Infecundity and dominant lethal mutations induced in *Musca domestica* L. by sodium azide (NaN_3)

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Summary. Results of our study suggest that sodium azide is effective in the induction of sterility and dominant lethal mutations in both sexes of *M. domestica* L. When treated males were crossed with nontreated females, 100% dominant lethal mutations and 72.3% infecundity were found, whereas in the crosses of nontreated males and treated females, 82.5% dominant lethal mutations and 33.1% infecundity were found. This showed that males are more sensitive to sodium azide than females.

Sodium azide has been found to be a very effective mutagen in bacteria and tester species of *Salmonella*[1,2]. In these microorganisms it induces mutations by base substitution. Goldsmith et al.[3] were the first to investigate chemical damage caused to insects' ovaries. Later on, Painter and Kilgore[4] recorded the inhibition of growth and the development of ovaries in *M. domestica* L. by an anti-tumour compound, 5-fluoroorotic acid. Howland et al.[5] fed *Trichoplusia ni* adults on 2% metepa, tris(2-methyl 1-aziridinyl) phosphine oxide and obtained 83% infecundity by crossing the treated females with treated males. Roach and Buxton[6] obtained 100% infecundity when *Conotrachelous neubhar* adults were treated with tepa, tris(1-aziridinyl) phosphine oxide. The failure of eggs laid to hatch is the expression of dominant lethal mutations produced either in sperms or ova or in both. Generally, death of embryos or zygotes occurs during early cleavages before the eggs hatch[7,8]. The induction of dominant lethals in insects' reproductive cells does not hinder the maturation of treated cells into gametes or their participation in zygote formation, but prevents further development of the zygotes into viable progeny[9]. The percentage of dominant lethals was calculated as embryos alive after treatment expressed as a percentage of the control[10], i.e.

$$100\left(1-\frac{\text{live embryos per treated female}}{\text{live embryos per control female}}\right).$$

Keeping in view the wide variety of uses of sodium azide in agriculture, medicine, and industry, it was thought desirable to study its effects on the fertility of insects. The purpose of the present work is to explore the possibilities of sterilizing *M. domestica* L. by using as a sterilant sodium azide, which is a respiratory inhibitor.

Materials and methods. Adult flies were collected from sweet shops near the University campus with the help of polythene bags. These flies were placed in plastic jars which had muslin cloths over their mouths tied with rubber bands. These jars were in turn placed in a B.O.D. incubator at 26 ± 2 °C with an alternative photoperiod of 12 h. Twice a day adult flies were fed with fly food (a mixture of sugar and nonfat dry powder milk in equal amounts, dissolved in water). Each jar was provided at the bottom with a black muslin cloth soaked in fermented solution on which eggs would be deposited. The fermented solution was prepared from 1 part saturated sugar solution and 12 parts of saturated yeast solution mixed with 200 parts of water. Collected eggs were placed on the top of wheat bran wetted with fermented solution in jars to obtain a fly culture. Flies were sexed within 16 h of their emergence in order to apply the chemical to the desired sex.

Treatment. Newly emerged flies were fed for 24, 48 and 72 h on treated diet (prepared by mixing 50 mg of fly food with 1 cm^3 aqueous sodium azide solutions of various concentrations i.e., 1 mg/ml, 0.5 mg/ml, 0.3 mg/ml and 0.1 mg/ml) and then provided with a normal diet. 4-day-old flies were allowed to mate. For experimental purpose only those pairs were taken which remained in copula for more than 15 min, because for the proper transfer of sperms at least 15 min are required. In order to know the correct percentage hatchability and age of larvae, collected eggs were allowed to hatch on black muslin cloth soaked in fermented solution or on damp black filter paper. The eggs laid by the female of each pair were counted daily to assess fecundity.

After treatment different kinds of crosses (MT × FNT and MNT × FT) were examined to estimate the percentages of infecundity, hatchability, pupal formation, adult emergence and dominant lethal mutations.

Results. In the present experiment, males were found to be more sensitive as compared with females. When on emergence flies were given treated diets (concentration 1 mg/ml) males died after 48 h, while females were alive even after 72 h of continuous feeding on treated food.

a) Effect on fecundity. A loss of fecundity, which is equivalent to depression in egg production, was found in sodium azide treated pairs. When treated males (MT) were allowed to mate with nontreated females (FNT), a decrease

Table 1. Effects of SA on percentages of: infecundity = 1, egg, hatchability = 2, pupal formation = 3, adult emergence = 4, and dominant lethal mutations = 5 in *Musca domestica* L.

Concentration	Eggs/F	1	2	3	4	5
Control	423	-	96.4	64.1	58.0	-
MT × FNT (feeding treatment 48 h)						
1 mg/ml	117	72.3	38.4	-	-	100
0.5 mg/ml	157	62.8	68.1	36.3	32.4	79.2
0.3 mg/ml	160	62.1	74.3	42.5	40.6	73.4
0.1 mg/ml	164	61.2	77.4	44.5	42.6	71.3
FT × MNT (feeding treatment 48 h)						
1 mg/ml	283	33.1	57.5	22.2	14.8	82.5
0.5 mg/ml	307	27.4	73.9	35.8	32.8	59.3
0.3 mg/ml	390	7.8	92.8	48.4	45.8	26.9
0.1 mg/ml	420	0.7	94.7	47.6	44.7	24.0

Average of 5 replicates.

