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Developmental Time, Mortality and Weight of Immature Fleshfly *Bercaea cruentata* (Diptera: Sarcophagidae) Larvae Exposed to Mercury

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فترة النمو ونسبة الموت و الوزن للأطوار الغير بالغة لذبابة اللحم التابعة لعائلة ذباب اللحم من رتبة ثنائية الأجنحة و التي عرضت يرقاتها للزئبق

فهد المسند

خلاصة: بلغت فترة النمو الكلية لذبابة اللحم نوع Bercaea cruentata (Meigen) من الطور اليرقي الأول إلى طور الحشرة الكاملة ٢٠،٥ و ٢٠،٥ و ٢٠،٩ و ٢٠،٩ و ٢٠،٩ و ٢٠،٩ و ١، ٢١ يوماً للذكور و ٣، ٢٠ و ٣و٢٠و ٧، ٢٠و ٤، ٢٠و ٥، ٢٠و ٨، ٢٠ يوماً للإناث عندما ربيت اليرقات على لحم بقري مفروم معامل بكلوريد الزئبق عند ست تراكيز من الزئبق ٠و ١٠٠و ٢٠٠و ٣٠٠و ٤٠٠و ٥٠٠ ميكروجرام زئبق لكل جرام من الوزن الجاف للحم البقري المفروم. كما لم يسجل أي إرتباط معنوي ($p>0.05$) بين التراكيز المختلفة للزئبق و كلا من فترات النمو (لليرقات- للخادرات- لليرقات و الخادرات معا) و نسب الموت (لليرقات-للخادرات-لليرقات و الخادرات معا) و أوزان الخادرات و الذباب المكتمل. بشكل عام أظهرت نتائج هذه الدراسة قدرة الأطوار الغير بالغة لذبابة اللحم B. cruentata على تحمل تراكيز عالية من الزئبق.

ABSTRACT: The total developmental time of the fleshfly, *Bercaea cruentata* (Meigen) reared on ground beef media treated with mercuric chloride solution (Hg concentrations of 0, 100, 200, 300, 400 and 500 µg/g dry ground beef), from first-instar larvae to adult was 20.5, 20.5, 20.9, 20.9, 20.9 and 21.1 days and 20.3, 20.3, 20.7, 20.4, 20.5 and 20.8 days for males and females, respectively. There was no significant correlation ($p>0.05$) between mercury concentrations and developmental time (larvae, pupae and total), mortality (larvae, pupae and total) and weights (pupae and adults). The immature stages of the fleshfly *B. cruentata* proved to be highly tolerant to mercury.

Keywords: fleshfly, *Bercaea cruentata*, mercury, larvae, tolerance.

Mercury (Hg) is one of the most dangerous heavy metal pollutants of the environment (The Study Group on Mercury Hazard, 1971; Williams, 1984; Schmidt and Ibrahim, 1994). It is used widely in agriculture as a fungicide, herbicide, fertilizer and as a seed dressing. It is also used by various industries for example, in the production of batteries, paints, electrical appliances and in the paper and pulp industry, dentistry and plastic products (Waldron, 1980; Eriksson, 1990).

The fleshfly, *Bercaea cruentata* (Meigen) has a world-wide distribution from the Indian sub-continent through Africa and Europe to both North and South America (Amoudi, 1993). It is of medical importance as it is commonly allied with facultative intestinal and traumatic myiasis in man and animals (Lapage, 1968; James and Harwood, 1969).

High levels of heavy metals, including Hg have been found in various organisms commonly infested by fleshfly larvae (Lodenius, 1981; Pavel and Povolny, 1993, 1994), including snails (Brooks *et al.*, 1992; Pavel and Povolny, 1993, 1994), earthworms (Beyer *et al.*, 1987; Pavel and Povolny, 1992, 1994), fungi (Laaksovirta and Lodenius, 1979; Lodenius, 1981) and turtles (Davenport and Wrench, 1990). Moreover, fleshfly larvae accumulate Hg from food into their tissues (Nuorteva and Hasanen, 1972; Nuorteva *et al.*, 1978, 1980) and high concentrations of the metal have been observed in these flies (Nuorteva and Nuorteva,

1982). Nevertheless, very little attention has been paid to the effect of heavy metals on fleshflies. Accordingly, the objective of the present study was to determine the effects of Hg on developmental time, mortality and weight of immature *B. cruentata*.

Materials and Methods

B. cruentata larvae were collected from Riyadh City in December 1997 using a plastic jar (20 cm deep x 15 cm diameter) containing 500 g decaying ground beef. These larvae were used to establish a laboratory colony that was maintained on ground beef in an environmentally controlled room at 25°C, 60-65% RH, and a 15:9 (L:D) h photoperiod as described by Amoudi *et al.* (1992).

