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FAHAD A.M. AL-MISNED

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Effect of cadmium on the longevity and fecundity of the blowfly *Chrysomya albiceps* (Wiedemann) (Diptera: Calliphoridae)

FAHAD A. M. AL-MISNED

Department of Zoology, College of Science, King Saud University, P.O.Box 2455, Riyadh 11451 Saudi Arabia

ABSTRACT

First-instar larvae of the blowfly *Chrysomya albiceps* were reared on ground beef media containing 0, 100 and 200 µg Cd/g of dry media. Adult longevity and fecundity were studied. When treated males were mated with treated females, the duration of pre-oviposition, oviposition and post-oviposition periods, longevity, egg batches per cage, total number of eggs per female and the percent of egg hatch were found to be affected by increasing cadmium concentrations. The duration of pre-oviposition and post-oviposition periods were significantly prolonged, whereas the duration of the oviposition period and longevity for both sexes shortened. Egg batches per cage, the total number of eggs per female and the percent of egg hatch decreased with increasing cadmium concentrations. Similar results were obtained by mating untreated males with treated females and treated males with treated females, except longevity of males was not affected. Untreated females mated with treated males showed no significant effects on ovipositional activities, longevity of females, the number of egg batches per cage, the total number of eggs per female and egg viability, but the longevity of males was shortened. It is concluded that cadmium has strong negative effects on the blowfly *Chrysomya albiceps*, especially on longevity and fecundity.

Keywords: cadmium; *Chrysomya albiceps*; longevity; fecundity.

INTRODUCTION

Over the last 30 years the environmental cadmium concentration has increased to toxic levels to several organisms (Quimby *et al.* 1979; Martoja *et al.* 1983; Schmidt *et al.* 1991, 1992, Schmidt & Ibrahim 1994). Cadmium is biomagnified in the food chain (Lindqvist 1988), and could therefore lead to serious developmental and physiological problems such as high mortality rates, deformations and toxic effects in higher trophic levels.

Several studies have been conducted to investigate the effect of cadmium on insects (Postma *et al.* 1994; Postma & Davids 1995, Rayms-Keller *et al.* 1998). Cheng (1980) fed larvae of *Drosophila melanogaster* Meigen on media mixed with cadmium >1000 ppm and found that adult flies died early without laying

eggs. Williams *et al.* (1987) found that egg-viability in chironomids was significantly lower in water containing 100-300 ppm cadmium compared to the control. Schmidt *et al.* (1991, 1992) and Schmidt & Ibrahim (1994) reported that high concentrations of cadmium had significant effects on the development, reproductive capacity and viability of the grasshopper *Aiolopus thalassinus*. Most recently Al-Misned (2001) studied the effect of cadmium in larval media on developmental time, mortality and pupal and adult weights of *Chrysomya albiceps* (Wiedemann). Transport of cadmium throughout the life stages of the same species was also described by Al-Misned (in press).

Chrysomya albiceps (Wiedemann) (Diptera: Calliphoridae) occur in different regions of the world: Africa, Southern Europe, Southwest Asia and East and Northwest India (Zumpt 1965), South America (Baumgartner & Greenberg 1985). It is ubiquitous in Saudi Arabia, commonly found in hospitals, homes, slaughterhouses, markets, garbage and gardens (Buttiker *et al.* 1979). Aspects of biology and behavior of *C. albiceps* were described by Zumpt (1965), Prins (1982) and Al-Misned *et al.* (2002).

This species is of veterinary importance as it is a carrion-breeder, and causes secondary myiasis in sheep (Zumpt 1965, Oldroyd & Smith 1973, Erzinclioglu & Whitcombe 1983, Leite *et al.* 1983). *C. albiceps* third instar larvae are predacious upon dipterous larvae (Zumpt 1965), and larvae of *C. marginalis* (Wiedemann) (Coe 1978), while the third instar of this species behaves as a predator on the pupae of their own species (Tantawi *et al.* 1996).

The present study was conducted to complete a previous study on the effect of cadmium on immature stages of *Chrysomya albiceps* (Wiedemann) (Al-Misned 2001). The present study investigated the effects of cadmium in larval media on adult stage parameters, the longevity, ovipositional activities and fecundity of *C. albiceps* females.

