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Impact of Low Concentration Fluoride on Toxification and Biological Traits of Silkworm, *Bombyx mori*

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The impact of low concentration fluoride on toxification was studied by treating 3rd instar silkworm larvae of Qiufeng × Baiyu (Fluoride resistant strain) and Jinshong × Haoyue (Fluoride sensitive strain). The 5th instar silkworm larvae of Zhenong-1, Shu-12, Qiufeng × Baiyu, Chunhua × Qhiushi, Jinshong and Haoyue were fed mulberry leaves treated with sodium fluoride at different concentrations to determine its effect on fecundity. Harmful effects of low concentration fluoride at early instars on growth, development and economic characters of *Bombyx mori* are related to its resistance. Harmful effect on resistant strain Qiufeng × Baiyu manifested on prolongation of instar duration of 15 and 6% in 70 ppm, where as the sensitive breed Jinshong × Haoyue manifested with 100% mortality in 50 ppm. The impact of low concentration fluoride on fecundity resulted in considerable increase in formed eggs (9%) and laid eggs (9%) in Zhenong-1 and Shu-12 among treated population over control. The present observations emphasis that fluoride apart from exhibiting harmful effects on silkworm growth and development, however, its low concentration do play a vital role in inducing some positive effect on fecundity and cocoon traits.

Key words: Fluoride, Toxification, *Bombyx mori*, Fecundity, Cocoon traits

Introduction

The air-borne fluoride emitted from many industrial and coal burning process and increased level of fluoride in water has great impact on plants and animals resulting in fluorosis (Chen and Wu, 1994). In recent years, the increased industrial emission of fluoride into the atmosphere has seriously affected silkworm cocoon production in China (Liu, 1982; He, 1984; Shong, 1985; Wo, 1986; Si, 1988; Lu *et al.*, 1998) and Japan (Kuribayashi, 1977). However such reports are not available in India except some studies on oral administration of sodium fluoride and its effect on physiology and biological feature of silkworm *Bombyx mori* (Chandrakala *et al.*, 1998; Aftab Ahamed *et al.*, 1999; Aftab Ahamed and Chandrakala, 1999).

It is also well known that low concentration of fluoride can alter insect population, fecundity, growth rate and mortality (Mayer *et al.*, 1988; Chen and Wu, 1994). Fluoride found to accumulate on the mulberry leaves at high level due to air pollution not only injure the leaf tissue (Kuribaryashi, 1977) also induce mortality in silkworm after feeding (Liu, 1982; Chandrakala *et al.*, 1998). Even when silkworms fed contaminated leaves with low level of fluoride significantly affect the cocoon quality and quantity (He, 1984; Shong, 1985; Wang and Bian, 1988; Chandrakala *et al.*, 1998). Mechanism of fluoride toxicity has also been studied in the blood of four different fluoride tolerant varieties of *B. mori* (Chen, 1993). The racial differences in the tolerance of toxic effects of fluoride and breeding fluoride resistant races were studied in China (Chen and Wu, 1994). All these studies are confined to late age silkworm to determine the effect of fluoride on toxicity and biological feature of *B. mori*. The present study was undertaken to determine the effect of fluoride on young age silkworms with reference to toxicity and biological traits of some Chinese bilvoltine breeds.

Materials and Methods

The Chinese bivoltine *Bombyx mori* breeds Qiufeng ×

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Baiyu, Jinshong × Haoyue, Zhenong-1, Shu-12, Chunhua × Qhiushi, Jinshong and Haoyue were obtained from the Department of Sericulture, Zhejiang University, China. Since, Qiufeng × Baiyu and Jinshong × Haoyue known as fluoride resistant and fluoride sensitive strains respectively were selected along with other strains for comparative study. The hatched larvae of all the strains were reared under optimum environmental conditions. The third instar larvae of Qiufeng × Baiyu (Fluoride resistant) and Jinshong × Haoyue (Fluoride sensitive) for first experiment and fifth instar larvae of Zhenong-1, Shu-12, Chunhua × Qhiushi, Qiufeng × Baiyu (Fluoride resistant), Jinshong and Haoyue (Fluoride sensitive) for second experiment were selected randomly and grouped into different batches. Each group consisted of three replications each with 100 larvae. A known quantity of sodium fluoride (NaF) was dissolved in small quantity of water and then diluted to 15, 20, 30, 50 and 70 ppm solution in distilled water. Freshly collected mulberry leaves from the field were soaked in the above concentration of fluoride solutions for 10 min individually and dried at room temperature.

