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Evaluation of Relative Fluoride Toxicity and Its Impact on Growth, Economic Characters and Fecundity of the Silkworm, *Bombyx mori* L.

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A detailed evaluation of relative toxicity of fluoride to the 5th instar larvae of PM and NB_4D_2 races of silkworm and the effects of lethal, sublethal and prevailing levels of fluoride in groundwater on the growth, economic characters and fecundity of the silkworm, *Bombyx mori* (Lepidoptera: Bombycidae) were studied. The feeding of mulberry, *Morus alba* leaves treated with lethal and sublethal doses of fluoride to PM and NB_4D_2 races of silkworms from the beginning of the 5th instar to the end of the feeding period resulted in significant reduction in growth, single cocoon weight, single shell weight, silk index, average filament length, and fecundity when compared to controls. These changes were more pronounced on exposure to lethal dose than sublethal dose and in general, the changes induced by fluoride were more striking in NB_4D_2 than PM, indicating the greater resistance of PM to higher fluoride levels. Groundwater quality in sericulturally important villages of Karnataka and Andhra Pradesh States has been studied with special reference to the presence of fluoride. On exposure to dose of prevailing levels in the waters of sericulturally important areas, the changes observed on growth, economic characters and fecundity were slightly lower when compared to controls and the decrease was found to be insignificant ($P \geq 0.05$). It was concluded that, though minute dose (4.0 ppm) of fluoride did not have any toxic impact, it is toxic at higher concentrations to silkworms.

Key words: Fluoride toxicity, Silkworm, *Bombyx mori*, *Morus alba*, Lethal dose, Sublethal dose, Economic characters, Fecundity

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Introduction

In recent years, high concentrations of fluoride in groundwater, used for drinking and irrigation poses a great problem in most of the States of India (Susheela, 2001; Muralidharan *et al.*, 2002). Out of 6 lakh villages in India, at least 50% of them may have fluoride content in drinking water exceeding 1.0 mg/l concentration of fluoride (up to 10 ppm) have been observed in dug and tube well waters which are being used for drinking and irrigation purpose in different parts of Karnataka and Andhra Pradesh States (Valdiya, 1987). Fluoride toxicity due to drinking of fluoride containing water was first described in humans (Shortt *et al.*, 1937). Fluoride concentration in groundwater as well as in soil is much higher than the permissible limits and the same has direct bearing on plants and animals (WHO, 1984). In addition, the atmospheric fluoride emanating from extremely scattered rural/urban industries like cement, brick, tile, coal burning, fertilizers *etc.*, has caused serious economic losses in the agriculture field. The negative effect of growth and development in plants and silkworms with fluoride was reported (Davies *et al.*, 1992; Chen and Wu 1995; Aftab *et al.*, 1999). Sericulture being an agro-based cottage industry has a direct bearing on the fluoride rich water as well as with atmospheric pollution of fluoride.

Silkworm cocoon production has added new dimensions in national economy of India, besides providing employment to millions of people in rural areas. In recent, however, the increased industrial emission of various toxicants including fluoride into the atmosphere has seriously affected silkworm cocoon production (Nath, 1993). The deleterious effects of fluoride on silkworms have been well documented in China, Japan and India (Fujii and Honda, 1972; Kuribayashi, 1977; Shong, 1985; Wu, 1990; Chen, 1993; Chen and Wu, 1994; Aftab and Chandrakala, 1999; Aftab *et al.*, 1999). Previous reports of fluoride toxicity to silkworms have been primarily with shorter duration of 24 hrs to 96 hrs and the quantity of residues accumulated on the mulberry leaves through atmospheric pollution (Fujii and Honda, 1972; Chen and Wu, 1995; Wu *et al.*, 1998). However, no information is available on detailed relative toxicity evaluation of fluoride to two races of silkworms *viz.*, PM (multivoltine) and NB_4D_2 (bivoltine) with longer period of exposure *i.e.*, for entire fifth instar larvae. Considering this, it was thought worthwhile to undertake detailed relative fluoride toxicity studies to the silkworm and to conduct a survey of fluoride levels present in groundwater in order to know the toxic impact on growth, economic characters and fecundity of the silkworm, *Bombyx mori.*

Materials and Methods

Insect

Economically important multivoltine race *viz.*, Pure Mysore (PM) and bivoltine race *viz.*, NB_4D_2 silkworm, *B. mori* larvae of uniform size, weight and age groups (fifth instar) were used as test material in the present study. Freshly hatched larvae of *B. mori* were reared up to IV moult on leaves of *Morus alba* (M_5 variety) in an environmental chamber as per the standard rearing conditions (Krishnaswami, 1978).

