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# Effect of Fluoride on the Reproductive Output of *Drosophila Melanogaster*

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*Abstract-* ***Drosophila melanogaster*** **is popularly used to study the effect of toxicity of chemicals. Exposure to fluoride may affect the reproductive potential of animals including humans. Therefore, a study was conducted to assess the effect of sub-lethal concentration of Sodium Fluoride (NaF) on the reproductive output of *Drosophila melanogaster*. It was found that 1.0 parts per million (ppm) of NaF was lethal for the adult flies and there was significant fall in the number of 3rd instar larvae, pupae and ecloded flies in different sublethal concentrations of NaF (0.2 ppm, 0.4 ppm, 0.6 ppm, 0.8 ppm). The study concluded that NaF (commonly used in pesticides), can cause developmental alterations in non-target insects like *Drosophila melanogaster*, thereby suggesting its role in developmental toxicity.**

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## I. Introduction

*Drosophila melanogaster* is popularly used as a model to study toxic potential of any chemical (Jatav et al., 2011), due to its easy maintenance in the laboratory and presence of about 50% homology with mammalian proteins (Pomerai et al., 2008). Many studies have been conducted using *Drosophila melanogaster* in laboratory conditions to reveal well defined effects of various insecticides and pesticides on the life cycle, hatchability and emergence of the fly (Nazir et al., 2001; Nazir et al.,2003, Gupta et al., 2005; Das et al., Podder 2010). There are four different stages in the life cycle of *Drosophila melanogaster*, i.e. egg, larva, pupa and adult. The eggs hatch into first instar larvae which moult twice into second and third instar larvae. Third instar larvae pupate and finally metamorphose into adult flies.

It has already been reported that fluoride containing chemicals like cryolite and NaF can cause alterations in the compound eye morphology and developmental stages in *Drosophila melanogaster* (Podder et al., 2012; Dutta et al., 2014). In the recent years, several investigations demonstrated that fluoride can induce oxidative stress and modulate intracellular redox homeostasis, lipid peroxidation and protein carbonyl content, as well as alter gene expression and cause apoptosis (Barbier et al., 2010).

Exposure to fluoride present in pesticides may affect the population of non-target organisms. Adverse effects of fluoride on fertility, fecundity and reproduction has been reported in several insects (Gerdes et al., 1971; Gong and Wu, 1991) and in *Drosophila melanogaster* (Khatun et al 2017). According to Freni (1994), lower birth rate in humans may be linked to intake of fluoride rich groundwater. The present study was undertaken to document the effect of sub lethal dose of NaF on the reproductive output of *Drosophila melanogaster*.

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## II. Methods

*Drosophila melanogaster* were cultured in standard cornmeal medium.

Two sets of cultured bottles were kept in triplicates: 1) Control , and 2) NaF treated.

1) Control set: Flies were cultured in normal cornmeal medium
2) NaF treated set: Flies were cultured in cornmeal medium in which NaF was mixed in different concentration i.e. 0.2ppm, 0.4ppm, 0.6ppm, 0.8ppm, and 1.0ppm.

Four adults of the same age group (two males and two females obtained from single line stock culture) were added into each bottle and left undisturbed so that flies of next generation could emerge from the pupae. These flies were counted. This denoted the reproductive output of the initially added flies. 3rd instar larvae and pupae were also counted to find out which developmental stage was most affected.

The statistical analysis of the count data was performed using Analysis of Variance (ANOVA). Because normality of data is a prerequisite for ANOVA, the count data were log transformed to ensure normal distribution.

## III. Results and Discussion

In the present study, the life cycle of *Drosophila melanogaster* was completed in nine days (at temperature ~ 28°C). AL-Saffar et al. (1995) found that development time steadily declined for *D. melanogaster* as temperature was raised from 15°C to 30°C. Maximum development rates have historically been observed between 30°C and 28°C (Davidson 1944, Ashburner and Thompson 1978). The generation time is roughly 10 days from fertilized egg to eclosed adult at 25° C (Fernández-Moreno et al., 2007).

Flies could not survive in medium with 1.00 ppm of NaF (Table 1, 2 and 3). Exposure of *Drosophila melanogaster* to sub-lethal doses (0.2, 0.4, 0.6 and 0.8 ppm) of NaF did not affect the duration of life cycle. However, Podder and Roy (2013) observed a distinct delay in emergence of flies in different concentrations of cryolite (sodium aluminum fluoride) as compared to control. Another study also demonstrated developmental delay in *Drosophila melanogaster* after chronic exposure to NaF (Dutta et al., 2014).

