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Effect of diazepam on the growth rate of *Chrysomya albiceps* (Fabricius) (Diptera: Calliphoridae) for the forensic entomology purposes

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Abstract: This study was conducted to examine the effect of diazepam on the development rate of *Chrysomya albiceps*. Larvae grew on the rabbit carcasses treated with lethal dosage of diazepam. Development rate of larvae and pupae were estimated by means of length, width and weight, whereas in adult flies length of costal vein in wing and tibia in hind leg as well as weight of the body were estimated. Results indicated that length of the larvae during most times 48, 72, 96 hours in treated group were significantly longer than untreated, but during 120, 168 hours, there were no significant differences. There were significant differences between larval lengths during different times. For each of larval width and weight treatment with diazepam did not affect significantly, whereas time had a significant effect. Treated with diazepam increased length and width of pupae significantly, but did not effect on the weight. For adult stage, costal vein length in treated group was longer than untreated significantly. The length of tibia in treated group was significantly longer than untreated. But, treatment with diazepam did not affect significantly. It was concluded that the presence of malathion altered the development rate of C. megacephala and thus disrupted normal postmortem intervalestimation.
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1. Introduction:

A primary objective of forensic examination for the corpses is determination of the minimum postmortem interval (PMI). Larvae of necrophagous fly species are used as forensic tools for the determination of PMI, because flies are the first insects reach the corpse within minutes to a few hours after death (Anderson, 2002), where females lay their eggs on the dead body and larvae feed on decaying tissues. Studying the biological characteristics of carrion fly species, including their development can be used to estimate the age of these insects (Erzinclioglu,1996) and then the minimum PMI (Amendt *et al*., 2011). Although the developmental rates of many carrion flies are known (O'Flynn, 1983; Anderson, 2000) some biotic factors have been shown to effect on their growth rates (Archer, *et al.*, 2005; Donovan *et al*., 2006). These factors include temperature (Nassu *et al.,* 2014), atmospheric humidity (Greenberg, 2002), larval density, body location and the presence of drugs in a corpse which effects on development rate, and failure to consider such factors may lead to errors in minimum PMI estimatation (Goff & Lord, 1994; Carvalho, 2010). This is known as forensic entomotoxicology, which is a relatively new branch of forensic entomology that studies the use of necrophagous insects as alternative specimens for the detection of drugs and toxic substances in animal tissue, and investigates the effect of these substances in the development of insects (Introna *et al.*, 2001). The use of necrophagous insects as specimens for drug detection is largely accepted, because some deaths undiscovered until the body is wholly or partially skeletonized. In such cases, analysis of toxicology using body fluids and tissues are almost impossible. Fly larvae involved in processing the corpse tissues would likely ingest any chemical metabolites from the corpse into their own tissues, which may influence on their development rate that can affect the estimation of the PMI (Estrada *et al.*, 2006; Fremdt *et al.*, 2007). *Chrysomyaalbiceps* is commonly found in cadavers in many parts of the world (Niederegger & Spiess, 2012) and is used in forensic entomology cases for postmortem interval determination (Al-Mesbah *et al*., 2011). The objective of this study was to investigate the effect of diazepam on the development rate of *Chrysomyaalbiceps* after feeding on rabbit cadaver exposure to the lethal dose.

2. Materials and Methods

Experimental design

This study was conducted in autumn during the period from 23/11/2017 to 7/12/2017 in Jeddah city

which located on the west coast of the Kingdom of Saudi Arabia (latitude 29.21 north & longitude 39.7 east), in the middle of the eastern shore of the Red Sea. Diazepam was supplied through the pharmacy of king Abdulaziz university hospital affiliated to the ministry of health in Jeddah in the Kingdom of Saudi Arabia. Sex domestic rabbits weighing 3.75-3.96 kg each were used. They were divided into two groups, three rabbits on each. The first group was treated with oral suspension of lethal dose of diazepam; 9 mg/kg body weight, according to (NTP Chemical repository, 1991), and rabbits in other group were treated with distilled water (as control). The rabbits were mechanically scarified by cervical dislocation. The carcass of each rabbit was placed inside a special metal cage, with surface of 2 cm^2mesh to keep out scavengers and allow insects access. The cage floored with soil. Then, caged carcasses were distributed into two lines which were located 10 m apart from each other.

