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Influence of Cadmium on Growth, Survival and Clutch Size of A Common Indian Short Horned Grasshopper, *Oxya fuscovittata*

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Abstract: Newly hatched nymphs of an Indian short horned grasshopper *Oxya fuscovittata*(Marschall) [Orthoptera: Acrididae] were fed ad libitum on foods treated with three sub lethal concentrations of $CdCl_2$ i.e. 25 ppm in oat or dose 1 (d1), 50 ppm in oat or dose2 (d2) and 100 ppm in oat or dose3 (d3) until they reached the adult stage for a complete generation. Growth was measured in terms of specific growth rate (SGR), average daily growth (ADG), percent weight gain (PWG) and Growth rate (GR). It was observed that growth retardation occurred significantly with the increment of doses in both sexes. Adult life spans significantly reduced in males but in case of females a significant difference was observed only in case of higher two doses (d2 and d3). Percent survival was significantly lower in d3. Similar trends followed in case of clutch size. We conclude that $CdCl_2$ exposure is likely to disrupt survival, growth and clutch size of *O. fuscovittata.*

Key words: Cadmium chloride, Specific growth rate, Average daily growth, Percent weight gain, Growth rate

List of Abbreviations:

Growth Rate (GR) = WT/TA (where WT= dry weight gained, T= duration of feeding period in days and A= mean dry weight of the insect during feeding period). Specific Growth Rate (% SGR) == (ln W_{t2} - ln W_{t1}) X 100/T(where Wt_1= initial weight, Wt2= final weight, T= number of days). Average Daily Growth (% ADG) = (W_{t2}-W_{t1}) X 100/ (W_{t1} X T) (where Wt_1= initial weight, Wt2= final weight, T= number of days). Percent Weight Gain (PWG) = (Final weight - initial weight / initial weight) X 100.

INTRODUCTION

Anthropogenic activities are causing levels of heavy metals to increase in the environment. Metal concentrations in biota generally are low, except in the vicinity of metal pollution sources [1]. However, because the residence times of metals in the biosphere are long, even the transport of small amounts through biota is of ecotoxicological interest. Among the pollutants of the environment, cadmium (Cd) has become more risky and its toxicity to men and animals is well documented [2,3]. Cadmium has received considerable attention over the past years as a result of increased environmental burdens from industrial, agricultural, energy and municipal source [2]. A general increase in the levels of cadmium threatens the health of terrestrial and aquatic organisms and therefore has become a major topic of toxicological research [4]. Although not essential for plant growth, cadmium ions are readily taken up by roots and translocated into the leaves in many plant species [5]. Foliar absorption and direct stem uptake also represent potential modes of entry [6,7]. Cd ions accumulate at higher levels in leaves than in other parts of plant [5].

Arthropods especially insects speed up the cycling of nutrients in terrestrial ecosystems [8]. Insects consume large amounts of food and are frequently eaten by other animals. Therefore they are an important link in the transport of metals between trophic levels. Roberts and Johnson [9] sampled invertebrates and their diet from the area of an abandoned lead-zinc mine in the United Kingdom. They found cadmium levels higher in herbivorous invertebrates than in the vegetation on which they fed (But not markedly so). There were much higher levels of cadmium in carnivorous invertebrates,

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suggesting that cadmium might have a capacity for accumulation in food chains. Reports on the acute and chronic toxic effects of Cd on insects are frequent in the literature. Several studies have demonstrated pleiotropic chronic effects of Cd on insect physiology, negatively affecting processes such as growth, development, reproduction and / or hatchability [10,11].These findings have prompted the suggestion of using model insects to assess heavy metal pollution effects in terrestrial as well as in aquatic environments. Since many pollutants occur together in the environment and exert a synergistic effect, it would be difficult to identify the effect of an individual toxicant in the field situation. Hence most of the studies on the chronic effects of Cd in soil insects have been performed in the laboratory.