MATERIALS AND METHODS

Stock colony

Egg-batches of 6th generation blowfly *C. albiceps* were obtained from an adult fly colony from Riyadh City (Saudi Arabia) within 0-6 h of egg deposition. The colony was maintained (Zoology Department, College of Science, King Saud University) in an environmental chamber at 25 ± 1°C, 60-65% RH, and a 15:9 (L:D) h photoperiod as described by Amoudi *et al.* (1992). Larvae were reared in ground beef medium containing cadmium as described by Al-Misned (2001).

Larval and pupal development

One hundred first-instar larvae were removed from the colony within 0-6 h of egg hatching and placed into glass rearing jars (11cm diameter) containing contaminated ground beef. The ground beef was artificially contaminated by homogeneously mixing fresh ground media with solutions containing different concentrations of cadmium to obtain the following Cd concentration series: 0 (control), 100 and 200 µg/g dry ground beef. The rearing jars were kept in an environmental chamber under the same conditions as the environmental chamber colony. After four days a covering media with 3-4 cm deep moistened sawdust with distilled water was added to each jar. The jars were covered by cotton cloth held by a rubber band to permit ventilation. Six replicate glass rearing jars were used at each treatment. Larvae were checked daily during the wandering phase through the sawdust strata until pupation to determine larval development time. Fresh pupae were collected daily from each Cd concentration, counted and weighed together, using an AC-100 Mettler balance with a limit of 0.1 mg (Mettler instruments, Zurich, Switzerland). The pupae were then kept in clean jars provided with moistened sawdust. The jars were covered with cotton cloth held by a rubber band until the emergence of adults to determine the pupal development time. Total number of pupae for each treatment was counted and larval morality rates were calculated. In order to obtain adult males and females from 100 and 200 µg Cd treatments as well as the control, according to the results obtained by Al-Misned (2001), replicate jars for rearing larvae for these treatments first were prepared for one and two days respectively, before replicate jars of the controls. Upon adult emergence, flies were immobilized by chilling for 3 minutes according to the technique described by Parrish & Bickely (1966), counted by sex, weighed and transferred to breeding cages to study longevity and fecundity. Total numbers of adults that enclosed from pupae for each Cd concentration were counted and pupal mortality rates calculated.

Longevity and fecundity

Longevity and fecundity of adults from both treated and untreated larvae were studied as following:

- Males from treated larvae × females from treated larvae.
- Males from untreated larvae × females from treated larvae.
- Males from treated larvae × females from untreated larvae.

Within 24 hours of emergence, twenty females and twenty males (both of the same age) from each treatment, including the control, were held in each of a

series of oviposition cages (13 × 8 × 10"). Two opposite sides of each cage were covered with fine wire mesh to provide light. A 13 cm diameter hole in the 3rd side of the cage was provided with a cotton-cloth sleeve for access to retrieve dead files and exchange food dishes. To study longevity and fecundity three replicate cages were used for each treatment. Each cage was provided sugar cubes in a petri dish and a distilled water waterer. A slice of fresh or frozen beef liver was provided in each cage for 12 h as a source of protein and egg deposition medium. After 12 h the liver was examined for the presence of egg batches. Egg batches were removed from the liver medium by means of a fine hairbrush to small petri dishes containing 2% sodium hydroxide solution to dissolve the agglutinated material responsible for cementing the egg masses and to separate eggs for counting (Parrish & Bickley, 1966) with a stereoscopic microscope with 10× magnification. To determine adult longevity dead flies were removed, sexed and counted daily until the last fly died. The number of egg batches was calculated per cage, because all females in each cage deposit the eggs in one batch on the liver substrate. The mean of eggs laid per female for each replicate cage was calculated by dividing the total number of eggs laid by the initial number of females (Fletcher *et al.* 1990). In each treatment, the mean number of egg batches per cage was calculated. The deterrent index was calculated according to Lundegren (1975) (B-A) 100/(A + B), where A and B are the number of eggs laid in the cadmium treatment and control, respectively. Mean longevity for each sex was calculated by multiplying the number of flies that died each day by the number of days they had lived, summing these values and dividing with the initial number of flies. The duration of minimum preoviposition period from emergence of the female adult to the first egg batch deposited and the duration of maximum oviposition period from first egg batch to last egg batch deposited for each replicate cage was claculated; the mean duration at each treatment was calculated using the data from the three replicates. The mean duration of minimum post-oviposition period for females which had lived after the last egg batch deposited was calculated at each treatment.