Measuring growth rate of *Chrysomyaalbiceps*:

About 15 individual of life larvae or pupae were randomly collected from each the two groups of carcasses. Larvae were put in near-boiling water (85-90°C) for 2-3 minutes, for killing and prevent larval shrinkage. Each of larvae and pupae were washed with distilled water followed by a normal saline; NaCl 0.9%; and were dried with a filter paper. They were weighed with a delicate balance to measure wet weight in mg. For measuring larval length, they were viewed laterally, and measured between the most distal point of the head and the most posterior abdominal segment. Larval width was measured across the intersection of the fifth and sixth abdominal segments. For measuring pupal length, they were viewed ventrally and their lengths were measured between the most anterior to the most posterior points. Width measurements were obtained by measuring samples across the intersection of the first and second abdominal segments (according to Mullany *et al*., 2014). After eclosion, flies were placed in the freezer for 5 minutes to slow down and then killed with ethyl acetate and kept in 75% ethyl alcohol. Before weighting, flies were taken out from ethanol and allowed to dry for 10 minutes. For each sample, the left wing and left rear leg were removed. The length of the costa (one of the peripheral wing veins) and tibia (one of the sections of the leg) were measured to give an indication of adult size and to determine if any differences existed between treatments due to drug exposure during earlier life stages (according to George *et al*, (2009) The measurement of samples was achieved under the dissecting stereo microscope from Leica Company (Leica M205 C stereomicroscope). Digital photographs were taken with Leica IC80 HD camera adapted to a Leica M205 C stereomicroscope.

Data on temperature and relative humidity in the study area were obtained from a center of the General Authority for Meteorology and Environment Protection in Jeddah.

Statistical analysis

Method of factorial experiments analysis was used in this study which achieved in randomized complete block design with 15 replicates for two factors. These factors were; drug with two levels (treatment and non-treatment) and time in hours which included seven levels, represented by the different times in which the study was conducted. The statistical analysis included "F test", and its results summarized in "ANOVA (analysis of variance) table", and then "Dancun's test" was used to compare means of significant factors, according to Snedicor (1958).

3. Results

For studying development rate of *Chrysomyaalbiceps*, the apparent measurements were conducted every 24 hours from the onset of the first larval instar. These measurements namely length, width and weight for larvae and pupae which fed and developed on the rabbit carcass treated with diazepam and compared to those fed on untreated carcasses. The adult insect measurements included length of the costal vein in left wing and tibia of the hind left leg, as well as the body weight. During this experiment temperature was fluctuating between 23.5^{O}C and 30.1^{O}C, and relative humidity was 71.1%.

Effect of diazepam on development rate of larvae of *Chrysomyaalbiceps*

The effect of two factors was studied. These factors were; drug with two levels (treatment and non-treatment), and time in hour which included seven levels, represented by the different times in which the study was conducted. The effect of these factors on larval length, width and weight was measured and a separate statistical analysis was performed for each of these measurements. Taking into account that within first 24 hours larvae were found only on untreated carcass.

For larval length, results of the statistical analysis showed that (table 1) during most times 48, 72, 96 h, larvae in the treated group (6.154, 12.327, 14.27 mm) were significantly longer than untreated (4.745, 10.563, 12.93 mm). During 120, 168 h, there was no significant difference in the length of the larvae in untreated group (12.906, 12.34 mm) and treated group (13.953, 12.578 mm). However, within 144 h, the length of untreated larvae (13.914 mm) was significantly longer than the treated larvae (11.742mm).

There were significant differences between larval lengths during different times (table 1). In the larvae which fed on untreated carcasses, the longest larvae

significantly was within 144 h (13.914 mm) which did not differ significantly from those in 96 h (12.93 mm) and 120 h (12.906 mm). The larval length in 168 h (12.336 mm) was not different significantly from those in 96 and 120 h, but both were higher significantly than in 72 h (10.563 mm). The smallest larvae were in 24 h (3.885 mm) and 48 h (4.745 mm). For treated group the longest larvae significantly were in 120 h (13,953 mm) and 96 h (14,266 mm), each was longer significantly than those in 72 h (12,327 mm), 144 h (11.742 mm) and 168 h (12.578 mm). The shortest larvae significantly were in 48 h (6.154 mm).