Acridid grasshoppers occupy a significant position in the food chain representing up to 20-30% of arthropod biomass [12]. Being a primary consumer they play a key role in the ecosystem. The short horned (acridid) grasshopper *Oxya fuscovittata* (Marschall) [Orthoptera: Acrididae] is a terrestrial phytophagous insect that feeds on a variety of plant species. They can be preyed upon by other insectivorous vertebrates. This species can accumulate Cd while feeding on green plant leaves, which might be transferred to next trophic levels through the food chain. The primary objective of the present study was to examine how increased concentrations of Cd in food throughout a complete life cycle affects the ability of exposed insects to successfully develop and reproduce.

MATERIALS AND METHODS

Rearing of the Test Insects: Acridids of interest were obtained from the laboratory colony of the Dept. of Zoology, Visva-Bharati University, Santiniketan, West Bengal; India. Identification of the species of interest was confirmed by the Zoological Survey of India Kolkata. In order to obtain a colony of the acridid species of interest, mass culturing had been done adopting the strategies proposed by Hinks and Erlandson [13] with slight modifications according to Haldar *et al.* [14].

To reduce natural mortality, 30 insects at most were placed in one cage. Cages were specially designed for the purpose of rearing at normal laboratory conditions (temp 32±1° C and relative humidity 70-80%). Each cage of wooden frame with nylon mesh measured (70 X 40 X 30) cm^3. The floor of the cage was wooden and in the middle of the floor there was a hole measuring (5 X 5) cm^2 with a valve for clearing of fecal matter and the exuviae were counted regularly taken up from the cage. Fine freshly washed and sterilized sand in standard enamel trays measuring (26 X 21 X 5) cm^3 were placed on the floor of the cages. Sufficient amount of distilled water was sprinkled daily to keep the sand moist.

Food and its Contamination: For the control experiments, the young hoppers (F1) from untreated parents were fed on white oats. To study the effects of cadmium consumed through food, oats were contaminated with 25, 50 and 100 mg $CdCl_2$ / Kg. Then three doses were set for the experiment, namely d1 (25ppm $CdCl_2$ in oat) or low dose, d2 (50ppm $CdCl_2$ in oat) or medium dose and d3 (100ppm $CdCl_2$ in oat) or high dose. For contamination in white oats, various amounts of $CdCl_2$ were dissolved in 20 ml of double distilled water and mixed with known amounts of white oats and pallets were prepared. After drying, contaminated pallets were given to hoppers. Little amount of green grass (i.e. *Cynodon dactylon*) was supplemented in each set as natural food source.

Contamination of Insects: The experiment was started with nymphs hatched in same day. Same amount of contaminated food (d1,d2,d3) were offered daily to each of the three sets for consumption of insects ad libitum during the experimental period.

Parameter Studied: Percent survival was calculated as percentage of individuals reaching adult stage [15]. Growth was measured in terms of growth rate (GR) according to Waldbauer [16]. But De Silva and Anderson [17] preferred a log value for more accuracy. Hence according to them specific growth rate (% SGR) was measured. Now, to understand the daily trend of growth average daily growth (% ADG) was calculated. To obtain total growth, percent weight gain (PWG) was also measured. Formulae of the measured parameters are given below:

1. GR= Wt/TA (where Wt= dry weight gained, T= duration of feeding period in days and A= mean dry weight of the insect during feeding period.
2. % SGR= $(\ln W_{t2} - \ln W_{t1})$ X 100/T
3. % ADG = $(W_{t2}-W_{t1})$ X 100/ $(W_{t1}$ X T) (where Wt_1= initial weight, Wt2= final weight, T= number of days) and
4. PWG = (Final weight - initial weight/initial weight)X100.

Apart from these, clutch size and life span of adult male and female individuals were recorded.

Statistical Analysis: All the data are presented as means ±SD. One way analysis of variance (ANOVA) and Duncans' multiple range test (DMRT) were carried out to compare the values between different doses (d1,d2,d3) using Microsoft excel 2000.

