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# Effect of Diazepam on the Developmental Parameters of Musca domestica

## *Diazepamın Musca Domestica'nın Gelişimsel Parametreleri Üzerine Etkisi*

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# EFFECT OF DIAZEPAM ON THE DEVELOPMENTAL PARAMETERS OF MUSCA DOMESTICA

## ABSTRACT

The objective of the present study was to investigate the some population dynamics and morphological parameters of *Musca domestica* Linnaeus, 1758 (Diptera: Muscidae) under the influence of diazepam. For this purpose, larvae of *M. domestica* were reared on diet with different dose of diazepam. We evaluated effect of diazepam on the some life history parameters i.e., pupal, and adult weight, the number of adult and pupae, and developmental rate of *M. domestica*. This study was carried out at the Entomology Laboratory of Ondokuz Mayıs University in 2021. The obtained results showed the pupal and adult weight, the number of adult and pupae, and development durations, were negatively associated with diazepam concentration. The findings showed that diazepam accelerates larval and pupal development period. Rising from the results, special attention should be taken in the application the toxicological analysis of insect larva data of fatal diazepam or other drug-related cases. Also, when analyzing entomological evidence for drug or chemical-related death, it is important to keep in mind its effects on Postmortem Interval (PMI) prediction.

***Keywords:*** Entomotoxicology, Flies, Forensic Entomology, Population Dynamics, Musca domestica.

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# DİAZEPAMİN MUSCA DOMESTİCA'NIN GELİŞİMSEL PARAMETRELERİ ÜZERİNE ETKİSİ

## ÖZ:

Bu çalışmanın amacı *Musca domestica* Linnaeus, 1758 (Diptera: Muscidae)'nin diazepam etkisi altındaki bazı populasyon dinamiklerini ve biyolojik parametrelerini araştırmaktır. Bu amaçla, *M. domestica* larvaları farklı dozlarda diazepam içeren besinle yetiştirilmiştir. Diazepamin *M. domestica*'nın bazı yaşam öyküsü parametreleri; pupa ve yetişkin ağırlığı, yetişkin ve pupa sayısı ve gelişim hızı gibi üzerindeki etkisini değerlendirdik. Bu çalışma, Ondokuz Mayıs Üniversitesi Entomoloji Laboratuvarı'nda 2021 yılında gerçekleştirilmiştir. Elde edilen sonuçlar pupa ve ergin ağırlığı, pupa ve ergin sayısı ve gelişme sürelerinin diazepam konsantrasyonu ile negatif ilişkili olduğunu göstermiştir. Bulgular, diazepamın larva ve pupa gelişim sürecini hızlandırdığını göstermiştir. Sonuçlardan yola çıkarak,

diazepam veya diğer uyuşturucuyla bağlı ölüm olaylarında böcek larva verilerinin toksikolojik analizi uygulamasında özel dikkat gösterilmelidir.

Ayrıca, uyuşturucu veya kimyasala bağlı ölüm için entomolojik kanıtları analiz ederken, bunun Postmortem Interval (PMI) tahmini üzerindeki etkilerini akılda tutmak önemlidir.

***Anahtar Kelimeler:*** Entomotoksikoloji, Sinekler, Adli Entomoloji, Populasyon Dinamiği, Musca Domestica.

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## 1. INTRODUCTION

Death investigations contain the analysis of gastric contents, blood, and urine. If it has been a long time since death, it can be difficult to use samples such as blood or urine. Insect evidence may be important tool incriminal investigations, particularly when examining suspected poisoning cases. A samples of the larval skin debris, maggots, and empty pupae, collected from the corpse can assist in forensic entomological investigations (Goff et al., 1988).

Insects, which have been on the planet for over 400 million years, are considered one of the most enduring and successful life forms, and constitute the basis of the global ecosystem (Amendt, 2004). Insects are colonized to the corpse immediately after death and generally used to estimate minimum Posmortem Interval (minPMI) and cause of death. Flies could also give evidence for the presence of toxins and drugs in the corpse (Kintz et al., 1990; Gautam et al., 2013). Insects generally involved in the forensic investigations are the Calliphoridae (blow flies), Sarcophagidae (flesh flies) and Muscidae (house flies) (Joseph et al., 2011). The larvae of carrion-feeding flies can metabolize chemical substances, poisons, and drugs and accumulate chemical substances ingested by the deceased people. These chemical substances may impact the growth rate and development of insects. Thus, entomological specimens collected from the corpse provide an alternative source of toxicological specimens that may assist in determining cause of death (El-Kady et al.,1994). The pharmacokinetics of chemicals and drugs in flies depend on their feeding behaviors, species, and developmental stages (Miller et al., 1994). Therefore, the knowledge of population dynamics, local insect communities, and growth rates is essential for entomotoxicological research (Gosselin et al., 2011).

