American-Eurasian Journal of Toxicological Sciences 2 (1): 20-24, 2010
ISSN 2079-2050

Toxicity Evaluation of Fluoride in Silkworm *Bombyx mori* L.

[1]S. Vidyunmala, [1]A. Farhana Begum, [2]K. Nagalakshmamma and [1]A. Vijaya Bhaskara Rao

[1]Department of Sericulture, Sri Krishnadevaraya University, Anantapur-515 003, India
[2]Department of Seribiotechnology, Sri Padmavathi Mahila Viswavidyalayam, Tirupathi, India

Abstract: Toxicity evaluation of a pollutant will be highly useful in the final evaluation of designing 'safe level' or 'tolerable level' of pollution to the terrestrial biosphere and thus will have the way in establishing limits and levels of acceptability by biotic components. Keeping inview of this a static test was conducted to evaluate the toxicity of fluoride in silkworm *Bombyx mori*., L. As the 96 hr acute toxicity or short term test is the most commonly employed toxicity test, silkworms were treated orally with various doses of fluoride for 96 h and the percent mortality was recorded. The 96 h LD_{50} value was found to be 27.5 mg/kg b.w.

Key words: Fluoride · Toxicity · Silkworm

INTRODUCTION

Fluorides make their presence in the environment via soil, air, water. Ultimately their presence leads to the contamination of terrestrial ecosystems [1]. The fluorides from soil, air, water and fertilizers are well known to interfere with the efficient functioning of plants and animals. Many reports exhibited that the serious effects on the mulberry and silkworm cocoon rop production. Metabolic and physiological derangements are well documented in silkworm on exposure to fluoride toxicity. Physiological status leading to what is known as metabolic suppression which result in affected cocoon production [2-10].

Since the terrestrial habitats are more prone to hazardous pollutant like fluoride, toxicity evaluation of a pollutant will be highly useful in the final evaluation of designing safe level (or) tolerable level of pollution to the terrestrial biosphere and thus will have the way in establishing limits and level of acceptability by the biotic components. It also helps in programming preferential sericulture programmes depending upon the quality of environment and quality of pollutant.

LD_{50}, a common measure of toxicity is the lethal concentration that causes death in 50 per cent of the treated animals. LD_{50} is generally expressed as the dose in milligram (mg) of chemical per kilogram (kg) of body weight, LD_{50} is often expressed as mg of chemical per volume of medium. The organism is exposed to chemicals are considered highly toxic when the LD_{50} is small and practically non-toxic when the value is large. However the LD_{50} does not reflect any effects from long term exposure (i.e., cancer birth defects or reproductive toxicity).

For evaluating, toxicity of a substance there are different parameters such as lethal, sub-lethal median lethal dose, safe dose etc. The toxicity can be evaluated by exposing the animals to different doses to successive batches of the animals for fixed time and after suitable intervals by accounting the number of animals dead (or) alive [11]. However, the toxicity depends on factors like seasonality [12] and body size, [13] its development stage, the time of exposed, sex, age, nutritional status and environmental parameters like temperature, hydrogen ion concentration (pH) humidity light etc. In evaluating toxicity in silkworm, the young age silkworm (I instar to III instar) larva are sensitive to chemical substances even a lot lower dose. Hence, the toxicity evaluation has to be done in either IV or V instar. Several reports are available on toxicity evaluation of chemical substances on various insects including *B. Mori* [14-20]. LD_{50} Values are important to evaluating the toxicity level and also to determine the sub-lethal doses, the present studies begin with 96 hrs LD_{50} PM X CSR2. The present study is useful to observe the effect of fluoride and behavioral changes in silkworm on exposure to acute and sub-acute doses of fluoride.