Fluoride selected

Commercially available anhydrous sodium fluoride (NaF) AR grade procured from S. D. Fine-Chem Ltd., Mumbai, India was used as a toxicant in the present study. The sodium fluoride was dissolved in distilled water to various concentrations (ppm) of range for further study.

Groundwater survey for fluoride levels

Field visits to different sericulturally important villages in the districts of Kolar (Karnataka State) and Anantapur (Andhra Pradesh State) were made to collect groundwater samples for evaluation of prevailing fluoride levels. Water samples were collected in polyethylene bottles (one litre) for fluoride analysis. A total of 120 groundwater samples were collected (*i.e.*, 6 samples per village). The fluoride levels in the collected waters from different villages, which was used for cultivation of mulberry, was estimated by the ion selective electrode method, as described by Harwood (1969).

Experimental procedure

Immediately after the IV moult, individual silkworm larvae (PM and NB_4D_2) of uniform weights and age were collected from the rearing tray and divided into six batches, comprising of 100 larvae in each batch. They were maintained in an environmental chamber at $24 \pm 1^{\circ}C$ with $75 \pm 5\%$ relative humidity (Krishnaswami, 1978). Mulberry leaves were dipped for few minutes in lethal, sublethal and a dose based on prevailing levels of fluoride in waters of sericulturally important areas of Karnataka and Andhra Pradesh States, removed immediately and let stand for few minutes for the water to evaporate before being given to silkworms. Later, the silkworms were separately fed four times a day until spinning on (i) fresh leaves treated with distilled water, which served as control (ii) leaves treated with lethal dose of fluoride (iii) leaves treated with sublethal dose of fluoride (iv) leaves treated with a dose based on prevailing levels of fluoride in the waters of Karnataka and Andhra Pradesh States.

Evaluation of fluoride toxicity

Immediately after the IV moult, individual silkworms (PM and NB_4D_2) of uniform size and age groups were collected from the rearing colony and divided into batches comprising of 100 larvae in each batch and maintained in an environmental chamber at $24 \pm 1°C$ and $75 \pm 5\%$ relative humidity (Krishnaswami, 1978). Mulberry leaves were dipped in various doses ranging from 30 to 120 ppm for 10 min, removed and allowed for few minutes for the water evaporation. The treated mulberry leaves were fed four times a day until spinning of each batch to one dose ranging from 50 ppm to 120 ppm and 30 ppm to 100 ppm fluoride for PM and NB_4D_2 races of silkworms respectively. These ranges were obtained on trial and error basis. Mortality was recorded in all the doses of fluoride taken every 24 hrs up to spinning. A batch of PM and NB_4D_2 races were separately reared with distilled water treated mulberry leaves, which served as control. The experiment was repeated thrice. The mortality rates observed at each dose obtained from the mean of three replicates were converted as per cent mortality and from it the probit mortality rate was observed (Finney, 1971). The probit mortality, expected probits, working probits, log doses, and weighing co-efficients of the PM and NB_4D_2 races of silkworms in different doses (ppm) of fluoride at 216 hrs (in case of PM) and 168 hrs (in case of NB_4D_2) of exposure was derived as per the method of Finney (1971) in order to obtain LD_{50} values, fiducial limits and regression equation for the fifth instar larvae of silkworms. The 95% fiducial limits were used to determine the statistical significance of any observed differences in LD_{50} values. LD_{50} values for 216 hrs and 168 hrs of fluoride were taken as lethal dose to study the growth, economic characters and fecundity of PM and NB_4D_2 races of silkworms. However, knowledge in the dose of a toxicant that kills 50% of the test animals in a fixed period of time could become insufficient to assess various responses of the animal to the toxicant (Nobbs and Pearu, 1976). Further studies on acute toxicity have significant limitations such as the occurrence of adaptation of test animal to the imposed toxicity (Stockner and Anita, 1976). Perkin (1979) felt the need for sublethal studies because distinct changes involving a sequence of events in the response of test animal could occur in sublethal dose. So, about one fifth of the 216 hrs and 168 hrs LD_{50} of fluoride was taken as the sublethal dose of fluoride for further studies.