Entomototoxicology, which is a new branch of forensic entomology, that allows qualitative and quantitative analysis of toxic substances, chemicals, and drugs in the corpse with the use of entomological evidence obtained from the crime scene (Hall et al., 1993; Liu et al., 2009; Dayananda and Kiran, 2013; Chophi et al., 2019).

Several studies have indicated that the presence of toxins and drugs could change the growth rates of insects and affect estimation of PMI (Carvalho et al., 2001; Elshehaby et al., 2019; Al-Keridis et al., 2022a; Al-Keridis et al., 2022b, Lamia et al., 2011; Bhandari et al., 2015; Al-Shuraym, 2021; Liu, 2009; Al-Shareef et al., 2021; Nuorteva et al., 1982; Kintz et al., 1990; Bourel et al., 2001; El-Samad et al., 2011; Baia et al., 2016).

Diazepam is a benzodiazepine and misuse of diazepam is associated with increased mortality (Pawar, 2021). Diazepam can help to treat seizures, anxiety, symptoms of alcohol withdrawal, and muscle spasms (Carvalho et al., 2001). Understanding the different impacts of drugs may have on cadaveric insects is of major importance because the impacts of drugs on flies' development vary from species to species.

The house fly *Musca domestica* Linnaeus, 1758 (Diptera: Muscidae), is a opportunistic and, cosmopolitan species and found throughout the world in close association with human activities. *Musca domestica* is a mechanical vector of pathogens that affect both humans and animals (Graczyk et al., 2001; Förster et al., 2007; Scott et al., 2014; Asiri, 2017). Moreover, house flies are the most important pests in livestock and poultry production and could lead to the economic loss in the livestock industry, (Scott et al., 2014; Asiri, 2017; Hussein et al., 2017; Miranda et al., 2019). Several studies observed the presence larvae and adults of houseflies on carcasses. *M. domestica* usually arrives at corpses after the blowflies, thereby it may serve as a possible forensic indicator. *M. domestica* can cause myiasis. When myiasis caused by houseflies larvae is generally used as evidence of insufficient care or neglect of the elderly and children (Wang et al., 2018). This study aimed to evaluate the effect of diazepam on the development and some life history parameters of *M. domestica.*

## 2. MATERIALS AND METHODS

### 2.1. Sample Collection and Insects Rearing

Adult of *Musca domestica* was collected from the campus land from June through August 2020 using aerial insect nets and atraps and transferred to to the Entomology laboratory of Ondokuz Mayıs University (41° 15′ N, 36° 19′ S) within 1 hour of collection. Adult flies (approximately 600) were cultured in clear rectangular plastic cages at a photoperiod of 14:10 (L:D) h, 50 ± 1.5% relative humidity (RH), and 26.2 ± 0.5°C. Adult flies were provisioned with table sugar, and water *ad libitum* (Hogsette and Coler, 2002). The houseflies were breeding method, with modifications proposed by (Holl and Gries, 2018). Males and females were held together in the same cages. The eggs collected were used to sustain the colony.

The larval oviposition substrates were a mixture of wheat bran, yeast, dry milk powder, and sugar, and water placed in a 100 ml plastic cups. We used flies from the same generation of the colony at all replicates to reduce the genetic variability among the samples. Larvae that hatched from the eggs were maintained under the same environmental conditions as the adult flies The pupae were kept in 500 ml glass jar containing sawdust till the adult emerging out.

### 2.2. Experimental Design

Diazepam in tablet form (Nervium 5 mg tablet Saba Pharmaceutıcals Industry and Trade Inc) was dissolved in 5 ml of distilled water (1 mg/ml Diazepam stock solution). To set the experiment, three plastic cups of different concentrations were prepared, and one cup was set as a control (an untreated diet). Three replicates of each concentration were used in each trial. From the stock solution, different amounts were mixed with diet to prepare different concentrations 1.5 ppm, 2 ppm, and 3 ppm, respectively. Forty first-instar larvae were added to 40 g of a diet composed of the different concentrations of diazepam inside 300 ml plastic cups. Larval and pupal durations, adult and pupal weight, larval and pupal survival were recorded.

