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Effect of Cadmium on Biology of Tobacco Caterpillar *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae)

Sharmila Chouhan*†, S. C. Verma and Meena Thakur*****

*Department of Environmental Science, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan-173 230, H.P., India

**Department of Seed Science and Technology. Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan-173 230, H.P., India

***Department of Entomology. Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan-173 230, H.P., India

†Corresponding author: Sharmila Chouhan

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ABSTRACT

The present study was conducted to investigate the impact of cadmium on biology of *Spodoptera litura* F. and its accumulation in three consecutive generations. The influence of Cd on fecudinty, fertility, incubation period, larval period, adult longevity and total development period was studied at seven different concentrations viz., 1, 5,10,15, 20, 30 and 40 mg/kg apart from control. Pure culture of *S. litura* from field collected adult was maintained under laboratory conditions on castor leaves. Fecundity and fertility was low in the highest dose of 40 mg/kg which decreased with generations. Incubation period prolonged with increasing cadmium concentration and generations. The variable doses of cadmium had significant influence on total larval period of the insect. Longest mean larval period of 23.39 days was recorded at 40 mg Cd/kg which differed statistically from its lower concentration. The duration of pupal period decreased with increasing Cd doses as well with generations. Thus, increased cadmium concentrations resulted in prolongation of total developmental period of the insect. Irrespective of generations, total developmental period was 33.97 days in the highest concentration (T_7) as compared to control (28.61 days). The accumulation of cadmium in the sixth instar larvae of *S. litura* increased with increase in dose of heavy metals in artificial diet and with increase in generations. The accumulation of cadmium in agroecosystem might influence the growth and development of *S. litura* through host plants to some extent.

INTRODUCTION

Environmental pollution is one of the severe problems the world is facing today. Among the various pollutants, heavy metals are major environmental pollutants. These are discharged into the atmosphere from the burning of fossil fuels, release of industrial wastes and use of agrochemicals. Natural processes and anthropogenic activities have resulted in the accumulation of heavy metals in soils to levels toxic to plants, animals and humans (Florea & Busselberg 2006, Boyd 2010). Land disposal of sewage and industrial effluents has been implicated as the chief source of heavy metal enrichment of pasture lands and agricultural fields, particularly near sewage treatment works in urban areas (Mapanda et al. 2005). Leafy vegetables viz., cabbage, cauliflower, spinach, broccoli etc. are commercially grown throughout the world and consumers' demand for better quality vegetables is increasing. The production of leafy vegetables using wastewater for irrigation is practiced in many cities, but little is known on the contamination of these vegetables with heavy metals. Heavy metals not only affect the growth and development of plants directly, but also affect the phytophagous pests feeding on them indirectly through the changes in structure and chemical constitutions of plants. Vegetable crops are attacked by sundry of insect pests which are directly or indirectly affected by heavy metals, particularly Ni and Cu (Heliovaara et al. 1987 and Zvereva et al. 2003). Among various insect pests attacking vegetable crops, *Spodoptera litura* Fabricius is a cosmopolitan and polyphagous pest of several crops. Since it is a vigorous feeder with high fecundity and number of generations per year it causes heavy loss to agricultural production. Effect of heavy metals on population and ecological parameters of some pests has been reported by few workers. Heavy metals viz., Ni is known to influence the growth and immune response of the pest. It is known to accumulate in the fifth and sixth larval instar when larvae were fed on artificial diets amended with different concentrations of the metal for three generations (Sun et al. 2007). Himachal Pradesh is well known for off-season commercial vegetable crop production. Since last two decades the state has emerged as an industrial hub resulting in various environmental problems.

Heavy metal pollution is an increasing problem in vegetable crops in the State. In the present study, cadmium accumulation in different developmental stages of *S. litura* and its effect on fecundity, fertility, duration of different developmental stages was investigated by feeding the larvae on artificial diet treated with different doses of cadmium and observations recorded for three generations. The results can provide some understanding on the impact of heavy metal pollution on natural control of phytophagous insect-pests by affecting the duration of developmental stages and by inducing growth abnormalities.