In first experiment mulberry leaves soaked in different concentrations of NaF were fed to newly exuviated 3rd instar larvae until 3rd moult. These silkworm larvae were fed with mulberry leaves devoid of fluoride in subsequent instars. After treatment the toxic symptoms of larvae were recorded. The larval duration was calculated from the beginning of 3rd instar until the end of 5th instar. Weight of newly 3rd and 4th moulted and 5th instar full grown larvae were recorded. Post-cocooning parameters, such as female and male cocoon weight, shell weight and cocooning rate were calculated. Fecundity parameter like formed eggs, laid eggs and laid egg percentage were calculated.

The procedure for the second experiment was the same as that of the first experiment except feeding of NaF treated mulberry leaves of 50 ppm concentration from first feed of 5th instar to maturation. The resultant pupae of this experiment were allowed for ecloson under controlled environment. Fecundity parameters like formed eggs, laid eggs and laid egg percentage were calculated. The control group was fed with mulberry leaves soaked in distilled water.

All of the data collected in this experiment were subjected to SSR (Studentised Significant Ranges) multiple range test to determine the significance between corresponding parameters of the treated groups and control groups following the method of Zhong (1992). The percent index was calculated for each parameter of treated groups over the corresponding parameters of control groups. In addition, the treated and non-treated mulberry leaves in the present experiments were sent to laboratory, Zhejiang University, China for determination of fluoride concentration before feeding. The actual corresponding fluorine ion concentration of untreated and treated mulberry leaves for first experiment is presented in Table 1. For second experiment only one concentration of 50 ppm NaF was considered and estimated fluorine content from untreated and treated mulberry leaves was 18.5 and 71.9 mg/kg respectively.

Results and Discussion

Silkworm larvae of third instar fed with mulberry leaves soaked in different concentrations of NaF solution were resulted in significant decrease of 9 and 10% in weight after 3rd moult in 70 ppm group in Qiufeng × Baiyu and 30 ppm group of Jinshong × Haoyue respectively. The 3rd instar duration and total larval duration (Table 2) was significantly increased at 7 and 5% in 50 ppm, 15 and 6% in 70 ppm of Qiufeng × Baiyu, respectively and 6 and 4% in 20 ppm, 14 and 7% in 30 ppm of Jinshong × Haoyue, respectively. Cocooning rate was also significantly decreased in Jinshong × Haoyue of 20 and 30 ppm treatments at 29 and 83% respectively and 100% mortality was noticed in 50 ppm treatment, where all silkworms died without entering moult after treatment (Table 2). Differences in food-uptake, sluggishness, growth and development of different silkworm strains against varied concentration of NaF were observed in the present investigation. This breed specific variation thus caused might

Table 1. Estimated fluorine ion concentration from untreated and treated mulberry leaves at different concentration of NaF

Groups	Qiufeng × Baiyu		Jinshong × Haoyue	
	Concentration (ppm)	Determined (mg/kg)	Concentration (ppm)	Determined (mg/kg)
Control	00	16.4	00	16.4
I	20	42.9	15	36.4
II	30	53.8	20	42.9
III	50	73.0	30	53.8
IV	70	88.5	50	73.0

Table 2. Effect of fluoride on larval parameters by oral administration of 3rd instar silkworm larvae of *Bombyx mori*