Egg viability

To study the egg viability from the first and second egg batches, three replicates of a known number of eggs from each treatment (> 50), were randomly chosen from the eggs laid in each treatment and placed on moistened black filter paper in a covered petri dish. The eggs were held in an environmental chamber under the same conditions of the colony until hatching was completed. The number of

Statistical analysis

Statistical analyses were conducted using the MINITAB (version 10) computer program (Minitab Inc. 1989). Relationships between all parameters and different cadmium concentrations were tested using Edwards' (1985) method. The number of egg batches per cage and the number of eggs laid per female in each treatment were compared using (ANOVA) (Kanji, 1993). Assumptions for ANOVA were tested. The student's t test was used to compare period parameters, longevity, egg batches per cage and total number of eggs per female between the control and treated groups (Kanji, 1993).

RESULTS AND DISUSSION

Larval and pupal development

Larval developmental time was extended, and larval and pupal mortality rates were increased with increasing cadmium concentrations in the larval media. There were losses in pupal and adult body weight. Similar observations have been reported by Al-Misned (2001).

Ovipositional activities

In treatments and controls, copulation commenced 3-4 days after adults emerged. Table 1 shows the duration of pre-oviposition, oviposition and post-oviposition periods of females from Cd-treated larvae and untreated control. Data recorded for treated males × treated females indicated that the mean duration of the pre-oviposition period ranged between 12.7 days for control larvae and 15.3-28.3 days for treated larvae; a statisticaly significant difference. Females from 100 and 200 µg/g Cd-treated larvae showed a significant ($p > 0.01$) increase of pre-oviposition period in comparison with the control. The correlation between cadmium concentration in larval media and pre-oviposition period was $r = 0.93$. In treated males × treated females, the oviposition period decreased from 17.7 days to 1.7 days for larvae treated with 200 µg Cd/g dry media, significant differences ($p < 0.001$). Similarly the high dose (200 µg Cd) caused significant prolongation ($p < 0.05$) of the post-oviposition period of treated females in comparison with the control. In general, the oviposition period was decreased ($r = -1.00$) and the post-oviposition period was increased ($r = 0.96$) with increasing cadmium concentration in the larval media.

In untreated males × treated females, females from 200 µg/g Cd-treated larvae had an extended pre-oviposition period ($p < 0.01$) whereas the oviposition period was shortened ($p < 0.01$) in comparison with the control group as in treated males × treated females. The post-oviposition period was not affected ($p > 0.05$).

In contrast, for treated males × untreated females, the data indicated that there was no significant effect ($p > 0.05$) of male Cd-treated larvae on the pre-oviposition, oviposition and post-oviposition periods of Cd-untreated larvae in comparison with the control group.

Table 1. Ovipositional activites and longevity of *C. albiceps* adults from larvae reared on media containing various concentrations of cadmium

Observation (Days) Means ± SE	Control	Cd concentration (μg/g dry media) 100	200
Males from treated larvae × females from treated larvae			
Pre-oviposition period[a]	12.7 ± 0.33	15.3 ± 0.33**	28.3 ± 1.20**
Oviposition period[b]	17.7 ± 0.67	9.7 ± 0.33**	1.7 ± 0.67***
Post-oviposition period[c]	4.3 ± 1.20	5.9 ± 1.01	8.2 ± 1.34*
Longevity ♂	23.6 ± 0.94	20.5 ± 0.76**	17.9 ± 0.93***
Longevity ♀	24.4 ± 0.88	22.9 ± 0.91	21.6 ± 1.32
Males from untreated larvae × females from treated larvae			
Pre-oviposition period[a]		16.0 ± 0.58*	27.7 ± 0.88**
Oviposition period[b]		8.7 ± 0.88**	3.7 ± 1.76**
Post-oviposition period[c]		6.1 ± 1.05	6.3 ± 0.98
Longevity ♂		23.7 ± 0.94	23.4 ± 0.93
Longevity ♀		23.5 ± 0.91	21.0 ± 1.39*
Males from treated larvae × females from untreated larvae			
Pre-oviposition period[a]		12.7 ± 0.67	12.0 ± 0.58
Oviposition period[b]		18.0 ± 0.58	17.7 ± 0.33
Post-oviposition period[c]		4.2 ± 0.99	5.6 ± 1.18
Longevity ♂		20.8 ± 0.76*	18.1 ± 0.92***
Longevity ♀		25.1 ± 0.94	24.9 ± 0.92

Significantly different form control at: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

[a] Number of days from emergence of adult females to first egg-batch deposited (mean was calculated for three replicates).