Determination of growth and cocoon characters

For determination of growth (g dry weight/10 larvae), the sacrifice method described by Maynord and Loosli (1962) was used to measure the growth of laboratory animals (Gerking, 1952; Pandian and Raghuraman, 1972) was employed in the present study. After weighing, the test individuals were transferred into experimental trays, while the others were weighed and killed for estimating growth. After completion of spinning, cocoon characters such as single cocoon weight (g), single shell weight (g) and average filament length (m) were determined for PM and NB_4D_2 races exposed to lethal, sublethal and prevailing level of fluoride in the ground water, as per the method of Krishnaswami *et al.* (1973). The silk index was calculated by the method of Joshi (1985).

Determination of fecundity

After assessment of cocoon characters, the remaining cocoons were kept separately under controlled condition at 24 ± 1°C. Female and male moths emerging from the cocoons of control and experimental batches were allowed for pairing. The paired moths were left undisturbed for few hours, decoupled and the mated female moths were allowed individually under inverted glass funnel to lay eggs and the eggs were then counted.

Statistical analysis

The mean of six individual values was subjected to statistical analysis and paired Student's 't' test was applied to compare the differences between the control and experimental groups. The significance level was derived at $P \leq 0.05$ in all cases.

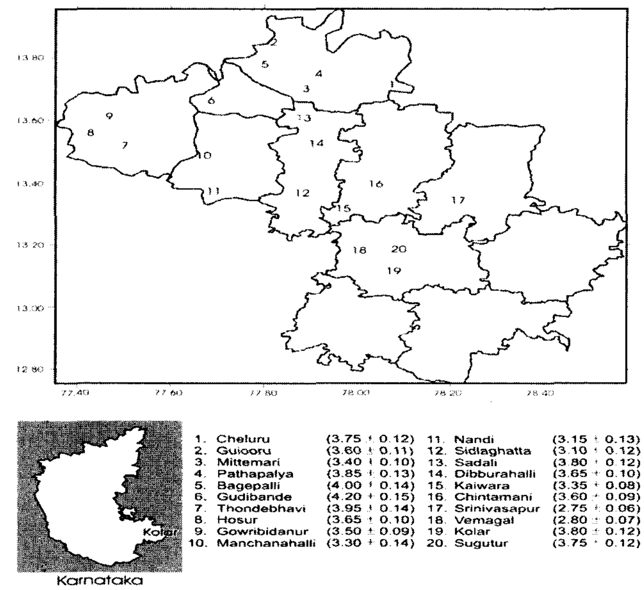

Fig. 1. Map showing the areas of survey conducted to collect groundwater samples in 20 villages in the district of Kolar, Karnataka State. The values indicated in the parenthesis are the levels (ppm) of fluoride present in groundwater.