With respect to the larvae width, the results of the statistical analysis in table (2) clarified that there were no significant differences due to the drug treatment, but there were significant differences in larvae width during different times. For untreated group, the biggest larval width significantly was at 144 h (3.139 mm), 120 h (2.96 mm) and 96 h (2.7 mm), but there were no significant differences between larval width at 96 h, 72 h (2.255 mm) and 168 h (2.335 mm), while the smallest larval width significantly was at 24 h (0.561 mm) and 48 h (0.718 mm). For the treated group, the biggest width significantly was in 96 h (3.251 mm), 120 h (3.232 mm) and 144 h (2.837 mm). The larval width at 144 h did not differ significantly from 72 h (2.387 mm), which in turn did not differ significantly from 168 h (2.261 mm). The smallest larval width significantly was within 48 h (1.045 mm).

For the larval weight, results demonstrated that treatment with dizepam did not affect significantly on larval weight, whereas time had a significant effect (table 3). In non-treated group the biggest weight was in 96 h (0.096 mg) which was not differ significantly from those in 120 h (0.059 mg) and 144 h (0.06 mg) which in turn did not differ significantly from 72 h (0.026 mg) and 168 h (0.045 mg). The lowest larval weight significantly was during 24 h and 48 h (0.003 and 0.005 mg; respectively). While in treated group the biggest weight was in 96 h (0.071 mg), which was not differ significantly from 120 h (0.055 mg), 144 h (0.049 mg) and 168 h (0.049 mg), which in turn did not differ significantly from those at 72 h (0.035 mg) and 48 h (0.016 mg).

Table (1): Duncan's test results to compare the average length of larvae of *C. albiceps* during different times under diazepam.

Treatment	**Time (hour)**							**Mean of treated**
	24	**48**	**72**	**96**	**120**	**144**	**168**	
Non- treated	3.885Ad	4.745Bd	10.563Bc	12.930Bab	12.906Aab	13.914Aa	12.336Ab	10.183A
Treated	00.00Bd	6.154Ac	12.327Ab	14.266Aa	13.953Aa	11.742Bb	12.578Ab	10.14A
Mean of hours	1.942e	5.450d	11.445c	13.598a	13.430a	12.828ab	12.457b	10.164
L.S.RFOR2 0.378,0.497 • Length was estimated in millimeters								
L.S.RFOR7 0.815,1.045 • Different letters indicate the significant differences								
L.S.R FOR 14 1.221,1.558 • Capital letter for vertical comparison of averages								
• Small letters for horizontal comparison of averages								

Table (2): Duncan's test results to compare the average width of larvae of *C. albiceps* during different times under diazepam treatment.

	Time (hour)							
Treatment	**24**	**48**	**72**	**96**	**120**	**144**	**168**	**Mean of treated**
Non- treated	0.561Ac	0.718Aac	2.255Ab	2.700Aab	2.963Aa	3.139Aa	2.335Ab	2.096A
Treated	0.000Ae	1.045Ad	2.387Abc	3.251Aa	3.232Aa	2.837Aab	2.261Ac	2.145A
Mean of hours	0.280d	0.882c	2.321b	2.975a	3.098a	2.988A	2.298B	2.120
L.S.RFOR2 0.181,0.237 • Width was estimated in millimeters								
L.S.RFOR7 0.389,0.499 • Different letters indicate the significant differences								
L.S.R FOR 14 0.583,0.743 • Capital letter for vertical comparison								
• Small letters for horizontal comparison								

Table (3): Duncan's test results to compare the average weight of larvae of *C. albiceps* during different times under diazepam treatment.