[b] Number of days from first egg-batch to last egg-batch deposited (mean was calculated for three replicates).

[c] Number of days from last egg-batch deposited to last female dead (mean was calculated for females which had lived after the last egg-batch deposited).

Longevity

The longevity of both males and females of *C. albiceps* from Cd-treated and untreated larvae are presented in Table (1) and Fig. 1. For treated males × treated females, longevity was decreased with the increase of cadmium concentration in the larval media ($r = -0.99$; $p < 0.05$) for both males and females. Adult females lived longer than males in both the control and treatments. Adults from 200 µg/g Cd-treated larvae lived an average of 17.9 days for males and 21.6 days for females, whereas control males lived an average of 23.6 days and females, 24.4 days. Adult mortality of both males and females from larvae reared on media treated with cadmium are presented in Fig.1. Percent mortality for adult males of control was about 32% at the 20th day, and mortality was 42 and 75% for the cadmium treated groups. Similar results were obtained for females. The lifespan of treated females was shortened. This effect appeared to be dependent on the cadmium concentration; 38% of females from 100 µg Cd/g-treated dry media died within 20 days of emergence and 63% at 200 µg Cd. The percentage of dead females before first egg-batch deposition for control, 100 and 200 µg Cd/g dry larval media were 2, 10 and 77%, respectively. The percentage of females alive after the last egg-batch for control, 100, 200 µg Cd/g was 16.6, 26.7 and 18.3%, respectively. In all cases mentioned above, males were more susceptible to the effect of cadmium and tended to have a shorter longevity than females.

Data of untreated males × treated females indicated that the high dose (200 µg Cd) caused a significant shortening in the longevity ($p < 0.05$) for females. In treated males × untreated females, there was a significant shortened longevity ($p < 0.001$) for males in comparison with the control group.

The adverse effect of cadmium on longevity observed in the present study was in agreement with several previous studies. Cheng (1980) found that the adult flies of *Drosophila melanogaster* Meigen resulting from larvae fed on media containing more than 1mg Cd/g, died early. Quimby *et al.* (1979) reported that the alligatorweed flea beetle, *Agasides hygrophila* Selman and Vogt, fed on alligatorweed exposed to 1.0 mg Cd/L in hydroponic culture were sensitive to leaf accumulations of 8.7 µg Cd/g leaves and that mortality rates were increased. Schmidt *et al.* (1991, 1992) observed a significant shortness in lifespan of F_1 and F_2 adults of *Aiolopus thalassinus* developed from eggs laid in Cd-treated soil, or adults developed from F_1 nymphs fed on food treated with various concentrations of $CdCl_2$ until the end of adult life. In contrast, Quimby *et al.* (1979) reported that there is no effect of cadmium on longevity of the mitsedge moth Bactra verutana Zelier, when larvae were fed on a plant diet containing 6.5 µg Cd/g. Also Kazimirova *et al.* (1997) reported that the longevity of Ceratitis *Capitata* and *Coptera occidentalis* was negatively affected by cadmium. This difference could be

explained by intrinsic species susceptibility to metals. Also, it could be due to other factors such as differences in food types, the period of feeding, size, age, developmental stage, sex, bioavailable form of metals and treatment levels (Nuorteva & Nuorteva 1982, Hare 1992, Al-Misned 2001, 2002 in press).