### 2.3. Statistical Analysis

Statistical analysis was done using SPSS 22 software (SPSS Inc., Chicago, IL). One way ANOVA tests with Tukey post-hoc tests were used to evaluate differences between groups. $P < 0.05$ was achieved to indicate statistical significance.

## 3. RESULTS

### 3.1. Larval and Pupal Developmental Period

Results revealed that treatment with diazepam influenced the the larval and pupal development times of flies. We determined significant declines among larval ($F=12.770$; $p<0.000$) and pupal ($F=6.231$; $p<0.000$) development periods, with the shortest period at concentration 3 (3ppm) and the longest period at control group (Table 1).

**Table 1.** Larval and pupal development durations of *M. domestica* at different diazepam concentrations.

| Concentration (ppm) | Larval Duration (days) (Mean ± SE) | Pupal Duration (days) (Mean ± SE) |
|---|---|---|
| Control | 6.00 ± 0. 068 bc* | 5.17 ± 0. 444 b |
| 1.5 | 5.83± 0.166 b | 4.50 ± 0.000 ab |

| | | |
|---|---|---|
| 2.0 | 5.33 ± 0. 166 ab | 4.00 ± 0.000 a |
| 3.0 | 5.00 ± 0.000 a | 4.00 ± 0.000 a |
| | F = 12.770; df = 3.8; P < 0.000 | F = 6.231; df = 3.8 P < 0.000 |

*Means in the same column followed by different letters are significantly different by Tukey test at the 5% significant level.

### 3.2. Pupal and Adult Weight

Mean pupal weight and adult decreased with increasing drug concentration (Table 2). Significant differences were determined for pupal weight (F=171.621; p<0.00), female weight (F=53.189; p<0.000) and male weight (F= 11.365; p<0.000) of flies between the diazepam-treated and control group (Table 2). Female and male weight significantly decreased when the concentration increased.

**Table 2.** Pupal and adult weight (g) of *M. domestica* at different diazepam concentrations

| Concentration (ppm) | Pupal Weight (Mean ± SE) | Female Weight (Mean ± SE) | Male Weight (Mean ± SE) |
|---|---|---|---|
| Control | 0.0179 ± 0.002 d* | 0.0028 ± 0.006 c | 0.0020 ± 0.009 c |
| 1.5 | 0.0148 ± 0.003 c | 0.0019 ± 0.008 b | 0.0019 ± 0.001 c |
| 2.0 | 0.0111 ± 0.004 b | 0.0018 ± 0.001 b | 0.0017 ± 0.001 b |
| 3.0 | 0.0076 ± 0.002 a | 0.0013 ± 0.001 a | 0.0011 ± 0.001 a |
| | F = 171.621; df=3.321; P < 0.000 | F = 53.189; df = 3.137, P < 0.000 | F = 11.365; df = 3.111; P < 0.000 |

*Means in the same column followed by different letters are significantly different by Tukey test at the 5% significant level.

### 3.3. Larval and Pupal Survival

The effects of diazepam treatment on survival rates of pupae and adults are presented in Table 3. Statistical analysis revealed a significant difference in the numbers of surviving pupae (F= 15.847; p<0.001), males (F= 4.632; p<0.000) and females (F=89.333, p<0.000) between diazepam-treated and control groups. In our study, pupal and adult mortality rates increased with increasing drug concentration (Table 3).

**Table 3.** Numbers of pupa and adult of *M. domestica* at different diazepam concentrations.

| Concentration (ppm) | Number of Pupae (Mean±SE) | Number of Females (Mean±SE) | Number of Males (Mean±SE) |
|---|---|---|---|
| Control | 37.33 ± 0.333 d* | 20.66 ± 0.666d | 14.00 ± 0.577 d |
| 1.5 | 30.33 ± 0.881c | 17.00 ± 1,000c | 9.66 ± 1.666 c |
| 2.0 | 22.33 ± 1,201b | 5.33 ± 1. 201b | 8.66 ± 1.027 b |
| 3.0 | 15.66 ± 0. 484 a | 5.00 ± 0.000 a | 6.00 ± 1.527 a |
| | F = 15.847; df=3.8; P < 0.000 | F = 89.333; df = 3.8 P < 0.000 | F=4.632; df=3.8 P<0.000 |

*:Means in the same column followed by different letters are significantly different by Tukey test at the 5% significant level.