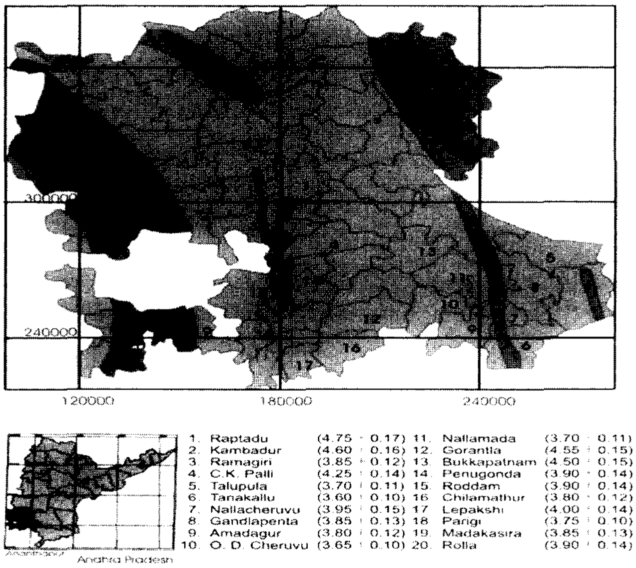

Fig. 2. Map showing the areas of survey conducted to collect groundwater samples in 20 villages in the district of Anantapur, Andhra Pradesh State. The values indicated in the parenthesis are the levels (ppm) of fluoride present in groundwater.

Results

Groundwater survey for fluoride levels

The water samples collected from the sericulturally dominant villages possessed fluoride concentrations ranging from 2.80 to 4.00 ppm and 3.60 to 4.75 ppm in 20 villages of Kolar district (Karnataka State) and in 20 villages of Anantapur district (Andhra Pradesh State) respectively (Fig. 1 and 2). Based on the prevailing levels of fluoride in the waters, an approximate average dose of 4.00 ppm was considered to know the impact of this dose on silkworm growth, cocoon characters and fecundity.

Evaluation of fluoride toxicity

The data on per cent mortality, probit mortality, expected probits, working probits, log doses and weighing co-efficients of the PM and NB_4D_2 races of silkworms in different dosages (ppm) of fluoride at 216 hrs (in case of PM) and 168 hrs (in case of NB_4D_2) of exposure are presented in Tables 1, 2, Fig. 3 and 4. The percent mortality plotted against log dosage of fluoride gave sigmoid curves, whereas probit mortality plotted against log dosage of fluoride gave straight lines (Fig. 3 and 4). The 216 hrs LD_{50} of fluoride toxicity observed for PM race was 82.37 ppm with fiducial limits at 95% was 79.90752 – 84.86745 and with regression equation value 9.241739 + 7.433986 (Table 1). The 168 hrs LD_{50} of fluoride toxicity for NB_4D_2 was 52.00 ppm with fiducial limit at 95% was 49.02464 – 53.21042 and with regression equation value of 5.807729 + 6.324549 (Table 2).

Determination of growth and economic characters

The data pertaining to growth, single cocoon weight, single shell weight, silk index and average filament length are presented in Table 3. As compared to control, the growth and cocoon characters revealed a significant reduction in PM and NB_4D_2 races on exposure to lethal and sublethal doses of fluoride. But the percentage of reduction was comparatively more during lethal intoxication both in PM and NB_4D_2 races under fluoride stress. Further, between the two races, the percentage of reduction observed were found to be more pronounced in NB_4D_2 race than in PM (Table 3). On exposure to the prevailing levels of fluoride (4.00 ppm) in the water used for cultivation of mulberry plants, the growth and cocoon characters did not affect much for both races of PM and NB_4D_2 (Table 3) and the decrease in growth and cocoon characters was found to be insignificant ($P \geq 0.05$).

Fecundity

The number of eggs laid by the female moth of PM and NB_4D_2 races of silkworms were found to be significantly low when compared with the controls on exposure to

Table 1. 216 hrs percent, probit mortality, expected probits, working probits, log dosage, weighing co-efficients of Pure Mysore (PM) race of silkworm, *Bombyx mori* fed with *Morus alba* leaves treated for different dosages of fluoride. Each value is a mean of three replicates

Sl. no.	Dose (ppm)	No. of animals tested	No. of animals dead	% mortality	Probit mortality	Expected probits	Working probits	Log dosage	Weighing co-efficients
1	50	100	10	10.00	3.52	3.60	3.72	1.70	0.302
2	60	100	17	17.00	4.01	4.10	4.05	1.78	0.471
3	70	100	26	26.00	4.48	4.50	4.35	1.85	0.581
4	80	100	42	42.00	4.80	4.90	4.80	1.90	0.634
5	90	100	55	55.00	5.39	5.20	5.13	1.95	0.627
6	100	100	68	68.00	5.92	5.50	5.47	2.00	0.581
7	110	100	83	83.00	6.28	5.80	5.94	2.04	0.503
8	120	100	100	100.00	8.09	6.00	6.66	2.08	0.439