Treatments	3rd instar duration (hr)	3rd moulting percentage	Weight of newly 3rd moulted larva (g)	Weight of newly 4th moulted larva (g)	Weight of full grown larva (g)	Total larval duration (hr)	Cocooning percentage
Qiufeng × Baiyu Control	56.3 ± 0.579 (100)	100 ± 0.000 (100)	0.1803 ± 0.005 (100)	0.8490 ± 0.008 (100)	4.69 ± 0.29 (100)	349.3 ± 28.36 (100)	98 ± 3.46 (100)
I	56.7 ± 0.579 (101)	100 ± 0.000 (100)	0.1911 ± 0.009 (106)	0.8330 ± 0.033 (98)	4.75 ± 0.16 (101)	361.7 ± 0.579 (104)	98.67 ± 2.31 (101)
II	57.7 ± 0.579 (102)	100 ± 0.000 (100)	0.1742 ± 0.009 (97)	0.8763 ± 0.001 (103)	4.62 ± 0.51 (99)	362.7 ± 0.579 (104)	96.67 ± 1.15 (99)
III	60.0 ± 1.000 (107)**	100 ± 0.000 (100)	0.1789 ± 0.007 (99)	0.7572 ± 0.007 (89)	4.63 ± 0.03 (99)	365.3 ± 1.528 (105)	99.33 ± 1.15 (101)
IV	64.7 ± 2.082 (115)**	100 ± 0.000 (100)	0.1645 ± 0.008 (91)*	0.8197 ± 0.026 (97)	4.79 ± 0.22 (102)	371.3 ± 3.215 (106)	97.33 ± 2.31 (99)
Jinshong × Haoyue Control	73. ± 1.000 (100)	100 ± 0.000 (100)	0.1822 ± 0.008 (100)	0.8608 ± 0.038 (100)	4.31 ± 0.15 (100)	388.7 ± 1.528 (100)	97.92 ± 1.91 (100)
I	75.3 ± 1.528 (103)	98.33 ± 0.794 (98)	0.1737 ± 0.009 (95)	0.8535 ± 0.005 (99)	4.23 ± 0.13 (98)	395.7 ± 5.033 (102)	96.25 ± 1.25 (98)
II	77.7 ± 1.528 (106)**	75.42 ± 7.939 (75)*	0.1734 ± 0.004 (95)	0.8446 ± 0.005 (98)	4.51 ± 0.14 (105)	404 ± 4.359 (104)**	69.58 ± 9.71 (71)**
III	83.3 ± 1.528 (114)**	27.50 ± 7.603 (28)**	0.1635 ± 0.006 (90)*	0.7880 ± 2.603 (92)	4.30 ± 0.14 (100)	416 ± 1.000 (107)**	17.08 ± 7.22 (17)**
IV	96 ± 1.000 (133)**	00 ± 0.000 (00)**	-	-	-	-	-

*: Significant at 0.5% level and **: Significant at 1% level.
SD: Standard Deviation (M ± SD); percentage over control in parentheses.

be due to the molecular mechanism that underlies in tolerance to fluoride as the compound known to damage the cells and cause inhibition of enzyme activity, interfering in normal growth and development (Chen and Wu, 1994; Meng *et al.*, 1994). Although, resistant strain Quifeng × Baiyu tolerates the fluoride poison and toxicity, prolongation of larval duration and delay in setting moult was noticed in all the treated groups. Where as the sensitive strain Jinshong × Haoyue intolerant to the fluoride poison lead to cent percent mortality in 50 ppm treatment (Table 2)

Significant decrease in the weight of newly 3rd moult exuviated larvae in 70 ppm of Quifeng × Baiyu and 30 ppm of Jinshong × Haoyue, where as non-significant decrease in the weight of 4th moult exuviated larvae and full grown larvae in both the strains (Table 2) indicates that larvae can recovered from early instars fluoride toxicity and convalescence from fluorosis by feeding of fluoride free leaves in subsequent instars.