	Time (hour)							
Treatment	**24 hours**	**48 hours**	**72 hours**	**96 hours**	**120 hours**	**144 hours**	**168 hours**	**Mean of treated**
Non-treated	0.003± 0.001 Ac	0.005± 0.008Ac	0.026± 0.021 Ab	0.096± 0.158 Aa	0.059± 0.024Aab	0.060± 0.137Aab	0.045± 0.23Ab	0.042± 0.202A
Treated	0.000± 0.000 Ac	0.016± 0.013 Abc	0.035± 0.026 Aabc	0.071± 0.008 Aa	0.055± 0.015 Aab	0.049± 0.177Aab	0.049± 0.026Aab	0.039± 0.166A
Mean of hours	0.002± 0.002 d	0.011± 0.012cd	0.031± 0.021 bc	0.083± 0.113 a	0.057± 0.020 ab	0.055± 0.017b	0.047± 0.025b	0.041
L.S.RFOR2 0.181,0.237 • Weight in milligrams								
L.S.RFOR7 0.389,0.499 • Different letters indicate the significant differences between the averages								
L.S.R FOR 140.583,0.743 • Capital letter for vertical comparison of averages								
• Small letters for horizontal comparison of averages								

Effects of diazepam on development rate of pupae of *Chrysomyaalbiceps*

The effect of two factors was studied, one of them was treated with diazepam, and the other factor was the time of pupal appearance which included five different times. The study was conducted on the length, width and weight of pupae, and separate statistical analysis was performed.

The results showed that treatment with the drug in general had a significant effect on pupal length and as well as the time of the appearance (table 4). For untreated group, the length of pupae that appeared within 24 h (1.72 mm) was significantly shorter than at 48 h (9.341 mm), 72 h (9.101 mm), 96 h (9.151 mm) and 120 h (8.764 mm). In treated group, pupal length did not differ significantly during all the times 24 h (8.808 mm), 48 h (8.888 mm), 72 h (8.563 mm), 96 h (8.223 mm) and 120 h (8.589 mm). When comparing the pupal length at different times, we found that within 24 h pupae in treated group (8.808 mm) was significantly longer than in untreated group (1.721 mm). While the pupal length was not defer significantly in treated and untreated groups during the different times; 48 h (9.341, 8.888 mm), 72 h (9.101, 8.563 mm), 96h (9.151, 8.223 mm) and 120 h (8.764, 8.589 mm). Overall, the treated pupae (8.614 mm) were longer than untreated ones (7.616 mm) significantly.

Regarding to the pupal width, results of statistical analysis in Table (5) showed that treatment with diazepam had a highly significant effect, as well as the time of pupal appearance. For untreated group, the width of pupae at 24 h (0.596 mm) was significantly smaller than those at 48 h (3.057 mm), 72 h (3.039 mm), 96 h (2.970 mm) and 120 h (2.969 mm) which did not differ significantly. While in treated group, there were no significant differences during different times 24 h (3.082 mm), 48 h (3.081 mm), 72 h (2.910 mm), 96 h (2.744 mm) and 120 h (2.93 mm).

However, when comparing different times, it was found that within 24 hours pupal width in treated group (3.082 mm) was significantly bigger than in untreated (0.596 mm). During the rest of the times (48, 72, 96, 120 h) there were no significant differences in pupal width between untreated and treated groups (3.057, 3.081; 3.039, 2.910; 2.970, 2.744; 2.969, 2.930 mm, respectively). In general, the width of the treated pupae (2.949 mm) was significantly bigger than untreated (2.526 mm).

For the pupal weight, results clarified that treatment with diazepam did not affect significantly, while times had a highly significant effect (Table 6). For untreated group, pupal weight within 24 h (0.012 mg) and 48 h (0.024 mg) did not differ significantly, both were significantly smaller than those at 72 h (0.051 mg), 96 h (0.041 mg) and 120 h (0.053 mg). Whereas, in treated group bigger weight was at 120 h (0.0449 mg) which was not differ significantly from 72 h (0.045 mg) which in turn was not differ significantly from 96 h (0.033 mg) which did not differ from 48 h (0.025 mg). The lowest weight was at 24 h (0.0165 mg), but it was not differ significantly from 48 h. While the weight of pupae in untreated group was not differ significantly from treated groupduringdifferenttimes24h (0.012,0.0165mg),48h (0.024,0.025mg),72h (0.0508,0.045 mg), 96 h (0.041, 0.033 mg) and 120 h (0.0529, 0.0449 mg).

Table (4): Duncan`s test results to compare the average length of pupae of *C. albiceps* during different times under diazepam treatment.