MATERIALS AND METHODS

The study was conducted during 2012-2013. Adults of the insect were collected from the field manually. The pure culture of tobacco caterpillar (*Spodoptera litura* F.) was maintained under laboratory conditions (maximum temperature 32.05°C and minimum 17.2°C) on castor leaves. Cadmium was procured from Central Drug House (P) Ltd. New Delhi in the form of $CdCl_2$ (analytical reagent grade). The research experiment was designed with eight cadmium treatments including control, viz., 0 mg/kg (T_0 control), artificial diet impregnated with 1mg/kg (T_1), 5 mg/kg (T_2), 10 mg/kg(T_3), 15 mg/kg(T_4), 20 mg/kg (T_5), 30 mg/kg(T_6) and 40 mg/kg (T_7). Freshly hatched larvae from control were reared on chick-pea artificial diet which was prepared as per the method of Anonymous (1988). Different doses of cadmium were added to the diet. Each treatment was replicated thrice. In each replication, ten larvae of *S. litura* were released and each larva was kept in individual vials of size (6 ×4 cm) containing weighed quantity of artificial diet (Plate-3). The eggs laid by the first generation adults of each treatment were used as the starting point for the second generation and second generation eggs were used as starting point for the third generation with the same treatment.

Detection of Heavy Metal Accumulation in Different Generations of *S. litura*

Heavy metals were detected in the sixth instar larva of each generation. For this study, 10 individuals of sixth instar larva of each treatment of three successive generations were taken. The sample for detection of heavy metal was prepared as per the method of Sun et al. (2007) and detected using Inductively Coupled Plasma Atomic Spectrometer (ICP-6300 DUO) which estimates heavy metals to < ppb (or trillion).

Fecundity and fertility: Fecundity was recorded by rearing a pair of adults which emerged from larvae fed on artificial diet impregnated with different doses of cadmium in one chamber/cage and by counting the total number of eggs laid by the female during its entire life. The eggs were further observed for hatching to record per cent fertility.

Incubation period: The eggs laid by females emerged from larvae fed on treated diet were observed for egg hatchability and egg duration.

Larval period: The duration of larvae fed on artificial diet impregnated with different doses of cadmium and on untreated diet was recorded.

Adult longevity: The adults were kept in a separate cage and fed on honey solution. Total period from the day of emergence of the adult from larvae fed on artificial diet impregnated with different doses of cadmium till death was recorded.

Total development period: For calculating total development period, the duration of egg laying to hatching, larval to pupal and pupal to adult emergence was recorded.

Statistical analysis: The data emanating from the above experiment was subjected to statistical analysis through completely randomized design (CRD) and the significance of each treatment was calculated as suggested by Cochran & Cox (1964).

RESULTS AND DISCUSSION

Effect of cadmium (Cd) on fecundity and fertility of *S. litura* F. in different generations

Fecundity: Fecundity (number of eggs laid/female) of the females developed from larvae fed on artificial diet impregnated with different doses of cadmium was observed (Table 1). All the treatments differed statistically from each other with maximum number of 953.92 eggs per female recorded in T_1 (1 mg Cd/kg) and minimum 466.00 eggs per female in T_7 (40 mg Cd/kg). A difference of 327.44 eggs per female was recorded at T_0 (control) and T_7(40 mg Cd/kg). Irrespective of the treatments, when generations were compared, maximum number of eggs (958.67) were recorded in generation-1 followed by generation-2 (551.00 eggs/female) and generation-3 (389.92). The number of eggs laid decreased in all the treatments from generation-1 to generation-3 and the difference increased with increasing dose of cadmium. In treatment T_7, in generation-1, 958.67 eggs per female were recorded, the number decreased drastically to 123.67 in generation-3. Whereas, in control 953.00 eggs were recorded in generation-1 which decreased to 651.67 in generation-3. Thus, at higher concentration of cadmium and with increasing generations, the number of eggs laid decreased which could help in natural control of the pest. Similar to present findings, Gorur (2007) also reported that heavy metals affected reproduction in successive generations of insects. Daniel (1994) and Forrest (1996) reported that adult survival

Table 1: Effect of cadmium (Cd) on fecundity and fertility of *S. litura* F. in different generations.