RESULTS

Around 52-73% individuals attained adulthood in the experimental sets. Percent survival of insects gradually decreased with the increment of doses where d3 showed significant result ($F=13.67035; P<0.001$) (Table 1). Growths of both sexes were measured in terms of ADG, %SGR, GR and PWG (Table 1 and Fig. 1, 2). From the data it was observed that ADG varied approximately 45-62% in males and 88-124% in females, where as %SGR was near 3 in males and near 4 in females in most cases. It was also observed that the value of ADG and %SGR decreased significantly (Male ADG: $F=123.4934; P<0.001$; Female ADG: $F=431.4701; P<0.001$; Male %SGR: $F=337.1496; P<0.001$; Female %SGR: $F=1513.714; P<0.001$) along with increased doses in both sexes. GR and PWG also showed similar trends (Male GR: $F=62053.73; P<0.001$; Female GR: $F=56.02712; P<0.001$; Male PWG: $F=337.1496; P<0.001$; Female PWG: $F=1513.714; P<0.001$) (Fig. 1, 2).

The result also revealed both sexes in controls were in their perfect health and they showed average normal adult life span which is usually seen in insectariums. But a significant decrease (Male ALS: $F=79.2; P<0.001$; Female ALS: $F=119.3182; P<0.001$) of the same with increased doses were observed (Table 1). Moreover some of the females fed with the highest dose (d3) were unable to lay eggs due to short life

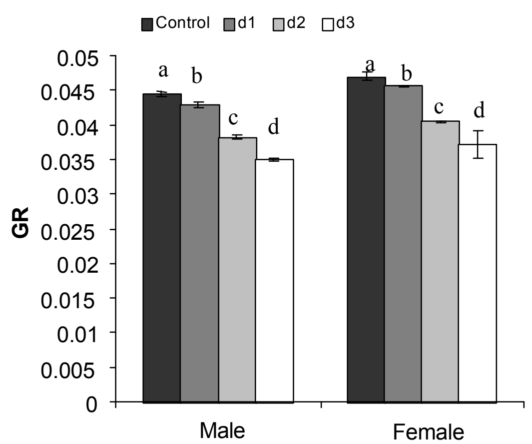

Fig. 1: Growth rate (GR) of male and female Oxya fuscovittata fed with control and cadmium treated diets (d1, d2 and d3). Each bar represents mean ± SD .Different superscripts denote significant differences between the mean values (p<0.001)

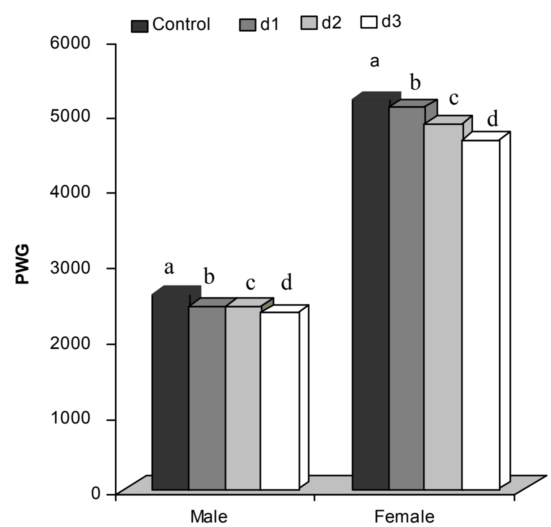

Fig. 2: Percent Weight Gain (PWG) of male and female *Oxya fuscovittata* fed with control and cadmium treated diets (d1, d2 and d3). Different superscripts denote significant differences between the mean values (p<0.001).Error bars are not shown as SD values are very low

Table 1: Average daily growth (%ADG), specific growth rate (%SGR), adult life span (ALS), percent survival and clutch size of *O. fuscovittata* fed with control and three sub lethal doses of cadmium chloride (d1, d2 and d3)