Sl. no.	nw	nwx	nwxx	nwy	nwyy	nwxy
1.	30.199	51.307	87.16	112.431	418.580	191.017
2.	47.144	83.829	149.061	190.792	772.134	339.257
3.	58.099	107.198	197.791	252.847	1100.390	466.527
4.	63.431	120.715	229.731	304.405	1460.841	579.311
5.	62.742	122.613	239.616	321.553	1647. 958	628.392
6.	58.099	116.198	232.396	317.627	1736.468	635.255
7.	50.260	102.600	209.448	298.745	1775.743	609.857
8.	43.863	91.199	189.620	291. 952	1943.234	607.022
	Snw	Snwx	Snwxx	Snwy	Snwyy	Snwxy
	413.837	759.660	1534.832	2090.352	10855.35	4056.636

LD_{50} value: 82.37 ppm.

Y value: 9.241739 + 7.433986.

Fiducial limit at 95%: 79.90782 – 84.86745.

Table 2. 168 hrs percent, probit mortality, expected probits, working probits, log dosage, weighing co-efficients of NB_4D_2 race of silkworm *Bombyx mori* fed with *Morus alba* leaves treated for different dosages of fluoride. Each value is a mean of three replicates.

Sl. no.	Dose (ppm)	No. of animals tested	No. of animals dead	% mortality	Probit mortality	Expected probits	Working probits	Log dosage	Weighing co-efficients
1	30	100	10	10.00	3.72	3.60	3.73	1.48	0.302
2	40	100	25	25.00	4.33	4.40	4.33	1.60	0.558
3	50	100	44	44.00	4.85	4.90	4.85	1.70	0.634
4	60	100	64	64.00	5.36	5.40	5.36	1.78	0.601
5	70	100	80	80.00	5.84	5.80	5.84	1.85	0.503
6	80	100	88	88.00	6.18	6.20	6.18	1.90	0.370
7	90	100	94	94.00	6.55	6.50	6.55	1.95	0.269
8	100	100	100	100.00	8.09	6.80	7.26	2.00	0.180

Sl.no.	nw	nwx	nwxx	nwy	nwyy	nwxy
1.	30.199	44.608	65.891	112.582	419.705	166.30
2.	55.788	89.376	143.185	241.395	1044.515	386.729
3.	63.431	107.767	183.094	307.577	1491.44	522.564
4.	60.052	106.782	189.874	321.759	1723.983	572.136
5.	50.260	92.735	171.105	293.569	1714.735	541.663
6.	37.031	70.473	134.117	228.667	1412.015	435.173
7.	26.907	52.583	102.760	176.322	1155.435	344.575
8.	17.994	35.988	71.976	130.547	947.115	261.093
	Snw	Snwx	Snwxx	Snwy	Snwyy	Snwxy
	413.837	795.662	1534.832	2090.352	10855.35	4056.636

LD_{50} value: 52.00 ppm.
Y value: 5.807729 + 6.324549.
Fiducial limit at 95%: 49.02464 – 53.21042.

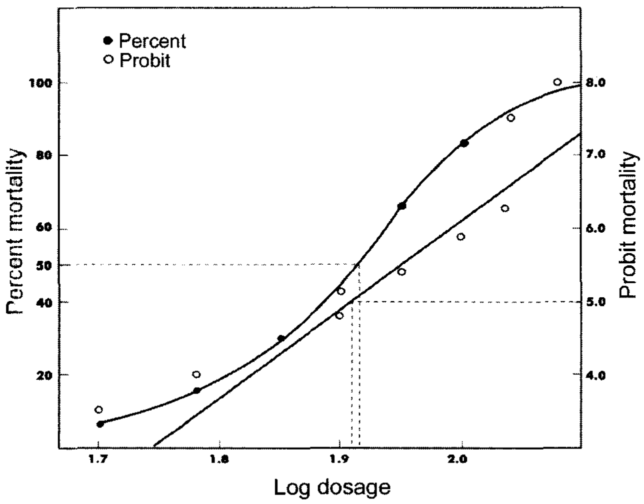

Fig. 3. 216 hrs percent and probit mortality of fifth instar PM race of silkworm, *Bombyx mori* as a function of log dosage of fluoride. Each point is a mean of three replicates. LD_{50} values are indicated both on per cent and probit mortality curves.