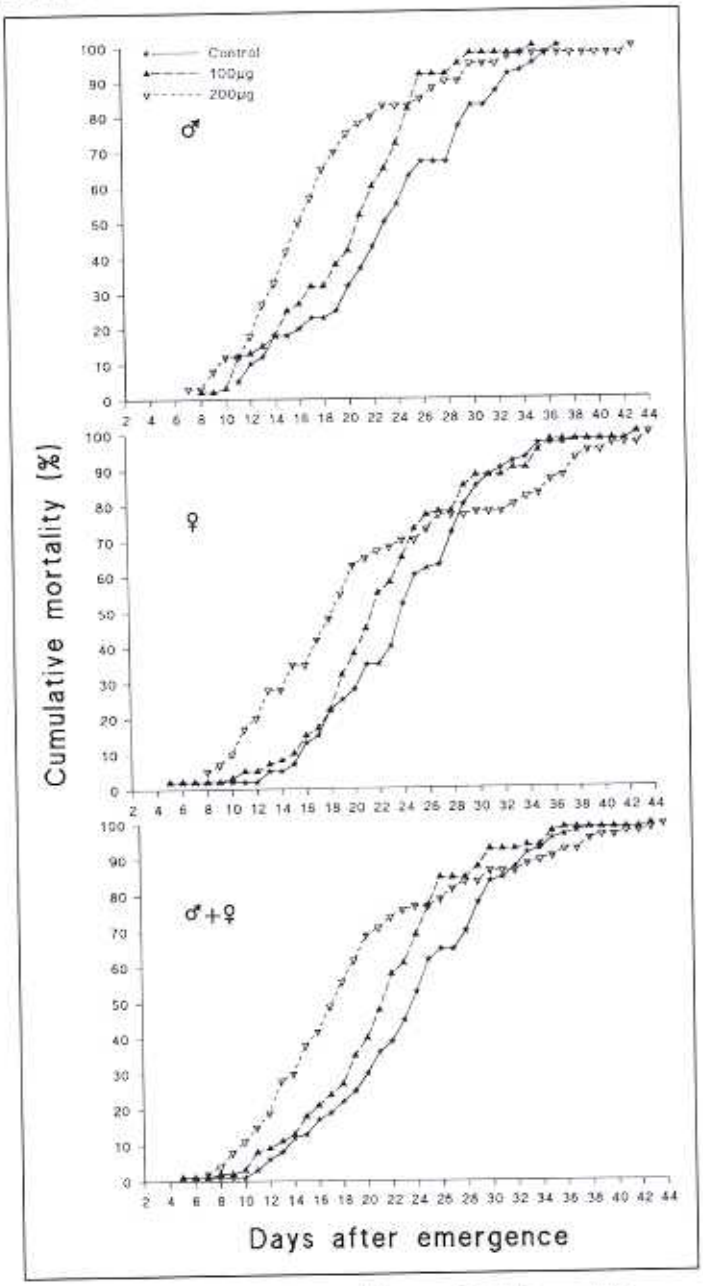

Fig.1. Effect of cadmium on lifespan of *C. albiceps* adults from larvae reared on media treated with cadmium

Fecundity

Results presented in Table (2) show that treatment of larvae with cadmium induced adverse effects on the number of egg batches per cage and fecundity.

In treated males × treated females, the two tested cadmium concentrations caused a highly significant decrease ($F = = 61.80$; $df = 2,6$; $p < 0.001$) in the number of egg batches per cage for females from treated larvae, and the correlation between cadmium concentration in larval media and the number of egg batches was highly negative ($r = -0.99$). Significant differences between total number of eggs per female among control, 100 and 200 µg Cd/g concentration ($F = 91.12$; $df = 2,6$; $p < 0.001$) were observed. The total number of eggs per female was decreased ($r = -0.99$) with increasing cadmium concentration. The highest concentration tested (200 µg Cd) decreased significantly ($p < 0.01$) the mean total number of eggs per female from 274.7 for control to 6.7. The two concentrations of 100 and 200 µg Cd/g dry media reduced oviposition by 42% and 95% respectively, compared to that of control.

In treated males × untreated females, data indicated that there was no significant effect ($p > 0.05$) of cadmium for males from treated larvae on the number of egg batches per cage and the total number of eggs per female in comparison with the control.

The effect of cadmium mixed in the larval media on the adults of different insects regarding subsequent egg production is contradictory in the literature. In the present study, it was observed that cadmium reduced fecundity. This finding is in agreement with Cheng (1980), who found that females of *D. melanogaster* died before laying eggs. Decreased oviposition was also observed in *Agasides hygrophila* fed on food mixed with cadmium (Quimby *et al.* 1979). Moreover, the numbers of eggs laid by adults were decreased in *A. thalassinus* (Schmidt *et al.* 1991, 1992). Martoja *et al.* (1983), by injecting cadmium chloride solution in *Locusta migratoria* (2 µg Cd/g body weight), reported that the decrease of fecundity can be explained by the presence of morphological abnormalities in ovaries, in addition to inhibition of oocyte growth and yolk. However, Quimby *et al.* (1979) found no effect of cadmium on the fecundity of *Bactra verutana*. Also the fecundity of *Ceratitis capitata* and *Coptera occidentalis* was not significantly influenced by cadmium (Kazimirova *et al.* 1997).