Egg batches were removed from the colony within 0-1 h of deposition. Within 30 min of hatching, 60 first-instar larvae were placed in rearing beakers (14 cm diameter) containing 100 g of ground beef (24.3 g dry weight) mixed homogeneously with mercuric chloride solution (25 ml) at Hg concentrations of 0 (control), 100, 200, 300, 400 and 500 mg/gm dry ground beef. Two replicate beakers were used at each concentration. After 4 days, the media were covered with 4 cm deep sawdust moistened with distilled water and the beakers were covered by cotton cloth, held by rubber bands to permit ventilation. Developmental time was estimated using six groups of third generation larvae.

The larvae were checked during wandering in the sawdust at 12-h. intervals until pupation to determine the larval developmental time. Fresh pupae were weighed (Mettler AC-100, Mettler Instruments, Zurich, Switzerland) and placed separately into 5x2.5 cm vials containing 10 mm of sawdust. The vials were covered with a cotton cloth held by rubber bands until adult emergence to determine the pupal developmental time. Upon emergence flies were etherized lightly, weighed, and sexed.

Statistical analyses were conducted using the MINITAB Computer Program. Relationships between developmental time, mortality and weight and Hg concentrations were tested using correlation coefficients. For univariate models, significance of the correlation coefficient, r, was tested with analysis of variance (Edwards, 1985). Student's *t*-test was used for comparisons between males and females.

Results

The total development time from first-instar larvae to adult of *B. cruentata* at five Hg concentrations and for controls are presented in Table 1. Developmental time was unaffected by increasing Hg concentrations. No correlations were detected between mean larval and pupal developmental time with Hg levels ($r=0.65$, $F=2.98$, $P>0.05$) and ($r=0.26$, $F=0.28$ $P>0.05$) for males, ($r=0.54$, $F=1.67$, $P>0.05$) and ($r=0.62$, $F=2.51$, $P>0.05$) for females, respectively. No differences were detected between the overall mean of larval and pupal developmental time for either sex ($t=1.52$, $df=10$, $P>0.05$) and ($t=1.39$, $df=10$, $P>0.05$), respectively. However, a significant correlation was detected between the mean total developmental time and Hg concentrations for males ($r=0.92$, $F=21.00$, $P<0.05$), while in females no significant correlation was found ($r=0.71$, $F=4.15$, $P>0.05$). However, there were significant differences between the overall mean of the total developmental time in both sexes ($t=2.28$, $df=10$, $P<0.05$).

Percentages of larval, pupal and total mortality were low for all Hg concentrations tested (Table 2). No correlations were observed between Hg concentration and larval, pupal and total mortality: ($r=0.44$, $F=0.96$, $P>0.05$), ($r=0.48$, $F=1.17$, $P>0.05$) and ($r=0.59$, $F=2.11$, $P>0.05$), respectively.

The mean weight of pupae and adults was not affected by increasing Hg concentrations (Table 3). No correlation between pupal weight and Hg levels was found ($r=0.03$,$F=0.00$, $P>0.05$) and ($r=-0.28$, $F=0.33$, $P>0.05$) for males and females, respectively. The lack of correlation extended to adult weight and Hg

TABLE 1

Developmental time (days) of* B. cruentata *larvae reared on ground beef media containing various concentrations of mercury.

Treatment (μg/g dry ground beef)	Number Used		Stages (days) Mean ± SD					
			Larva		Pupa		Total	
	♂	♀	♂	♀	♂	♀	♂	♀
Control	39	56	6.9 ± 0.97	6.8 ± 0.95	13.6 ± 0.67	13.5 ± 0.91	20.5 ± 0.91	20.3 ± 0.83
100	54	44	6.8 ± 0.78	6.9 ± 0.63	13.7 ± 0.44	13.4 ± 0.62*	20.5 ± 0.86	20.3 ± 0.72
200	47	50	7.5 ± 0.88	7.1 ± 0.82	13.5 ± 0.69	13.6 ± 0.58	20.9 ± 0.80	20.7 ± 0.95
300	39	47	7.2 ± 0.97	6.7 ± 0.93*	13.7 ± 0.61	13.7 ± 0.62	20.9 ± 0.85	20.4 ± 0.85*
400	48	42	7.1 ± 1.07	7.0 ± 0.98	13.8 ± 0.64	13.6 ± 0.55*	20.9 ± 0.93	20.5 ± 0.71*
500	48	44	7.5 ± 0.92	7.2 ± 0.94	13.6 ± 0.61	13.6 ± 0.50	21.1 ± 1.05	20.8 ± 0.88
Overall Mean ± SD			7.2 ± 0.29	7.0 ± 0.19	13.7 ± 0.11	13.6 ± 0.10	20.8 ± 0.25	20.5 ± 0.21

*Means between sexes in a particular concentration were significantly different ($P< 0.05$; *t*-test).

TABLE 2

Mortality of the immature stages of* B. cruentata *reared on ground beef media mixed with various concentrations of mercury.