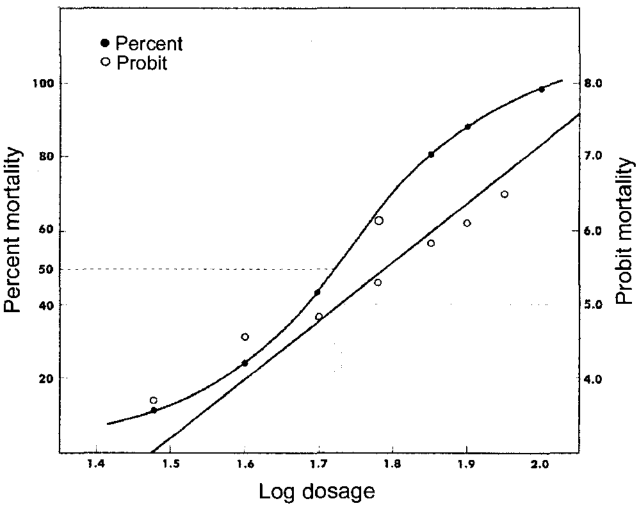

Fig. 4. 168 hrs percent and probit mortality of fifth instar NB_4D_2 race of silkworm, *Bombyx mori* as a function of log dosage of fluoride. Each point is a mean of three replicates. LD_{50} values are indicated both on per cent and probit mortality curves.

Table 3. Growth, single cocoon weight, single shell weight, silk index, average filament length, and fecundity in the control and fluoride treated PM and NB_4D_2 races of silkworm, *Bombyx mori*

	PM		NB_4D_2	
	Control	Experimental	Control	Experimental
Growth (g dry wt. /10 larvae)				
Lethal	23.175 ± 1.137	15.655 ± 0.427 (-32.45%) $P \leq 0.001$	44.350 ± 1.725	26.073 ± 0.795 (-41.21%) $P \leq 0.001$
Sublethal	23.175± 1.137	18.837± 0.548 (-18.72%) $P \leq 0.001$	44.350 ± 1.725	32.021 ± 0.912 (-27.80%) $P \leq 0.001$
Prevailing levels in ground-water	23.175 ± 1.137	22.248 ± 0.874 (-4.00%) NS	44.350 ± 1.725	41.600 ± 1.682 (-6.20%) NS
Single cocoon weight (g)				
Lethal	1.105 ± 0.092	0.683 ± 0.038 (-38.19%) $P \leq 0.001$	1.795 ± 0.230	0.825 ± 0.090 (-54.04%) $P \leq 0.001$
Sublethal	1.105 ± 0.092	0.837 ± 0.052 (-24.25%) $P \leq 0.01$	1.795 ± 0.230	1.156 ± 0.108 (-35.60%) $P \leq 0.01$
Prevailing levels in gound- water	1.105 ± 0.092	1.064 ± 0.079 (-3.71%) NS	1.795 ± 0.230	1.696 ± 0.203 (-5.52%) NS
Single shell weight (g)				
Lethal	0.141 ± 0.016	0.089 ± 0.006 (-36.88%) $P \leq 0.01$	0.328 ± 0.034	0.154 ± 0.021 (-53.05%) $P \leq 0.001$
Sublethal	0.141 ± 0.016	0.108 ± 0.008 (-23.40%) $P \leq 0.01$	0.328 ± 0.034	0.216 ± 0.015 (-34.15%) $P \leq 0.01$
Prevailing levels in ground-water	0.141 ± 0.016	0.136 ± 0.014 (-3.55%) NS	0.328 ± 0.034	0.311 ± 0.026 (-5.18%) NS
Silk index				
Lethal	1.000 ± 0.016	0.631 ± 0.006 (-37.00%) $P \leq 0.001$	1.000 ± 0.034	0.470 ± 0.021 (-53.00%) $P \leq 0.01$
Sublethal	1.000 ± 0.016	0.750 ± 0.008 (-25.00%) $P \leq 0.001$	1.000 ± 0.034	0.650 ± 0.015 (-35.00%) $P \leq 0.001$
Prevailing levels in ground-water	1.000 ± 0.016	0.977 ± 0.014 (-2.30%) NS	1.000 ± 0.034	0.955 ± 0.027 (-4.50%) NS
Average filament length (m)				
Lethal	485 ± 27	291 ± 16 (-40.00%) $P \leq 0.001$	1050 ± 53	450 ± 22 (-57.14%) $P \leq 0.001$
Sublethal	485 ± 27	359 ± 14	1050 ± 53	630 ± 35.00