MATERIALS AND METHODS

Silkworm *Bombyx mori*., L (PM X CSR_2) of V instar with an average weight of 1.5gm. were procured from RSRS, Anantapur, Andhra Pradesh and acclimatized to

Corresponding Author: A. Vijaya Bhaskara Rao, Department of Sericulture, Sri Krishnadevaraya University, Anantapur-515 003, E-mail: dravbrao@yahoo.co.in. Tel: +9440255089

laboratory and maintained the rearing conditions according to Krishnaswamy [21]. The temperature 24.0±1°C and humidity 75% ± 1 % were maintained throughout the rearing. During acclimatization fresh mulberry leaves V1 variety fed *adlibitum,* sodium fluoride (Merck, India Ltd., Mumbai) was used. A stock solution of 100 mg/ml was prepared in distilled water. An appropriate amount of fluoride was drawn from this stock solution for the further experiment. Fluoride was given to silkworm through oral dosage by spraying on mulberry leaves at different doses. Preliminary tests were conducted to find out the median tolerance limit (LD_{50}) of the silkworm for 96h by Probit analysis method [11]. The dose of fluoride at which 50% mortality occurred was taken as the median lethal dose (LD_{50}) for 96 h, which was found to be 27.5 mg/kg b.w.

The mean values were derived from the following the method of Finney Probit Kill Theory, (1971). The data was subjected to following statistical equations for arriving at LD_{50} values.

A graph was drawn between percent mortality and log doses of fluoride and a sigmoid curve was obtained.

A graph was drawn between probit mortality and log concentration of the pesticide and a linear curve was obtained.

To know the validity of data it was subjected to Dragstedt and Beheren's equation (Carpenter, 1975) as given below,

$$\text{Log } LD_{50} = \log A + \frac{50-a}{a-b} \times \log 2$$

Where,

A = Concentration of fluoride below 50% mortality.

a = Percent kill just below 50% mortality.

b = Percent kill just above 50% mortality.

The mean LD_{50} value was calculated from the values obtained from the above three methods namely percent, Probit mortality and Dragstedt and Beheren's method.

RESULTS AND DISCUSSION

The minimum dose at which zero percent mortality and maximum dose at 100% mortality was exhibited at 0.950 mg/kg b.w and 40.0 mg/kg b.w respectively in silkworm, *Bombyx mori*., L. LD_{50} obtained thorough sigmoid curve is 27.5 mg/kg b.w. (Fig. 1) and linear curve is 27.5 mg/kg b.w (Fig.2). The LD_{50} values were further verified by Dragstedt and Behren's method and the LD_{50} value obtained was 27.58 mg/kg b.w. Hence, the average LD_{50} for 96 h is estimated to be 27.52 mg/kg b.w.

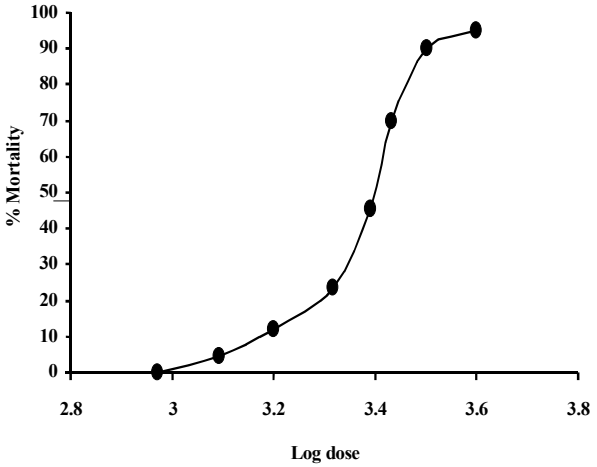

Fig. 1: The graph showing sigmoid curve between percent mortality (kill) of silkworm against log dose

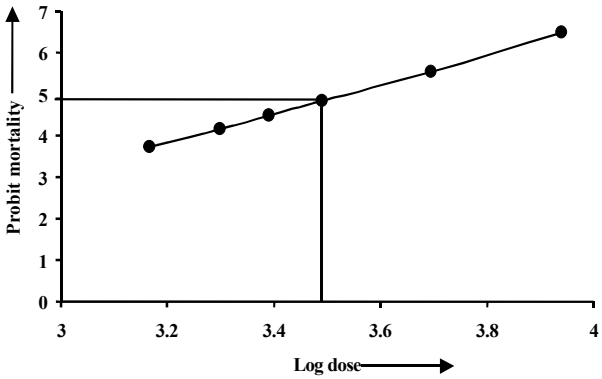

Fig. 2: The graph showing linear curve between probit mortality of silkworm against log dose