## 4. DISCUSSION

The current study was performed to investigate the effect of diazepam on the some life history parameters of *M. domestica*. In our study, the development time of *M. domestica* were affected by diazepam in a concentration-dependent manner. The duration of larval and pupal development of *Musca domestica* was significantly decreased compared to control. This was probably related to the drug interfering with the insect' physiology, metabolic processes, and therefore affecting their development (Introna et al., 2001). In the current experiment, relative to the developmental duration of control insects, the larval developmental duration was reduced by 4-24 h, pupal developmental duration was reduced by 16-28 h, and the total developmental duration was reduced by 20 h -2,17 days. Our result is consistent with Carvalho et al. (2001), who found that diazepam accelerate development rate of *Chrysomya putoria* (Wiedemann, 1830) and *Chrysomya albiceps* (Wiedemann, 1819) (Diptera: Calliphoridae). In another study, Bhandari et al. (2015), observed that the development periods of Calliphoridae and Sarcophagidae species were shorter in high concentrations diazepam treated groups as compared. These results concur with Al-Shareef et al. (2021), who observed that the total development period of *C. albiceps* which, fed on the diazepam-containing rabbit tissue, developed faster than the control group.

Different chemicals and drugs may significantly distrupt the physiology of carrion flies (Introna et al., 2001) and accelerate or delay developmental rates (Carvalho, 2010). In this respect, it is crucial to test the effects of different drugs on the fly's life cycle. For examples: Zolpidem tartrate also prolonged the developmental stages of *Sarcophaga ruficornis* Fabricius, 1794 (Diptera: Sarcophagidae) (Al-Keridis et al., 2022a), *Chrysomya rufifacies* (Macquart, 1842) (Diptera: Calliphoridae) and

*Chrysomya indiana* (Diptera: Calliphoridae) (Al-Keridis et al., 2022b) in a concentration-dependent manner.

Our study showed a significant negative relationship between drug concentrations and pupal and adult weight. Pupae, female and male weights decreased with increasing drug concentration. The heaviest pupae, female and male weights were recorded in the control group. These results are line with who Al-Keridis et al, (2022b), indicated that pupa and adult weights of *C. rufifacies*, *C.indiana* and *S. ruficornis* (Al-Keridis et al., 2022a), decreased as zolpidem concentration increased. Similarly, Al-Shuraym et al. (2021), who showed that the pupal and adult weight of *C. megacephala* and *Chrysomya saffranea* (Bigot,1877) (Diptera: Calliphoridae), decreased with increased concentration of zolpidem tartrate. This observation agreement with Baia et al. (2016), who found *C. megacephala* pupae of the 8 and 32 ng/g groups were heavier than the control, and 4 ng/g groups, and the adults of the 8, 16, and 32 ng/g groups were heavier than the 4 ng/g and control groups.

In our study, there is a negative relationship between pupa and adult numbers and drug concentrations. As the drug concentration increased, the number of pupae and adults decreased. Baia et al. (2016), showed that the mortality of *C. megacephala* larvae was increased when fed on Flunitrazepam treated compared to the control culture. Carvalho et al. (2001), found that pupae, adult rate, and mortality rates were significantly affected when larvae of *C. putoria* and *C. albiceps* were fed with diazepam-treated rabbit tissues.

## 5. CONCLUSION

In this study, the effects of different concentrations of diazepam on some life history parameters and development periods were investigated. The findings showed that diazepam accelerates development period and and as such can influence the colony of such insects. This drug also decreased the values of some life history parameters, the weight of pupae and adult, number of pupae and adult, depending on the drug concentrations. Thus, these morphological parameters can be considered important measurements that may provide useful data for postmortem interval estimation. Based on the results of the present study, it is clear that further analysis is needed, including different fly species, drugs, and concentrations, to establish a systematic database to support criminal investigations.

### Conflict of Interest:

The authors declare that there is no conflict of interest.

### Ethics:

This study does not require ethics committee approval

### Author Contribution Rates:

Design of the Study: MK (100%).

Data Collection: MK (100%).

Data Analysis: MK (100%).

Writing of the Article: MK (100%)

Submission and Revision of the Article: MK (100%)

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