Egg hatch

In treated males × treated females, egg hatch was 31.7 and 0% at concentrations of 100 and 200 µg Cd/g dry larval media respectively, compared with 70% in the control (Table 2). A negative correlation ($r = -1.00$; $p < 0.05$) between the egg hatch and concentration of cadmium was observed. In treated

Table 2. Fecundity and egg hatch laid by *C. albiceps* females from larvae reared on media containing various concentrations of cadmium

Cadmium concentration (μg/g dry media)	No. of egg batches/cage Mean ± SE	Total No. of eggs/♀ Mean + SE	Deterrent[a] index	Hatched eggs (%)
Males from treated larvae × females from treated larvae				
Control	8.0 ± 0.58	274.7 ± 20.00	-	70.0
100	3.7 ± 0.33**	112.3 ± 14.10**	41.96	31.7
200	1.3 ± 0.33**	6.7 ± 1.45**	95.24	0.0
Males from untreated larvae × females from treated larvae				
100	3.3 ± 0.67*	120.7 ± 10.70**	38.95	28.2
200	1.7 ± 0.33**	6.3 ± 0.88**	95.52	0.0
Males from treated larvae × females from untreated larvae				
100	7.7 ± 0.33	282.3 ± 14.90	0.00	76.2
200	8.3 ± 0.33	268.0 ± 10.40	1.23	69.7

Significantly different from control at: $^{*}p < 0.05$, $^{**}p < 0.01$

[a] Deterrent index = (B-A) 100/(A + B), where A and B are the number of eggs laid in the cadmium treatment adn control, respectively.

males × untreated females, there is no effect of cadmium for males from treated larvae on the hatchability.

This finding is in agreement with Schmidt *et al.* (1992), who found that the viability of eggs was decreased in *Aiolopus thalassinus* adults developed from nymphs fed on food treated with various concentrations of cadmium.

CONCLUSION

We conclude that the effect of cadmium on *C. albiceps* adults is exhibited when larvae were fed on media treated with cadmium. From the results of the present study, cadmium has negative effects on all parameters studied, especially longevity and fecundity. Toxic effects very similar to those observed in the present study might be expected after the ingestion of food contaminated with cadmium. A combination of laboratory and field investigations would be most useful in defining field effects.

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تأثير الكادميوم على عمر وخصوبة الذبابة الزرقاء *Chrysomya albiceps* (Wiedemann) التابعة لعائلة الذباب الملون Calliphoridae من رتبة ثنائية الجناح Diptera

فهد بن عبدالمحسن محمد المسند

قسم علم الحيوان – كلية العلوم – جامعة الملك سعود
ص. ب. 2455 – الرياض 11451 – المملكة العربية السعودية

خلاصة

تم دراسة تأثير الكادميون المضاف للغذاء اليرقي على عمر وخصوبة كل من الذكور والإناث المكتملة النمو للذبابة الزرقاء *Chrysomya albiceps* حيث غذي الطور اليرقي الأول لهذه الذبابة على غذاء يرقي يحتوي على تراكيز مختلفة من الكادميوم (0، 100، 200 ميكروجرام لكل جرام من الوزن الجاف). بعد بزوغ الذباب المكتمل الناتج من هذه اليرقات تمت تربيته في ثلاث مجموعات بالإضافة للمجموعة الضابطة في أقفاص خاصة لدراسة طول العمر وخصوبة كل من الذكور والإناث. في المجموعة الأولى ذكور معاملة مع إناث معاملة وفيها تأثر بوضوح طول عمر كلا الجنسين وكذلك خصوبة الإناث مع زيادة تركيزات الكادميوم. فترتا ما قبل وضع البيض وما بعد وضع البيض زادتا زيادة معنوية بينما فترة وضع البيض وعمر كل من الجنسين نقصتا. كما أن متوسط عدد البيض الذي وضعته الأنثى وكذلك نسبة فقس البيض نقصتا بزيادة تركيزات الكادميوم. في المجموعة الثانية ذكور غير معاملة مع إناث معاملة تم الحصول على نفس النتائج السابقة في المجموعة الأولى فيما عدا عمر الذكور لم يتأثر مقارنة بالمجموعة الضابطة. في المجموعة الثالثة ذكور معاملة مع إناث غير معاملة تم الحصول على نتائج مشابهة لنتائج المجموعة الضابطة فيما عدا عمر الذكور قصر بالمقارنة بعمر ذكور المجموعة الضابطة.