The toxicity studies of fluoride were also made by some workers on different animals. Ramakrishna *et al.*, [5] obtained V instar silkworm larvae LD_{50} as 82.37 ppm of fluoride at 216 hrs (in case of Pure Mysore) and LD_{50} 52.00 ppm of fluoride at 168 hrs (in case of NB_4D_2 race). According to Fieser *et al*., [22] for Water flea (*Daphnia magna*) 48 h LC_{50} was found to be 200 mgF/litre and in Caddis fly (*Chimars Marginata*) the 96 h LC_{50} was 44.9 mgF/litre of last intar [23]. Prawn (*Penaeus Indicus*) exhibited 96 h LC_{50} as 111.18 mgF/litre [24]. In Mysid shrimp (*Mysidopsis bahia*) the LC_{50} was 10.5 mgF/litre [25]. Vijayabhaskara Rao, A. [26] showed a 24 hr LD_{50} of 50.56 mgF/kg b.w. in albino mice, *Mus norvegicus albinus*, Pillai *et al.,* [27, 28] demonstrated a 24 hr LD_{50} of 54.4 mg /kg bw, for male mice and slightly less for female mice (51.6 mg F/kg b.w).

Fuji and Honda [29] reported that mulberry leaves contaning 10-15 ppm fluoride were lethal to silkworm larvae. Farhana Begum, A [30] reported a 24 hr LD_{50} as

Table 1: Mortality of silkworm larvae in different doses of fluoride at 96 h exposure periods

S.No.	Dose of fluoride (mg/kg b.w.)	Log dose	No. of larvae exposed	No. of larvae dead	% Mortality	Probit mortality
1.	0.950	2.97	50	0	0	--
2.	10.00	3.00	50	5	10	3.72
3.	15.00	3.17	50	10	20	4.16
4.	20.00	3.30	50	15	30	4.48
5.	25.00	3.39	50	20	40	4.75
6.	30.00	3.47	50	35	70	5.52
7.	35.00	3.54	50	45	90	6.28
8.	40.00	3.60	50	50	100	--

Table 2: LD_{50} values of silkworm larvae after 96 h treatment

S. No.	Name of the Method	LD_{50} value (in mg/kg b.w.)
1.	Percent mortality (Sigmoid curve)	27.50
2.	Probit mortality (linear curve)	27.50
3.	Dragsted and Beheren's method	27.58
	Mean	27.52
	Standard deviation ±	± 2.065

23.9 mg/kg b.w. V instar silkworm *Bombyx mori*., L. (PM X NB4D2) with fluoride. In the present study the 96 h LD_{50} was evaluated to be as 27.52 mg/kg b.w. for V instar silkworm PM X CSR2 silkworm, *Bombyx mori*., L. The variation in the LD_{50} value in the present study from the earlier studies indicates that different silkworm verities and localities exhibit wide range of fluoride tolerance. The results were also coincide with the studies of Yuin Chen [31] who reported that there was a considerable difference in the LC_{50} ranging from 19 to 693 mg F/kg of dried mulberry leaves for the same variety of silkworm maintained in different localities, there by indicating adaptation to atmospheric fluoride pollution. The results of the present study also support the statement in which it is noted that silkworm is highly sensitive to fluoride perhaps the higher metabolic rate of rapid incorporation of toxicants into the tissues would occur [32]. LD_{50} values are useful in determining the sub lethal and sub-sub lethal doses of fluoride.