There was significant reduction in the number of 3rd instar larvae and pupae in different concentration of NaF as compared to control ($F=36.29$, $P<0.05$) as shown in Tables 1 and 2. There was also significant reduction in the number of flies of the next generation in different concentrations of NaF as compared to control ($F = 42.33$, $P <0.05$). Greatest reduction in reproductive output was seen in fly culture with 0.8 ppm of NaF in the cornmeal medium (Table 3). Decreased fecundity has been reported in *Bombyx mori* and *D. melanogaster* following exposure to environmental fluoride (Gerdes et al., 1971; Chen, 2003a; Chen, 2003b; Khatun et al 2017).

There was no change in number when third instar larvae changed into pupae in the medium containing NaF, suggesting sub-lethal concentration of NaF did not affect the growth phase. This may be due to the reason that ingestion of NaF with food during the larval life might have activated the drug-metabolizing enzymes. Drug-metabolizing enzymes have been reported in *Drosophila melanogaster* by Pai (1983).

Further, fluoride induces oxidative stress in fluoride-intoxicated animals through generation of Reactive Oxygen Species (ROS) and lipid peroxidation (Chlubek, 2003). A significant depression was seen in the number of ecloded flies from the pupae. This might be because NaF could have caused the reduction of oxidative phosphorylation and Adenosine triphosphate (ATP) synthesis. The pupae require energy for morphogenesis and organogenesis. Reduction in the ability of ATP synthesis might have interfered with metamorphosis. NaF might also have interfered with hormones required for metamorphosis.

*Table 1:* Number of 3rd instar larvae of *Drosophila melanogaster* at the end of 4th day of exposure to different concentrations of NaF

| NaF Concentration | | | | | | | |
|---|---|---|---|---|---|---|---|
| Experimental set number | Initial number of flies | Control | 0.2ppm | 0.4ppm | 0.6ppm | 0.8ppm | 1.0ppm |
| 1 | 0.60(4) | 1.60(40) | 1.30(20) | 1.25(18) | 1(10) | 0.77(6) | All flies died |
| 2 | 0.60(4) | 1.51(33) | 1.20(16) | 1.11(13) | 1(10) | 0.69(5) | All flies died |
| 3 | 0.60(4) | 1.41(26) | 1.20(16) | 1.17(15) | 1.11(13) | 0.90(8) | All flies died |
| Mean±SE | | 1.5±0.05 | 1.2±0.03 | 1.17±0.04 | 1.03±0.03 | 0.8±0.04 | |

Values are mean±SE. The count data were log-transformed for statistical analysis (ANOVA). The count data are given in parentheses .

*Table 2:* Number of pupae of *Drosophila melanogaster* at the end of 5th day of exposure to different concentrations of NaF

| NaF Concentration | | | | | | | |
|---|---|---|---|---|---|---|---|
| Experimental set number | Initial number of flies | Control | 0.2ppm | 0.4ppm | 0.6ppm | 0.8ppm | 1.0ppm |
| 1 | 0.60(4) | 1.60(40) | 1.30(20) | 1.25(18) | 1(10) | 0.77(6) | All flies died |
| 2 | 0.60(4) | 1.51(33) | 1.20(16) | 1.11(13) | 1(10) | 0.69(5) | All flies died |
| 3 | 0.60(4) | 1.41(26) | 1.20(16) | 1.17(15) | 1.11(13) | 0.90(8) | All flies died |
| Mean±SE | | 1.5±0.05 | 1.2±0.03 | 1.17±0.04 | 1.03±0.03 | 0.8±0.04 | |

Values are mean ±SE. The count data were log-transformed for statistical analysis (ANOVA). The count data are given in parentheses.

*Table 3:* Reproductive output of NaF treated flies after nine days of exposure to different concentration of NaF

| NaF Concentration | | | | | | | |
|---|---|---|---|---|---|---|---|
| Experimental set number | Initial number of flies | Control | 0.2ppm | 0.4ppm | 0.6ppm | 0.8ppm | 1.0ppm |
| 1 | 0.60(4) | 1.6 (40) | 1.25(18) | 1.17(15) | 1.04(11) | 0.69(5) | All flies died |
| 2 | 0.60(4) | 1.49 (31) | 1.17(15) | 1.07(12) | 0.90(08) | 0.47(3) | All flies died |
| 3 | 0.60(4) | 1.54(35) | 1.20(16) | 1.14(14) | 1.04(11) | 0.77(6) | All flies died |
| Mean±SE | | 1.55±0.03 | 1.2± 0.02 | 1.13±0.03 | 0.99±0.05 | 0.64±0.09 | |

Values are mean ±SE. The count data were log-transformed for statistical analysis (ANOVA). The count data are given in parentheses.

## IV. Conclusions

The present study concluded that NaF, which is a regularly used in toothpaste, insecticides and in water fluoridation program, can cause developmental alterations in non-target insects like *Drosophila melanogaster*, hereby suggesting its role in developmental toxicity.

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*Conflict of interest:* The authors declare that they have no conflict of interest.

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