Treatment	**Time (hour)**					**Mean of treated**
	24	**48**	**72**	**96**	**120**	
Non- treated	1.721±3.300Bb	9.341±0.344Aa	9.101±0.185Aa	9.151±0.226Aa	8.764±0.210Aa	7.616±3.309B
Treated	8.808±0.292Aa	8.888±0.344Aa	8.563±0.419Aa	8.223±0.621Aa	8.589±0.463Aa	8.614±0.498A
Mean of hours	5.265±4.300b	9.114±0.406a	8.832±0.421a	8.687±0.658a	8.677±0.370a	8.115
L.S.R.FOR2 0.330,0.453 • Length was estimated in millimeters L.S.R.FOR5 1.230,1.566 • Different letters indicate the significant differences L.S.R. FOR 10 0.876,1.119 • Capital letter for vertical comparison of averages • Small letters for horizontal comparison of averages						

Table (5): Duncan`s test results to compare the average width of pupal stage of *C. albiceps* during different times under diazepam treatment.

Treatment	Time (hour)					Mean of treated
	24	48	72	96	120	
Non-treated	0.596±1.145Ab	3.057±0.131Aa	3.039±0.104Aa	2.970±0.130Aa	2.969±0.131Aa	2.526±1.099B
Treated	3.082±0.284Ba	3.081±0.118Aa	2.910±0.297Aa	2.744±0.293Aa	2.930±0.115Aa	2.949±0.269A
Mean of hours	1.839±1.514b	3.069±0.126ab	2.974±0.131a	2.857±0.231a	2.950±0.125a	2.738
L.S.R.FOR2 0.122,0.167 • Width was estimated in millimeters L.S.R.FOR5 0.398,0.524 • Different letters indicate the significant differences L.S.R. FOR10 0.323,0.412 • Capital letter for vertical comparison • Small letters for horizontal comparison						

Table (6): Duncan's test results to compare the average weight of pupal stage of *C. albiceps* during different times under diazepam treatment.

Treatment	Time (hour)					Mean of treated
	24	48	72	96	120	
Non-treated	0.012± 0.023Ab	0.024±0.027Ab	0.0508±0.003Aa	0.041±0.008Aa	0.0529±0.007Aa	0.036±0.023A
Treated	0.0165±0.011Ad	0.025±0.003Acd	0.045± 0.012Aab	0.033±0.001Abc	0.0449±0.010Aa	0.033±0.015A
Mean of hours	0.014±0.0181c	0.024±0.019bc	0.048±0.009a	0.037±0.011B	0.049±0.009a	0.034
L.S.R.FOR2 0042,0.006 • Weight was estimated in milligrams L.S.R.FOR5 0.017,0.018 • Different letters indicate the significant differences between averages L.S.R. FOR10 0.011,0.014 • Capital letter for vertical comparison • Small letters for horizontal comparison						

Effects of diazepam on development rate of adult stage of *Chrysomyaalbiceps*

The effect of diazepam and time of adult eclosion (represented by four different times) were studied on the length of costal vein in left wing, length of tibia in left hind leg and adult weight. A separate statistical analysis was performed for each of these measurements.

For the length of costal vein, the results demonstrated that each of the time of adult eclosion and treatment with diazepam was a highly significant effect (Table 7). In untreated group, the length of costal vein at 72 h (1.75 mm) was smaller than each of 24 h (6.50 mm), 48 h (6.47 mm) and 96 h (6.77 mm). For treated group, costal vein length did not differ significantly at 24 h (6.33 mm), 48 h (6.62 mm), 72 h (6.47 mm) and 96 h (6.56 mm). When comparing the effect of treatment with diazepam, it was clear that there was a significant difference in the length of costal vein within 72 h, where it was higher significantly in treated group (6.47 mm) than untreated (1.75 mm). While there were no significantly differences between treated and untreated groups during 24 h (6.33, 6.50 mm), 48 h (6.62, 6.47 mm), 96 h (6.56, 6.77 mm). In general costal vein length in treated group was longer than untreated (6.50, 5.37mm).