Dose	ADG±SD Male	ADG±SD Female	%SGR±SD Male	%SGR±SD Female	ALS±SD (in days) Male	ALS±SD (in days) Female	%Survival±SD	Clutch Size±SD
Control	62.06±1.05a	124.21±1.48a	3.43±0.114a	4.133±0.079a	33.00±1.00a	39.33±1.53a	73.11±3.67a	12.67±1.55a
d1	56.67±1.42b	118.29±1.58b	3.26±0.018b	3.988±0.027b	29.67±1.53b	36.33±1.53a	67.47±5.43a	11.33±0.58a
d2	50.58±0.35c	100.44±1.00c	2.91±0.150c	3.527±0.035c	22.67±2.08c	28.33±0.58b	60.37±3.90a	11.00±0.58a
d3	44.63±1.64d	88.26±1.23d	2.64±0.012d	2.611±0.016d	14.67±1.53d	20.33±1.53c	51.91±3.93b	7.67±0.58b

Notes:Means within a column bearing the same letter were not significantly different (p>0.05) span. Normal numbers of eggs were found in pods laid by the females kept as control. Although there was not so much variation of clutch size in d1 and d2, a significant decrease (F=23.04762; P<0.001) was observed in the individuals fed with d3 compared to control, d1 and d2 (Table 1).

DISCUSSION

The present study investigated some of the effects of elevated Cd concentrations on the survival, growth and clutch size of a terrestrial short-horned grasshopper *O. fuscovittata.*

For the present experiment it is evident that Growth was significantly retarded along with the elevated doses of Cd. It was also observed that survival and clutch size significantly decreased only in the highest dose, while adult life span showed significant differences in d2 and d3. All these adverse effects were observed may be due to the accumulation of Cd in the insect body. While working on the accumulation of heavy metals, Devkota and Schmidt [6] observed that in grasshoppers the accumulation factor was in the order Cd>Hg>Pb, where Cd was significantly in higher concentrations than Hg and Pb. Schmidt *et al.* [25] also found very high concentrations of Cd in midgut, malpighian tubules, muscles, fat bodies and gonads of adult *Aiolopus thalassinus* after feeding them Hg, Cd and Pb contaminated food. From these studies they inferred that herbivorous animals like most short-horned grasshoppers can magnify heavy metals, especially Cd in their bodies and may transfer them to higher trophic levels. Among heavy metals, insects living in terrestrial environments are mainly exposed to Cd through their food [11]. Negative effect of metal contamination on larval growth rate has been reported by Koricheva *et al.* [13]. This observed effect of pollution is characteristic for chewing insects. Effect of heavy metal treated diet on the development of insects was also studied by Mathew and Al Doori [18] on *Drosophila melanogaster.* Similar growth reduction has been reported for terrestrial hemimetabolous (*Oncopeltus cincta*, *O.fasciatus*) [21,3]and holometabolous insects (*L. dispar*) [19], as well as for aquatic holometabolous larvae (*C. riparius*) [28] chronically exposed to Cd. Vogel [31] determined heavy metal concentrations (Cd, Pb, Mn, Zn) in bark and wood of *Picea abies* from different locations and compared with that of the bark-beetles feeding on it. He had the opinion that in case of cadmium and zinc a positive correlation of metal concentration in beetles and food as well as an accumulation in the beetles could be demonstrated. A similar observation was reported by Schmidt *et al.* [24] in *A. thalassinus* . Though in the present study the accumulated level of Cd had not been measured, but we get enough support from these literatures to conclude that growth retardation and other adverse effects occurred due to the Cd accumulation inside the body of the grasshoppers of interest.

Although Lapointe *et al.* [14] reported that survival was not associated with Cu concentration in *Diaprepes abbreviates*, however in our study a gradual decrease along with higher doses was observed. Adult life span showed a tendency to decrease with increased doses. Schmidt *et al.* [25] and Cervera *et al.* [3] reported similar results where survival of *A. thalassinus* and *O. fasciatus* decreased with increased doses respectively.

Investigations on various insect species have shown a depression or an inhibition of reproduction due to metal [7,27,15,3]. Cheng [4] found that the adult flies of *Drosophila melanogaster* resulting from larvae fed on media containing more than 103 µg Cd/g, died early without laying eggs. During the present study it was found to be true because some females (about 43%) fed with the highest dose died prior to egg laying. We conclude that dietary cadmium exposure is likely to disrupt survival, growth and clutch size of *O. fuscovittata.*

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