Table 3. Continued

	PM		NB_4D_2	
	Control	Experimental	Control	Experimental
		(-26.00%) $P \leq 0.001$		(-40.00%) $P \leq 0.001$
Prevailing levels in ground-water	485 ± 27	460 ± 24 (-5.15%) NS	1050 ± 53	972 ± 47 (-7.43%) NS
Fecundity (No.)				
Lethal	365 ± 14	211 ± 6 (-42.19%) $P \leq 0.001$	527 ± 21	213 ± 7 (-59.58%) $P \leq 0.001$
Sublethal	365 ± 14	263 ± 8 (-27.94%) $P \leq 0.001$	527 ± 21	308 ± 10 (-41.56%) $P \leq 0.001$
Prevailing levels in ground-water	365 ± 14	353 ± 12 (-3.29%) NS	527 ± 21	499 ± 17 (-5.31%) NS

Percentage decrease relative to controls is given in parenthesis.
Values are means ± SD of six individual observations ($n = 6$).
NS denotes not significant with controls ($P \geq 0.05$).

lethal and sublethal doses of fluoride (Table 3). On exposure to the prevailing levels of fluoride in the waters, did not show any negative impact as eggs were laid by both races of PM and NB_4D_2 silkworms (Table 3). The decreases in fecundity were found to be more on exposure to lethal dose than sublethal dose of fluoride. The overall effect of fluoride on growth, cocoon characters and fecundity were race-wise in the order NB_4D_2 > PM; dose-wise were in the order lethal > sublethal > prevailing levels of fluoride.

Discussion

Toxicity of various toxicants on non-target organisms is now very well recognized. It is evident from the present study that fluoride was found toxic to the fifth instar larvae of PM and NB_4D_2 races of silkworms on exposure to lethal and sublethal doses. The lower levels of fluoride did not show much impact on both races of silkworms and showed no mortality. These findings indicate that the silkworms did not affect much, if lower levels of fluoride translocate to the mulberry leaves through the root system.

Fujii and Honda (1972) and Chen and Wu (1995) observed that fluoride was more toxic to silkworm, *B. mori*. Observations of Kuwana *et al.* (1967) and Fujii and Honda (1972) indicate that the silkworms are not only sensitive to various insecticides, but equally susceptible to fluoride. In order to know the toxic impact of fluoride on diapause and non diapause silkworms, a detailed relative fluoride toxicity studies were undertaken to derive LD_{50} and sublethal doses. In addition to this, a survey has been conducted to know the fluoride levels in the groundwaters, being used for cultivation of mulberry in the sericulturally important areas of Kolar and Anantapur districts. The present study clearly indicates that the lower levels of prevailing fluoride in the waters did not show any mortality and negative impact on growth and development of silkworms. However, on exposure to lethal and sublethal doses, some characteristic behavioural changes were also observed in silkworms exposed to fluoride. Silkworms maintained on leaves treated with only water (control) and prevailing dose behaved normally *i.e.*, they were active, healthy and their movements were well co-ordinated without any disturbance during feeding. But silkworms fed with leaves treated with lethal and sublethal doses of fluoride exhibited spontaneous contractures, which made the worms roll over one another, appearance of black specks on inter-segmental regions, vomiting of gastric fluid (on final days) and irregular excretion. The fifth instar larval duration was long (12 to 18 hrs) in the worms treated with the lethal dose compared with that of controls.