Treatment	No. of eggs/Female				Fertility (%)			
	Generation							
	1	2	3	Mean	1	2	3	Mean
T_0 (Control)	953.00(30.87)*	775.67(27.85)	651.67(25.53)	793.44(28.08)	87.83(69.58)	85.31(67.47)**	83.63(66.14)	85.59(67.73)
T_1 (1mg/kg)	955.00(30.90)	741.33(27.42)	603.33(24.56)	766.56(27.63)	87.61(69.41)	84.66(66.95)	83.47(66.05)	85.25(67.47)
T_2 (5mg/kg)	948.33(30.79)	678.33(26.04)	555.00(23.56)	727.22(26.80)	87.56(69.35)	84.12(66.57)	75.09(60.07)	82.26(65.33)
T_3 (10mg/kg)	966.00(31.08)	551.67(23.49)	431.00(20.76)	649.56(25.11)	87.87(69.62)	82.21(65.08)	73.65(59.17)	81.24(64.62)
T_4 (15mg/kg)	947.00(30.77)	511.67(22.62)	325.67(18.05)	594.78(23.81)	87.36(69.17)	76.95(61.39)	72.07(58.10)	78.79(62.89)
T_5 (20mg/kg)	943.33(30.71)	438.33(20.93)	248.33(15.76)	543.33(22.47)	89.14(70.77)	76.10(60.75)	67.00(55.07)	77.41(62.20)
T_6 (30mg/kg)	960.00(30.98)	385.00(19.62)	180.67(13.44)	508.56(21.35)	91.34(72.88)	69.51(56.50)	64.95(53.72)	75.26(61.04)
T_7 (40mg/kg)	958.67(30.96)	315.67(17.76)	123.67(11.12)	466.00(19.95)	90.70(72.27)	64.97(53.72)	61.92(51.90)	72.53(59.29)
Mean	953.92(30.89)	551.00(23.22)	389.92(19.10)	631.61(24.40)	88.67(70.38)	77.98(62.30)	72.72(58.78)	79.79(63.82)
	$CD_{(0.05)}$				$CD_{(0.05)}$			
	Treatment		: (0.25)		Treatment		: (1.82)	
	Generation		: (0.16)		Generation		: (1.11)	
	Generation x Treatment		: (0.44)		Generation x Treatment		: (3.15)	

*Figures in parenthesis are square root transformation; ** Figures in parenthesis are arc sine transformations.

and fecundity were reduced in blow-fly by constant exposure of cadmium. Moe et al. (2001) reported that reduction of fecundity in the adult stage seems to be caused directly by the cadmium accumulated during the larval stage.

Fertility: The percent fertility i.e., percent hatchability of eggs laid by the females developed from larvae fed on artificial diet impregnated with different doses of cadmium was observed (Table 1). Irrespective of generations, maximum fertility (85.25%) was recorded with artificial diet impregnated with 1 mg Cd/kg (T_1) which was statistically at par with control (85.59%) and differed statistically with rest of the treatments. Treatment T_2 resulted in 82.26 percent fertility of eggs which was statistically at par with T_3 (81.24 %). Minimum fertility (72.53%) was recorded with treatment T_7 which was statistically at par with T_6 (75.26%). Irrespective of treatments, on an average in generation-1, maximum fertility of 88.67 percent was recorded which was significantly different from generation-2 (77.98%) and generation-3 (72.72%). At T_0 (control) the percent fertility decreased from 87.83 to 33.63 and 87.61 to 83.47 percent, respectively in generation-1 and generation-3, whereas, at highest concentration of 40 mg Cd/kg (T_7) it decreased from 90.70 percent at generation-1 to 61.92 5 at generation-3. Thus, with increasing concentration of the metal in artificial diet and with increasing generations the percent fertility reduced significantly. According to studies conducted by Sheikh et al. (2010) on the toxicological effects of heavy metals on *culex pipiens*, the hatchability percent of eggs laid with the LC_{50} of $CdCl_2$, $CuSo_4$, $Pb(NO_3)_2$ and $Hg(NO_3)_2$ significantly decreased to 37, 73, 80 and 39 percent, respectively, compared to 97 percent for eggs laid by untreated females.