Typical symptoms of exposure to fluoride intoxication involving the determination of LD_{50} include were observed, the controlled silkworm exhibited in their usual manner i.e., the silkworm were very actively feeding and movements were well co-coordinated, silkworm were alert and at any slight disturbance, moved fast. The silkworm exposures to lethal doses of fluoride became irritable and hyper excited movements abnormal crawling movements were observed, other symptoms that have been observed are slowly becoming restlessness slowly becoming sluggish with sharp jerky movements on exposure to lethal dose. Finally the silkworm settles down at some place with the loss of equilibrium and caused to death. Following sub lethal dose exposure, symptoms of fluoride poisoning are not severe, include appearance of some hyper excitable movements and slight vomiting at the initial hours and decreases food consumption. Fluoride, as a complex mode of action and the knowledge of toxicity studies and behavioral observations are certainly useful to establish limits and levels of susceptibility of the silkworm *Bombyx mori*., to fluoride.

ACKNOWLEDGEMENT

The authors highly acknowledge the UGC, New Delhi, for providing financial support as Minor Research Project to Dr. A. Vijaya Bhaskara Rao.

REFERENCES

1. Kuribayashi, S., 1977. Effects of atmospheric pollution by hydrogen fluoride on mulberry tree and silkworm. J. Sericultural Sci., 46: 536-544.
2. Chen, Y.Y. and Y.C. Wu, 1994. Study on the effect of fluoride on growth, development and economic parameters of the silkworm, *Bombyx mori*., Sericologia, 34: 627-635.
3. Aftab, A.C.A. and M.V. Chandrakala, 1999. Effect of oral administration of sodium fluoride on food and water utilization in silkworm *Bombyx mori*., L. Insect Science Applications, 19: 193-198.