Treatment (μg/g dry ground beef)	% Mortality (n)		
	Larva	Pupa	Total
Control	17.5 (120)	4.0 (99)	20.8 (95)*
100	15.8 (120)	3.0 (101)	18.3 (98)*
200	14.2 (120)	5.8 (103)	19.2 (97)*
300	21.7 (120)	8.5 (94)	28.3 (86)*
400	16.7 (120)	10.0 (100)	25.0 (90)*
500	20.0 (120)	4.2 (96)	23.3 (92)*

*Number of flies reaching the adult stage.

concentrations per μg/gm dry ground beef ($r=0.29$, $F=0.36$, $P>0.05$) and ® = 0.43, $F=0.93$, $P>0.05$) for males and females, respectively.

Significant differences were observed between sexes with respect to overall mean weights ($t=11.40$; $df=10$; $P<0.001$) and mean adult weights ($t=13.34$, $df=10$; $P<0.001$), with pupal weights being smaller in females than in males (Table 3).

Discussion

The total developmental time of *Bercaea cruentata* from first-instar larvae to adult in the present study was extended when compared to the studies of Knipling (1936), Zumpt (1965) and James and Harwood (1969). However, developmental time was reduced when compared to the findings of Madubunyi (1986) for the same species (Table 4). These variations might have resulted due to differences in experimental temperatures and humidities.

TABLE 4

Total developmental time (days) from first instar larvae to adult of* B. cruentata *in different studies.

Developmental time/days	Authors
14-16	Knipling, 1936
8	Zumpt, 1965
11.8	James and Harwood, 1969
22.9	Madubnyi, 1986
20.4	Present study

Lucid from the present study is exposure of *B. cruentata* to high levels of Hg had no significant effect upon the fly's development. Some animals are able to excrete high proportions of metals under contaminated conditions and thereby regulate concentrations in the body. In addition, increased tolerance to the toxic effects of some metals can be acquired through previous sublethal exposures. Bryan (1971) has proposed three mechanisms for the loss of trace metals from invertebrates: excretion across the body surface, excretion *via* the gut and excretion *via* the urine. Food borne Hg ingested by larval *B. cruentata* over the whole period of rearing may have been discharged with the sloughed larval exuviae or pupal shells or might have accumulated in their bodies during the developmental period and subsequently excreted with faeces in the adult stage. Nuorteva and Nuorteva (1982) studied Hg bioaccumulation in blowflies (Calliphriodae) and concluded that larval Hg levels were 4.3 times higher than that of their food. In the adult stage however, Nuorteva *et al.* (1978, 1980, 1982) reported that the level of Hg was half of that present in larval rear food. The same authors also reported that the highe levels of Hg accumulates in the abdomen but seems t

TABLE 3

Weight of pupal and adult of* B. cruentata *larvae reared on ground beef media mixed with various concentrations of mercury.

Treatment (μg/g dry ground beef)	Number Used		Weight (mg* Mean ± SD)			
			Pupa		Adult	
Control	39	56	128.2 ± 9.56	114.5 ± 13.99	80.9 ± 8.00	72.4 ± 9.52
100	54	44	123.8 ± 11.07	108.0 ± 20.50	78.3 ± 8.74	68.9 ± 14.24
200	47	50	124.1 ± 11.83	113.9 ± 10.66	79.6 ± 8.18	71.8 ± 7.10
300	39	47	128.6 ± 12.41	111.5 ± 14.53	81.6 ± 9.03	70.3 ± 10.53
400	48	42	126.9 ± 14.16	111.7 ± 10.47	79.9 ± 8.42	70.5 ± 7.48
500	48	44	125.7 ± 11.50	110.3 ± 11.88	80.8 ± 8.17	69.6 ± 8.53
Overall Mean ± SD			126.2 ± 2.03	111.7 ± 2.38	80.2 ± 1.17	70.6 ± 1.32

*Means between sexes in all concentration were significantly different ($P< 0.001$; *t*-test).

be eliminated from that region more rapidly than from any other parts of the body. However, they did not observe any ill effects of Hg on blowflies and thus concluded that sarcosaprophagous insects are highly tolerant to Hg and have effective mechanisms for its elimination. Andrzejewska *et al.* (1990) in their study on the moth, *Spodoptera littoralis* have reported that the major route of excretion for toxic metals (up to 90%) was *via* the faeces. The same authors reported that pupal shells contained 18-80 % of analyzed heavy metals found in pupa stage, although the metal concentrations in the adult stage were much smaller than in caterpillars. Strong evidence of heavy metal adaptation in natural populations of the fruitfly, *Drosophila melangogaster* (Diptera), is available and has also been induced in laboratory strains (Magnusson and Ramel, 1986; Maroni *et al.*, 1987; Lauverjat *et al.*, 1989). The present study while enhancing our knowledge of the effects of Hg upon life cycle growth and development also provides strong indication that the species can not be employed as a biomonitor for environmental Hg pollution. Further research is needed to delineate the tolerance of *B. cruentata* to mercury and, possibly, to other heavy metals. Future studies should quantify Hg levels over the various life cycle stages in order to determine pathways for excretion and to identify major tissues involved in the bioaccumulation process.

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