The cocooning percentage index with reference to female and male cocoon weight and shell weight was declined in all fluoride treated groups over control (Table 3). Comparatively, the toxic effects of fluoride on cocoon characters of males were more obvious than those that of females in fluoride resistant variety Qiufeng × Baiyu. Further it was also reported that the cocoon weight and shell weight of the low fluoride resistant race Hang 8 were more susceptible to fluoride pollution and its toxic effects on cocoon characters of males were more distinct than females (Chen and Wu, 1994).

Oral administration of fluoride of different concentration for 3rd instar larvae of Quifeng × Baiyu and Jinshong × Haoyue was resulted in increase of 2% in laid eggs and 2% in formed eggs in Jinshong × Haoyue at 30 ppm. This clearly indicates that Jinshong × Haoyue being a sensitive strain to fluoride responded and resulted in increased fecundity. Whereas no considerable changes in fecundity was noticed in resistant variety Quifeng × Baiyu (Table 4).

Oral administration of fluoride of 50 ppm concentration for fifth instar larvae of 6 bivoltine strains was resulted in increase of 9% in laid eggs 9% in formed eggs in Zhenong-1 and 9% in laid eggs and 8% in formed eggs in

Table 3. Effect of fluoride on cocoon characters by oral administration of 3rd instar silkworm larvae of *Bombyx mori*

Treatments	Female			Male		
	Cocoon weight (g)	Cocoon shell weight (g)	Cocoon shell ratio (%)	Cocoon weight (g)	Cocoon shell weight (g)	Cocoon shell ratio (%)
Qiufeng × Baiyu	2.417 ± 0.117 (100)	0.443 ± 0.124 (100)	18.33 ± 0.703 (100)	1.853 ± 0.073 (100)	0.424 ± 0.032 (100)	22.84 ± 1.005 (100)
Control						
I	2.305 ± 0.132 (95)	0.427 ± 0.039 (99)	18.51 ± 0.925 (101)	1.743 ± 0.123 (94)**	0.403 ± 0.041 (95)	23.07 ± 1.014 (101)
II	2.281 ± 0.151 (94)	0.428 ± 0.035 (97)	18.79 ± 1.037 (103)	1.743 ± 0.110 (94)**	0.391 ± 0.023 (92)	23.30 ± 1.960 (102)
III	2.307 ± 0.110 (95)	0.433 ± 0.031 (98)	18.76 ± 0.916 (102)	1.761 ± 0.082 (95)**	0.401 ± 0.028 (95)	22.76 ± 0.975 (100)
IV	2.334 ± 0.108 (96)	0.448 ± 0.032 (101)	19.15 ± 0.769 (104)	1.736 ± 0.086 (94)**	0.405 ± 0.026 (96)	23.32 ± 0.864 (102)
Jinshong × Haoyue	2.206 ± 0.111 (100)	0.494 ± 0.036 (100)	22.40 ± 1.325 (100)	1.708 ± 0.096 (100)	0.446 ± 0.033 (100)	26.14 ± 1.343 (100)
Control						
I	1.983 ± 0.156 (90)	0.443 ± 0.172 (90)	22.28 ± 1.372 (99)	1.567 ± 0.135 (92)**	0.423 ± 0.036 (95)	26.10 ± 1.286 (100)
II	2.197 ± 0.141 (100)	0.494 ± 0.046 (100)	22.45 ± 0.896 (100)	1.725 ± 0.078 (101)	0.447 ± 0.034 (100)	25.93 ± 1.451 (99)
III	1.983 ± 0.269 (90)	0.446 ± 0.091 (90)	22.23 ± 3.096 (99)	1.685 ± 0.289 (99)	0.423 ± 0.094 (95)	24.64 ± 3.224 (94)*
IV	-	-	-	-	-	-

*: Significant at 0. 5% level and **: Significant at 1% level.
SD: Standard Deviation (M ± SD); percentage over control in parentheses.