Effect of cadmium (Cd) mediated artificial diet on the incubation period in different generations: The incubation period of the eggs laid by the females developed from larvae fed on artificial diet impregnated with different doses of cadmium was observed (Table 2). Irrespective of generations, in treatment T_1 and T_0 (control) the incubation period was of 3.00 days which was statistically at par with T_2 (3.11 days), T_3 (3.14 days), T_4 (3.33days) and control (3.00 days). Maximum incubation period (3.94 days) was recorded in T_7 which was statistically at par with T_6 (3.79 days) as well as T_5 (3.60 days). In generation-1, on an average the incubation period was of 3.00 days in all the treatments but in generation-2 a difference of 1.17 days was recorded in control (T_0) and highest dose (T_7) which increased to 1.67 days in the third generation. Irrespective of treatments it varied from 3.33 days in generation-1 to 4.70 days in generation-3.

Effect of cadmium (Cd) mediated artificial diet on the total larval period in different generations: Variable doses of cadmium had significant influence on total larval period of *S. litura.* (Table 3). The larva fed on artificial diet mediated with 1 mg Cd/kg (T_1) resulted in minimum larval period (17.06 days) which differed statistically with rest of the treatments. The treatments T_2, T_3, T_4 and T_5 had 17.60, 18.67, 19.98 and 20.87 days of larval period, respectively. The longest larval period of 23.39 days was recorded in T_7 which differed statistically with T_6 (22.19 days). Thus, a difference of 6.45 days was recorded in the total larval period at T_0 and T_7, irrespective of generations. Whereas, irrespective of treatments, significantly minimum larval period (18.91 days) was recorded in generation-1, followed by generation-2 (19.53 days) and generation-3 (20.32 days). The larval duration recorded in all the generations increased with dose of cadmium. In generation-1 at T_0, total larval period was 16.77 days which increased to 17.20 in generation-3, whereas in T_7 total larval period was of 22.23 days in generation-1

Table 2: Effect of cadmium (Cd) mediated artificial diet on incubation period of *S. litura* F. in different generations.

Treatment	Total larval period (days) Generation			
	1	2	3	Mean
T_0 (Control)	3.00	3.00	3.00	3.00
T_1 (1mg/kg)	3.00	3.00	3.00	3.00
T_2 (5mg/kg)	3.00	3.00	3.33	3.11
T_3 (10mg/kg)	3.00	3.10	3.33	3.14
T_4 (15mg/kg)	3.00	3.33	3.67	3.33
T_5 (20mg/kg)	3.00	3.80	4.00	3.60
T_6 (30mg/kg)	3.00	4.00	4.37	3.79
T_7 (40mg/kg)	3.00	4.17	4.67	3.94
Mean	3.33	4.37	4.70	4.13

$CD_{(0.05)}$ Treatment: 0.34; Generation: 0.21; Generation × Treatment: 0.59

Table 3: Effect of cadmium (Cd) mediated artificial diet on total larval period of *S. litura* F. in different generations.

Treatment	Total larval period (days) Generation			
	1	2	3	Mean
T_0 (Control)	16.93	16.93	16.97	16.94
T_1 (1mg/kg)	16.97	17.00	17.20	17.06
T_2 (5mg/kg)	17.33	17.60	17.87	17.60
T_3 (10mg/kg)	17.97	18.53	19.50	18.67
T_4 (15mg/kg)	18.97	19.80	21.17	19.98
T_5 (20mg/kg)	19.83	20.77	22.00	20.87
T_6 (30mg/kg)	21.03	22.23	23.30	22.19
T_7 (40mg/kg)	22.23	23.37	24.57	23.39
Mean	18.91	19.53	20.32	19.59

$CD_{(0.05)}$ Treatment: 0.04; Generation: 0.03; Generation × Treatment: 0.07

Table 4: Effect of cadmium (Cd) mediated artificial diet on pupal period of *S. litura* F. in different generations.

