No. of mortality in treated pupae	Mortality (%)	Net mortality (%)
1 (control)	30	30	30	—	0.0	0.0
1	30	30	28	2	6.6	6.6
1 (control)	60	30	30	—	0.0	0.0
1	60	30	26	4	13.3	13.3
1 (control)	120	30	30	—	0.0	0.0
1	120	31	18	13	41.9	41.9
3 (control)	30	30	30	—	0.0	0.0
3	30	30	29	1	3.3	3.3
3 (control)	60	30	30	—	0.0	0.0
3	60	31	27	4	12.9	12.9
3 (control)	120	30	30	—	0.0	0.0
3	120	28	22	6	21.4	21.4
5 (control)	30	30	30	—	0.0	0.0
5	30	30	29	1	3.3	3.3
5 (control)	60	30	30	—	0.0	0.0
5	60	28	25	3	10.7	10.7
5 (control)	120	30	30	—	0.0	0.0
5	120	29	24	5	17.2	17.2

Dipping treatment (period) = Significant at 5% level.
Critical difference=15.51
Dipping treatment (pupal age) =Insignificant.

Rubio(1966)noted 100% mortality in the Mexican fruit fly *Anastrepha ludens* at .05%conc. of hempa. The present authors found that in *Drosophila melanogaster* the toxic effect or the mortality increased with increase in the dosage, and this observation agrees with similar observations made by McFadden and Rubio (1966) on *Anastrepha* and Hafez *et al.* (1966a & b) on *M. d. vicinia* and *Anophelis pharoensis.*

The larval treatment of *Drosophila melanogaster* with hempa does not hold out any promise as a method of inducing sterility because of the high rate of mortality even in low dosage.

Adult Treatment

In the present study, the adult feeding treatment resulted in significant and effective sterility. Between the lower conc. of treatment (0.5% and 1.0%) the difference was found to be significant whereas, the difference between the higher conc. (1.0%and 2.0%) was insignificant. It was found to have a pronounced sterility effect on the males rather than females. A similar effect of hempa was noted by Davis and Eddy (1966) in *Fannia canicularis*, by Jackson and Brindley (1971)[1]in European corn borer *Ostrinia nubilalis* and Ansari and Khan(1971)[2] in *Musca domestica.* However Pausch (1969) recorded sterility in both sexes of *Fannia canicularis* at .25%conc. (in adult feeding treatment).

It may be mentioned here that while hempa produces greater effect on the femalese emerging from larval feeding treatment, but its effect is reversed in the adult treatment i. e. it is found to be more effective on males. This sort of reversal of effect has not been reported earlier in case of hempa. Wolfenbarger (1972) noted a similar reversal of action in case of pink boll worm *Pectinophora gossypiella* with Metepa. The reason for this reversal of action is not known, but it can be presumed that perhaps it is due to difference in the mode of administration of the chemical which results in the different rate of absorption, distribution, metabolism and

excretion.

Pupal treatment

Kaur & wentworth (1972) observed a maximum sterility of 63.5% in treated males and 56.2% in treated females by dipping *Musca autumnalis* pupae of 2-3 days age in a 4.5% acetone solution of hempa. McFadden & Rubio (1966) recorded complete sterility in Mexican fruit flies *Anastrepha ludens* by dipping pupae in a 5% solution of hempa for 1 minute only. Metwally (1972) noted also complete sterility in Khapra beetle *Trogoderma granarium* by pupal treatment. However, in the present insects *Drosophila melanogaster*, the authors could not achieve any spectacular success in pupal treatment. The authors noted a maximum net sterility of 46.5% when 3 day old pupae were dipped for a long period of 120 mins. in a 5% solution. Hempa did not affect the fecundity of the treated females in any way.

The maximum net mortality of 41. 9% was observed in the 1 day old pupae of *Drosophila melanogaster* dipped for 120 minutes.

The pupal treatment study reveals that the pupae of *Drosophila* are rather less sensitive to the sterility and mortality effect of hempa.

Summary

The effects of oral feeding of hempa on the larvae and adults of *Drosophila melanogaster* have been studied. It was found that when treated (in larval stage) female was paired with normal male the fecundity was increased with reduction in hatching percentage. Similarly treated male when mated with normal female recorded a fall in the fecundity with a corresponding rise in the hatching percentage. In the larval treatment Hempa was found to be more effective on females than males.

It produces a 100% mortality in larval treatment at .5%conc. The adult treatment produces a net sterility percentage of 99.5 at 2.0% conc., when treated male and female are mated, and the male was found to be more affected than females. Thus, there is a reversal of action of hempa between larval and adult treatment i. e. it is more effective on females in the larval stage. Such reversal of action has not been noted earlier in hempa.

Hempa applied against adults holds out a good promise as sterilant for male. It does not in any way affect the capacity of the sterile males to mate with the normal females in nature.

Pupal treatment produces a significant sterility but not complete sterility as claimed by others.

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