Table 4. Effect of fluoride on fecundity by oral administration of 3rd instar silkworm larvae of *Bombyx mori*

Treatments	Formed eggs (no.)	Laid eggs (no.)	Laid eggs (%)
Qiufeng × Baiyu	796 ± 34.76 (100)	716 ± 34.76 (100)	90.07 ± 3.05 (100)
Control			
I	697 ± 34.32 (97)	697 ± 34.32 (97)	89.37 ± 3.14 (99)
II	694 ± 37.88 (97)	694 ± 37.88 (97)	90.04 ± 3.11 (100)
III	684 ± 44.85 (96)	684 ± 44.85 (96)	90.21 ± 4.05 (100)
IV	692 ± 46.00 (97)	692 ± 46.00 (97)	90.14 ± 3.48 (100)
Jinshong × Haoyue	570 ± 31.87 (100)	570 ± 31.87 (100)	93.69 ± 2.75 (100)
Control			
I	539 ± 38.89 (95)	539 ± 38.89 (95)	92.95 ± 3.16 (99)
II	565 ± 36.40 (99)	565 ± 36.40 (99)	90.63 ± 3.55 (97)
III	582 ± 35.17 (102)	582 ± 35.17 (102)	91.53 ± 4.62 (98)
IV	-	-	-

*: Significant at 0.5% level and **: Significant at 1% level.
SD: Standard Deviation (M ± SD); percentage over control in parentheses.

Shu-12 strains. Decrease of 3% in laid eggs and 2% in formed eggs in Jinshong and decrease of 27% in laid eggs and 18% in formed eggs in Haoyue was observed than their respective controls(Table 5). This study clearly indicates that the harmful effect of fluoride on fecundity was not so serious as that of larval survivability. Interestingly, although low concentration of fluoride has positive effect on fecundity by increasing the number of eggs, but the

Table 5. Effect of fluoride on fecundity by oral administration of 5th instar silkworm larvae of *Bombyx mori*

Strains	Treatments	Formed eggs (no.)	Laid eggs (no.)	Laid eggs (%)
Zhenong-1	Control	489 ± 38.09 (100)	429 ± 32.31 (100)	87.84 ± 5.03 (100)
	T	532 ± 51.00 (109)	468 ± 35.56 (109)	88.21 ± 4.38 (100)
Shu-12	Control	544 ± 55.00 (100)	510 ± 50.00 (100)	93.73 ± 3.12 (100)
	T	588 ± 31.90 (108)	556 ± 30.19 (109)	94.47 ± 2.10 (101)
Qiufeng × Baiyu	Control	620 ± 38.65 (100)	550 ± 36.74 (100)	88.60 ± 2.91 (100)
	T	587 ± 46.65 (95)	520 ± 48.82 (95)	88.51 ± 3.53 (100)
Chunhua × Qiushi	Control	716 ± 38.08 (100)	641 ± 36.20 (100)	89.54 ± 2.30 (100)
	T	715 ± 26.05 (100)	666 ± 22.92 (104)	93.25 ± 2.44 (104)
Jinshong	Control	606 ± 42.62 (100)	565 ± 43.25 (100)	93.09 ± 3.19 (100)
	T	591 ± 56.47 (98)	548 ± 49.52 (97)	92.68 ± 3.35 (100)
Haoyue	Control	482 ± 30.03 (100)	444 ± 25.2 (100)	91.14 ± 2.06 (100)
	T	393 ± 65.29 (82)	325 ± 58.41 (73)	88.85 ± 6.03 (97)

*: Significant at 0.5% level and **: Significant at 1% level.
SD: Standard Deviation (M ± SD); percentage over control in parentheses.

rate of response varies with the silkworm strains. Hence as it is obvious from the present study that the concentration of fluoride might vary among different silkworms strains to derive the positive effect on fecundity. Meanwhile, Chen (1996, 2003) also opinioned that there is difference in fluoride effects on fecundity among different strains of *B. mori*. But the physiological and biochemical phenomenon that underlies in the positive effect of fluoride on fecundity need to be explored. Thus it is highly suggestive to screen dose, race/strain and age specific effect of fluoride in order to evolve either fluoride resistant silkworm strain or to exploit the positive impact of fluoride in improvement of fecundity.

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