Treatment	Total pupal period (days) Generation			
	1	2	3	Mean
T_0 (Control)	8.67	8.67	8.67	8.67
T_1 (1mg/kg)	8.67	8.67	8.33	8.56
T_2 (5mg/kg)	8.67	8.33	8.00	8.33
T_3 (10mg/kg)	8.33	8.00	7.33	7.89
T_4 (15mg/kg)	8.00	7.67	7.00	7.56
T_5 (20mg/kg)	7.67	7.33	6.67	7.22
T_6 (30mg/kg)	7.33	7.00	6.33	6.89
T_7 (40mg/kg)	7.00	6.33	6.00	6.44
Mean	8.04	7.75	7.29	7.69

$CD_{(0.05)}$ Treatment: 0.28; Generation: 0.15; Generation × Treatment: NS

which increased to 24.57 in generation-3. Thus, with increasing dose and increasing generations the larval period also increased. The present findings are in confirmation with the findings of Sildanchandra & Crane (2000) who reported that high cadmium concentration increases the larval duration of holometabolous insects. According to Barbosa et al. (1983), chronic exposure of gypsy moth larvae to cadmium led to decrease in pupal duration.

Effect of cadmium (Cd) mediated artificial diet on pupal period in different generations: Irrespective of generations, minimum pupal period (6.44 days) was recorded in T_7 i.e. artificial diet impregnated with 40 mg Cd/kg, whereas, maximum pupal period (8.56 days) was recorded with T_1 (1 mg Cd/kg), which was statistically at par with T_2 (8.33 days) as well as control (8.67 days) (Table 4). Artificial diet impregnated with 10 mg Cd/kg, 15 mg Cd/kg, 20 mg Cd/kg and 30 mg Cd/kg resulted in 7.89, 7.56, 7.22 and 6.89 days pupal period, respectively, which differed statistically with each other (Table 2). Maximum pupal period (8.04 days) was recorded in generation-1 which differed statistically with generation-2 (7.75 days) and generation-3 (7.29 days).

Effect of cadmium (Cd) mediated artificial diet on total developmental period in different generations: As evident from Table 5, in treatment T_1 when larva were fed on artificial diet mediated with 1 mg Cd/kg, the total life cycle of *S. litura* was 28.72 days which was statistically at par with T_2; (5 mg Cd/kg) i.e 29.08 days. The duration of total developmental period at T_3, T_4, T_5 and T_6 was 29.89, 31.13, 31.88 and 33.02 days, respectively. The larva fed on artificial diet mediated with 40 mg Cd/kg i.e. T_7 took maximum time (33.97 days) to complete its life cycle. Minimum period of total life cycle (29.99 days) was recorded in generation-1 followed by generation-2 (30.88 days) and generation-3 (31.50 days). At T_0 (control) the total developmental period recorded at the three generations was statistically similar i.e. 28.60 days at generation-1 and generation-2 and 28.63 days at generation-3 which was statistically similar to T_1 in all the generations. On an average, at T_7 a difference of 2.23 days was recorded in the total developmental period. The findings are in con-firmation with the finding of Smit et al. (2004) who observed that Zn @ 5mg Zn/dry food led to increase of developmental period of spring tail *(Folsomia Candida*). Zheng-Tian et al. (2011) reported that cadmium in food significantly led to increase in developmental period of *Pirata subpiraticus*. Significant prolongation of gypsy moth larval development was obtained after acute or chronic exposure to cadmium at a concentration of 100 µg/g (Gintenreiter et al. 1993 and Ilijin et al. 2010).

Accumulation of cadmium in the larvae of *S. litura* in different generations: The accumulation of cadmium was recorded in the 6th instar larvae of each generation which increased with increase in the dose of the cadmium and with increasing generations (Table 6). Low amount of cadmium i.e., 0.09, 0.06, 0.04 ppm was already present in the body of

Table 5: Effect of cadmium (Cd) on total developmental period of *S. litura* F. in different generations.