Regarding to the length of tibia in left hind leg, statistical analysis in table (8) showed that each of time of adult eclosion and treatment with diazepam was a highly significant effect. In 72 h, the length of tibia in treated group (2.18 mm) was significantly greater than untreated (0.62 mm), whereas there were no significant differences in the length of tibia between untreated and treated groups at each of 24 h (2.22, 2.28 mm), 48 h (2.2, 2.29 mm) and 96 h (2.29, 2.18 mm). Overall, the length of tibia in treated group (2.23 mm) was significantly longer than untreated (1.85 mm). As for the effect of the time on the length of tibia it was clear that in treated group length of tibia in 72 h (0.62 mm) was significantly less than 24 h (2.22 mm), 48 h (2.2 mm) and 96 h (2.29 mm). Whereas, there were no significant differences in the length of tibia at 24 h (2.28 mm), 48 h (2.29 mm), 72 h (2.18 mm) and 96 h (2.18mm).

As for the weight of adult fly, results in table (9) clarified that time of adult eclosion was a highly significant effect, while treatment with diazepam did not affect significantly. For untreated group, the fly weight at 96 h (0.0452 mg), was greater than the weight at 24 h (0.023 mg) and 72 h (0.0025 mg), but the lowest weight significantly was at 48 h (0.014 mg) which was not differ significantly from the weight at 24 h and 72 h. In treated group, the weight did not differ significantly at different times; 24 h (0.017 mg), 48 h (0.014 mg), 72 h (0.0217 mg), 96 h (0.0256 mg).

When compared the effect of diazepam on the weight of the adult fly during the different times it was found that there was a significant difference at 96 h, where the weight of flies in untreated group (0.0452 mg) was significantly greater than treated 0.0256 mg), while there were no significant differences in fly weight between untreated and treated groups at each of 24 h (0.023, 0.017 mg), 48 h (0.014, 0.014 mg), 72 h (0.0025, 0.0217 mg). In general, there were no significant differences in the weight of untreated (0.021 mg) and treated flies (0.020 mg).

Table (7) Duncan's test results to compare the average length of costal vein in left wing of *C. albiceps* during different times under diazepam treatment.

Treatment	**Time (hour)**				**Mean of treated**
	24	**48**	**72**	**96**	
Non- treated	6.50±0.521Aa	6.47±0.665Aa	1.75±2.816Bb	6.77±0.328 Aa	5.37±2.564B
Treated	6.33±0.348Aa	6.62±0.179Aa	6.47±0.346Aa	6.56±0.592Aa	6.50±0.493A
Mean of hours	6.41±0.452a	6.55±0.493a	4.11±3.120B	6.67±0.489 a	5.934
L.S.R. FOR2 0.782,0.62965 L.S.R. FOR7 0.51,0.387 L.S.R.FOR14 1.154,0.921			• Length was estimated in millimeters • Different letters indicate the significant • Capital letter for vertical comparison • Small letters for horizontal comparison		

Table (8): Duncan's test results to compare the average length of tibia in left leg of *C. albiceps* during different times under diazepam treatment.

Treatment	**Time (hour)**				**Mean of treated**
	24	**48**	**72**	**96**	
Non- treated	2.22±0.089Aa	2.2±0.089Aa	0.62±0.993Bb	2.29±0.0799Aa	1.85±0.871B
Treated	2.28±0.094Aa	2.29±0.114Aa	2.18±0.129Aa	2.18±0.194Aa	2.23±0.148A
Mean of hours	2.25±0.089a	2.28±00.0889a	1.40±0.993B	2.23±0.78a	2.040
L.S.R FOR2 0.172,0.130 L.S.R.FOR4 0.263,0.212 L.S.R. FOR8 3.88, 0.304			•Length was estimated in millimeters • Different letters indicate the significant • Capital letter for vertical comparison • Small letters for horizontal		

Table (9): Duncan's test results to compare the average weight of insect of *C. albiceps* during different times under diazepam treatment.