The data obtained from the present study clearly indicated that the growth, single cocoon weight, single shell weight, silk index, average filament length and fecundity in PM and NB_4D_2 races of silkworms were disturbed when subjected to fluoride stress. Fluoride remarkably reduced the normal growth and cocoon characters and the reduction was more pronounced at lethal exposure and marginal at sublethal exposure. He (1984); Chen and Wu (1994); and Chandrakala *et al.* (1998) reported similar inhibition of growth and cocoon characters on exposure to low level doses of fluoride with short duration up to 96 hrs of fifth instar. The mechanism of toxicant action on silkworms is still not fully understood and more detailed studies on the toxicity effects on non-target organisms are now needed. The site of action of most of the toxicants/pollutants in the nervous system (Bhosale *et al.*, 1988; Nath and Kumar, 1999). The administration of toxicant caused perturbations of normal physiological functions (Samaranayaka, 1974; Reddy *et al.*, 1989; Nath *et al.*, 1997). Recently, Bhatnagar *et al.* (2002) demonstrated the action of fluoride on nervous system and clearly described how the fluoride affects structure and functions of nervous system in mice. In the present study, the maximum and normal reductions in growth rates at both lethal and sublethal doses of fluoride is may be due to malfunctioning of detoxifying mechanism in silkworm body. The fluoride may cause some changes in the larval body, nervous system, or in the metabolic activity due to fluoride action.

The maximum and normal reductions in single cocoon weight, single shell weight, silk index and filament length at both lethal and sublethal doses of fluoride is might be due to the lack of silk protein synthetic mechanism and also may be due to some physiological alterations as a result of action of toxicants within the silkworm body (Dasmahapatra *et al.*, 1989). From the present study, it is clearly evident that, on exposure to a dose of prevailing levels in the waters of sericulturally important areas in Karnataka and Andhra Pradesh States, the changes observed on growth and cocoon characters were lower and the decrease was found to be insignificant ($P \geq 0.05$). This finding clearly shows that there is not much impact of low levels of fluoride on growth and economic characters of both races of PM and NB_4D_2, if very low concentration of fluoride translocates into mulberry plants from the groundwater.

Growth and fecundity, as one of the most important parameters to evaluate the toxicity of any chemical or physical agents in animal test systems (Luning, 1966; Sankaranarayanan, 1969; Gayathri and Krishnamurthy, 1979) and these serve as good indicators of various somatic effects caused by a chemical in the test substrate (Luning, 1966). In the present study, the authors have observed a reduction in fecundity in silkworms on exposure to lethal and sublethal doses of fluoride. The fecundity of an insect is one of the valuable index component and depends on the number of eggs produced, which in turn reflects the number of ovarioles present in the insects. Further, the fecundity of insects mainly depend on its hormonal system. The reduced fecundity in silkworms on exposure to fluoride in the present study is might be due to the effect of fluoride in the net flux of hormones, which in turn might have affected the development of ovarioles with marked reproductive abnormalities, resulting decrease in the total number of eggs laid.

The high degree of change in growth in NB_4D_2 race when exposed to lethal and sublethal doses of fluoride indicated its higher affinity for NB_4D_2 race, suggesting its high susceptibility to fluoride. The lower effects of fluoride on PM race compared with NB_4D_2 race indicated its lower affinity towards PM, suggesting that the PM silkworms have resistance and may metabolize the fluoride to a greater extent than NB_4D_2 race silkworms.

In conclusion, the present results indicate the effects of fluoride on the studied parameters and their relation to resistance against fluoride. The effects were more pronounced in NB_4D_2 race than PM race, indicating that PM race has greater resistance against fluoride and with this acquired resistance, the breeding of toxicant tolerant *B. mori* is possible.

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