Treatment	Total developmental period (days) Generation 1	2	3	Mean
T_0 (Control)	28.60	28.60	28.63	28.61
T_1 (1mg/kg)	28.63	28.67	28.87	28.72
T_2 (5mg/kg)	29.00	29.03	29.20	29.08
T_3 (10mg/kg)	29.30	29.87	30.50	29.89
T_4 (15mg/kg)	29.97	31.27	32.17	31.13
T_5 (20mg/kg)	30.50	32.10	33.03	31.88
T_6 (30mg/kg)	31.37	33.40	34.30	33.02
T_7 (40mg/kg)	32.57	34.07	35.27	33.97
Mean	29.99	30.88	31.50	30.79

$CD_{(0.05)}$ Treatment: 0.75; Generation: 0.46; Generation × treatment: 1.30

Table 6: Accumulation of cadmium in the larvae of *S .litura* F. in three generations.

Treatment	Cadmium (ppm) Generation 1	2	3	Mean
T_0 (Control)	0.09	0.06	0.04	0.07
T_1 (1mg/kg)	0.67	0.86	1.07	0.87
T_2 (5mg/kg)	3.21	4.70	7.06	4.99
T_3 (10mg/kg)	7.41	12.22	18.98	12.87
T_4 (15mg/kg)	12.43	21.48	28.54	20.82
T_5 (20mg/kg)	15.95	27.88	34.11	25.98
T_6 (30mg/kg)	24.81	36.81	47.33	36.31
T_7 (40mg/kg)	32.97	46.84	56.18	45.33
Mean	12.19	18.86	24.16	18.40

$CD_{(0.05)}$ Treatment: 0.61; Generation: 0.37; Generation × Treatment: 1.05

S. litura larvae in the control itself (T_0). In T_1 i.e., when diet was impregnated with 1 mg/kg of Cd, the accumulation in generation-1 was 0.67 ppm which increased to 1.07 ppm and on an average 0.87 ppm of Cd was recorded in the 6^{th} instar larvae at T_1, irrespective of generations. Whereas, in generation-1, generation-2 and generation-3 the concentration increased from 0.67 to 32.97 ppm, 0.86 to 46.84 and 1.07 to 56.18 ppm at T_0 to T_7, respectively. In generation-1 it was 12.19 ppm which was statistically lower than generation-3 (18.86 ppm) and generation-3 (24.16 ppm).

Thus, the heavy metal accumulation in the sixth instar larvae of *S. litura* increased with increase in dose of heavy metals in artificial diet and showed significant doses-dependent relationship with heavy metal doses in the artificial diet, which may be due to less detoxification, readily assimilation nature of heavy metals as well as loss of accumulated heavy metals through excretion and ecdysis mechanism. Cadmium is very readily assimilated, and the concentration in organisms is often more than five times higher than in their food (Hopkin1989). Gintenreiter et al. (1993) reported that cadmium accumulation in gypsy moth (*Lymantria dispar*) larval body reached much higher with an increase in concentration in food. In *S. litura* the accumulation of heavy metals viz., Zn and Ni with the increasing dose in artificial diet has been reported by Qiang et al. (2008) and Xia et al. (2007), respectively. The heavy metal zinc was accumulated in the larval hemolymph and fat body of *S. litura* and it was a dose-dependent relationship (Qiang et al. 2005).

CONCLUSION

The fecundity and fertility values in various treatments were lower than control and differed significantly. Incubation period and larval period increased with increase in dose and differed significantly from control and lower doses, whereas, pupal period decreased with the increase in concentration of heavy metals. Total developmental period also increased with the increase in doses of heavy metals and differed significantly from lower doses and control. Accumulation of cadmium in the sixth instar larva increased with the increase in doses in artificial diet in three successive generations. It can be concluded that growth and development of *S. litura* was dose dependent. The higher doses of cadmium in the artificial diet increased the generation time, whereas, lower doses shortened the generation time. Thus accumulation of cadmium in agroecosystem might influence the growth and development of *S. litura* through host plants to some extent.

FURTHER RESEARCH

Further studies can be conducted on Cd concentrations found naturally under field/soil condition and its accumulation in crops and herbivores (*S. litura*) under natural conditions can be studied.

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