Table 2. Effects of SA on F_1 generations from MT × FNT on percentages of: infecundity = 1, eggs hatchability = 2, pupal formation = 3, adult emergence = 4 and dominant lethal mutations = 5 of *M. domestica* L. (average of 5 replicates)

Concentration	Eggs/F	1	2	3	4	5
0.5 mg/ml	222	47.5	58.1	25.2	23.8	78.4
0.3 mg/ml	215	49.1	67.9	28.8	26.9	77.1
0.1 mg/ml	246	41.8	70.7	28.8	28.0	73.0

Abbreviations used: MT, Males treated; FT, females treated; MNT, males nontreated; FNT, females nontreated; SA, sodium azide.

in egg-laying was observed in the females, although they were not treated. The percentage infecundity was observed more in MT × FNT crosses (72.3) as compared to MNT × FT crosses (33.1) when they were given feeding treatments for 48 h (concentration 1 mg/ml). F_1 generations of treated pairs (MT × FNT) were also found to have various degrees of infecundity which depended on the doses applied to parent flies. Various degrees of infecundity induced in parents and F_1 generations at different doses are given in tables 1 and 2.

b) Effect on hatchability. Hatchability of eggs laid is an important criterion for assessing a potent chemosterilant. Percentage hatchability was observed to be 38.4 and 57.5 in the crosses of MT × FNT and FT × MNT respectively, when flies were fed for 48 h (concentration 1 mg/ml) in comparison to 96.4 for normal pairs. This also showed that males are more sensitive than females.

c) Effect on pupal formation. The percentage pupal formation was zero in MT × FNT crosses in comparison to 22.2% in crosses of FT × MNT. With normal pairs pupal formation was 64.1%. The percentage pupal formation at different doses is given in table 1.

d) Effect on adult emergence. The percentage emergence was found to be zero and 14.8 in the crosses of MT × FNT and FT × MNT respectively when flies were fed on treated food for 48 h (concentration 1 mg/ml). For normal pairs percentage emergence was 58.0.

e) Effect on dominant lethal mutations. Dominant lethal mutations showed a linear correlation with the concentrations of sodium azide applied. The higher the concentration applied and the longer the time given for feeding on treated diets, the more dominant lethal mutations were found, i.e. 100% dominant lethals were recorded in MT × FNT crosses, whereas 82.5% dominant lethals were found in FT × MNT crosses when flies were fed on treated diets for 48 h (concentration 1 mg/ml). Tables 1 and 2 depict the effects of different concentrations on the percentages of infecundity, egg hatchability, pupal formation, adult emergence and dominant lethal mutations induced in parent flies and F_1 generations. F_1 generations examined are only of MT × FNT crosses. This compound has not been identified as a chemosterilant in the literature before.

Discussion. Data presented in this paper show that sodium azide, even in small doses for a short duration of treatment (48 h), has a specific inhibitory effect on egg laying, hatchability, pupal formation and adult emergence. Goldsmith et al.[3] and Painter and Kilgore[4] were of the opinion that the damage caused by chemicals to ovaries is also responsible for causing depression in egg laying. In MT × FNT crosses, depression in egg laying may be due to an insufficient supply of sperms during mating. The present findings are very much in accordance with the findings of DeMilo et al.[11]. They reported that reduction in egg hatchability or emergence in F_1 generations in *Anthonomus grandis* was a less reliable indicator of sterilizing activity than reduction in oviposition, even when treated males were crossed with untreated females. The same results were also obtained by Borkovec et al.[12], when they treated *Anthonomus grandis* Boheman with 56 aziridinyl compounds.

Sodium azide did not produce complete infecundity in *M. domestica*, whereas several examples have been recorded of other chemicals with which 100% infecundity was obtained[6,13-15]. Sterility recorded in F_1 generations is similar to that observed by Stimmann and Gough[16] when they treated *Trichoplusia ni* with tretamine. Sodium azide was found to sterilize both sexes of the house fly as did apholate, aphomide and aphoxide studied on house flies, mosquitoes and stable flies[17]. Both in higher plant and bacterial systems azide appears to be a highly efficient and effective mutagen. However, its mechanism of action in vivo is not yet understood. Analyses of human leucocytes have shown no evidence of structural changes induced by sodium azide[18]. Its most noticeable effect was the transference of sterility or lethal genes from parents to offspring in the F_1 generation. Sodium azide can therefore be effectively used to reduce the population of house flies, which are a common household pest and occur whereever there is human habitation and unhygienic conditions. For practical reduction of population size, laboratory sterilized but sexually vigorous males are released in nature in numbers greater than those existing in the natural population. Sodium azide could be sprayed in the field for sterilization, but owing to its mutagenic effect this method is not appropriate.

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