4. Aftab, A.C.A., M.V. Chandrakala and V.G. Maribashetty, 1999. Influence of sodium fluoride on nutritional efficiency of a multivoltine race of the silkworm, *Bombyx mori*., J. Experimental Zool. in India, 2: 127-133.
5. Ramakrishna, S., B. Surendra Nath and Jayaprakash, 2004. Evaluation of Relative Fluoride Toxicity and its impact on Growth, Economic Characters and Fecundity of the silkworm, *Bombyx mori*., L. Intl. J. Industrial Entomol., 8(2): 151-159.
6. Ramakrishna, S. and Jayaprakash, 2007. Shifts in Protein metabolism in Hemolymph and Fat Body of the silkworm, *Bombyx mori*., L. in response of Fluoride Toxicity, Intl. J. Industrial Entomol., 15(1): 59-68.
7. Vidyunmala, S., A. Farhana Begum, B. Gangadhar and A. Vijaya Bhaskara Rao, 2009. Effect of Fluoride on Behavioral and some Biochemical aspects of the silkworm *Bombyx mori*., L. Academic J. Entomol., 2(2): 76-79.
8. Chen Yuin, Xin Du, Yuanxiang Jin and Hangzhou, 2005. Cytochemical evidence of anomalous dose-response of acid phosphatase activity in the blood but not the midgut of fluoride-treated silkworm larvae, *Bombyx mori*., Fluoride, 38(2): 91-174.
9. Yuyin Chen, 2003 [a]. Variable tolerance of the silkworm *Bombyx mori*., to atmospheric fluoride pollution. Fluoride, 36(3): 157-162.
10. Yuin Chen, 2003[b]. Difference in fluoride effect on fecundity among varieties of the silkworm *Bombyx mori*. Fluoride, 36(3): 157-162.
11. Finney, D.J., 1971. Probit Analysis. 3rd (ed) London, New York, Cambridge University Press, pp: 333.
12. Victor Perez-Landa., Maria Jesus Beluence and Javier Franco, 2008. The effect of Seasonality and Body size on the sensitivity of Marine Amphipods to toxicants, Bulletin of Environ. Contamination and Toxicol., 81(6): 548-552.
13. Ramakrishna, A., 1995. Studies on fluoride induced alterations in the freshwater mussel *Lamellidens marginalis* (LAMARK). Ph.D., Thesis, Sri Krishnadevaraya University, Anantapur, India.
14. Kuwana, Z., S. Nakamura and H. Sugiyanna, 1967. Effects of insecticides on the silkworm larvae, *Bombyx mori*., L. analysis of the insecticides silkworm larvae relationship by means of subcutaneous injections and topical and oral applications. Bulletin of Sericulture Experiment Station, 22(1): 123-180.
15. Kashi, K.P., 1972. Tolerance of silkworm (*Bombyx mori*., L.) larvae to organophosphorous insecticides. J. Sericlture Sci., 41: 301-304.
16. Singh, Y.P. and A.S. Srivastava, 1984. Relative toxicity of some insecticides to the larvae of crypto blades gnidiella Miller. J. Entomol., 46(3): 346-351.
17. Srivastava, A.S. and M.A. Massoli, 1985. Relative toxicity of some insecticides to the second instar larvae of *Legmantria abfuscenta* Walker. Entomon., 10(3): 241-244.
18. Christian, L., Mounty, C. John, Owens, E. Brod, Lewis Frank and M. Devis, 1986. Interaction studies of methyl parathion, carbofuran, permithrin and piperonyl butoxide applied to South Western corn borer Larvae (Lepidoptera pyralidae). J. Ecol. Entomol., 79(3): 531-541.
19. Thay, M. and P.T. Collins, 1986. Joint effect of temperature and insecticides on mortality and fecundity of Sitophilus pruzae, Cealeoptera cuculionidaeo and wheated maize. J. Econom. Entomol., 79: 909-914.
20. Venkata Reddy, S., N. Sivarami Reddy and R. Ramamurthi, 1989. Carbonyl effects on the growth and silk quantities of the silkworm *Bombyx mori*., L. Indian J. Sericulture, 28(2): 182-190.
21. Krishnaswamy, S., 1978. New Technology of silkworm rearing. Bulltin. No. 3. CSR and TI, Mysore, pp: 23.
22. Fieser, A.H., J.L. Sykora, M.S. Kostalos, Y.C. Wu and D.W. Weyel, 1986. Effect of fluorides on survival and reproduction Daphnia magna. J. Water Pollution Control Federation, 58(1): 82-86.
23. Camargo, J.A. and J.V. Tarazona, 1990. Acute toxicity to freshwater benthic macroinvertebrates of fluoride ion (F-) in soft water. Bulletin of Environ. Contamination Toxicol., 45(6): 883-887.
24. McCune, D.C., T.L. Lauver and K.S. Hansen, 1991. Relationship of concentration of gaseous hydrogen fluoride to incidence and severity of foliar lesions in black spruce and white spruce. Canadian J. for Res., 21: 756-761.
25. LcBlanc, G.A., 1984. Interspecies relationships in acute toxicity of chemicals to aquatic organisms. Environ. Toxicol. Chem., 3: 47-60.
26. Vijaya Bhaskara Rao, A., 1994. Fluoride induced alterations in mice, *Mus norvegicus albinus*-Impact of aluminum. Ph.D.. Thesis, S. K. University, Anantapur, India.

27. Pillai, K.S., A.T. Mathai and P.B. Deshmukh, 1988. Effect of sub acute dosage of fluoride on male mice. Toxicol. Lett., 44: 21-29.
28. Pillai, K.S., A.T. Mathai and P.B. Deshmukh, 1987. Acute toxicity of fluoride to mice, Fluoride, 20: 68-70.
29. Fuji, M. and S. Honda, 1972. The relative oral toxicity of some fluorine compound for silkworm larvae. J. Sericulture Sci., 41: 104-110.
30. Farhana Begum, A., 2003. Effect of fluoride on some physiological aspects and cocoon commercial characters of silkworm *Bombyx mori*., L.M. Phil Dissertation, S. K. University, Anantapur, India.
31. Yuin Chen, 2003. Variable tolerance of the silkworm *Bombyx mori.,* to atmospheric fluoride pollution, Fluoride, 36(3): 157-162.
32. Dame, R.F., 1972. The ecological energies of growth respiration, Assimilation in the inter tidal American oyster, *Crassostree virginica*. Marine Biol., 17: 243-250.