Treatment	**Time (hour)**				**Mean of treated**
	24	**48**	**72**	**96**	
Non- treated	0.023±0.018Abc	0.014±0.003Ac	0.0025±0.006Abc	0.0452±0.0572Aa	0.0025±0.006Abc
Treated	0.017±0.007Aa	0.014±0.003Aa	0.0217±0.013Aa	0.0256±0.013Ba	0.0217±0.013Aa
Mean of hours	0.020±0.00.0142b	0.0141±0.0035b	0.0121±0.0137b	0.0354±0.043a	0.0121±0.0137b
L.S.R.FOR2 0.011, 0.008 L.S.R.FOR4 0.016, 0.013 L.S.R. FOR8 0.024, 0.019			• Weight was estimated in milligrams • Different letters indicate the significant differences • Capital letter for vertical comparison of averages • Small letters for horizontal comparison of averages		

4. Discussion:

In this study we found that diazepam increased larval length during the early hours of the larval stage (48, 72, 96 h), while it did not affect during the late hours (120, 168 h). However, this drug had no significant effect on larval width or weight either during the whole larval stage or during different times. Our result was accordance with Mullany *et al*. (2014) who confirmed that when the larvae of *Calliphorastygia* fed on a substances containing metamphetamine, their length become longer significantly than larvae in control group. In addition, this study assured by Turner (2004) who reported that feeding larvae of *Calliphoravicina* on pig liver treated with deferent concentrations of paracetamol did not effect on the weight significantly.

The results of this study, however, partly agreed with George *et al.* (2009) who proved that when *Callihorastygia* larvae fed on pet mince containing deferent concentrations of morphine; 2, 10, 20 mg/kg at 22^{O}C, results did not shown any significant effect on the length or width for the larvae with four or seven

days old. In addition, Rumiza *et al*. (2008) reported that feeding the larvae of *Chrysomyamegacephala* on rat liver containing several concentrations of malathion (10, 25, 50 mm / kg body weight), did not effect on their length or weight. Likewise, Zou *et al*. (2013) confirmed that ketamine included in the food of *Luciliasericata* larvae did not effect on their length or weight.

In contrast, Fathy *et al*. (2008) confirmed that feeding of *Chrysomyaalbiceps* larvae on rabbit carcasses treated with lethal dose of codeine caused significant increase in their weight, indicating that codeine stimulated larval growth. Carvalho *et al*. (2001) also reported that when *Chrysomyaalbiceps* larvae fed on rabbit carcasses treated with a twice lethal dose of diazepam, the weight of larvae in treated group was less than control group after six hours of exposure. In addition, Kharbouch (2008) proved that feeding the larvae of *Luciliasericata* on a pig liver containing codeine caused significant increase in the weight of larvae aged 48-96 hours old. Likewise, Zhou *et al* (2014) found that after the larvae of *C. megacephala* fed on an artificial diet containing different concentrations of ketamine (0, 0.5, 25, 50, 100 µg/ g)) their length and weight in treated group was lower than control group.

In this study, it was clear that, diazepam had significant effect on the length and width of pupae, these measurements increased significantly in pupae of treated group. Whereas diazepam had no significant effect on pupal weight. Our study agreement with Mullany *et al*. (2014) who said that feeding of *Calliphorastygia* larvae on a substance containing metamphetamine, caused significant increasing in length and width of the pupae in the treated group, with no significant effect of on the weight. Goff *et al*. (1991) also showed that when feeding the larvae of the flesh fly *Boettcheriscaperegrina* on a rabbit carcass treated with various concentrations of heroin in the form of morphine (6, 12, 18, 24 mg / kg), the weight of pupae was not significantly affected. The results of current study were somewhat similar to that of Rumiza *et al.* (2008), where it was shown that feeding larvae of *Calliphorastygia* on a diet containing several concentrations of morphine (2, 10, 20 mg / kg) has no significant effect on length or weight of the resulting pupae.

The results of this study showed that feeding of *C. albiceps* larvae on the rabbit carcass treated with lethal dose of diazepam caused a significant increase in the length of costal vein in the left wing of adult fly. As well as, tibia of the hind left leg was significantly longer in the treated group, while the weight of adult fly was not affected significantly. This results were in agreement with Mullany *et al*. (2014) who confirmed that when feeding larvae of *Calliphorastygia* on food containing metamphetamine, the length of costal vein in the left wing and tibia of the left leg in the treated group were significantly longer than control. While these results differed from George *et al.* (2009) where it was found that after feeding larvae of *Calliphorastygia* on a diet containing different concentrations of morphine (2, 10, 20 mg / kg), the length of costal vein in the wing and tibia of the left leg does not affect significantly.

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