



E-ISSN: 2320-7078

P-ISSN: 2349-6800

www.entomoljournal.com

JEZS 2024; 12(3): 159-161

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Received: 07-04-2024

Accepted: 11-05-2024

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Effect of alprazolam on development of life cycle stages of blow fly *Lucilia cuprina* (Diptera: Calliphoridae)

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DOI: <https://doi.org/10.22271/j.ento.2024.v12.i3b.9329>

Abstract

The aim of the present study is to determine the effect of alprazolam on life cycle stages of *Lucilia cuprina*. *Lucilia cuprina* species were collected on the decaying meat. Changes in the life cycle stages was studied after exposure to alprazolam. The alprazolam treated food cause the effect on development of larvae. The larval and pupal development slows down with increase in the concentration of alprazolam. The flies emerged earlier from control and then from alprazolam treated food.

Keywords: Calliphoridae, Life cycle, *Lucilia cuprina*, alprazolam

1. Introduction

Forensic science is used to answer the questions related to crime investigations, forensic scientists study chemicals, substances, objects, tissue sample and impressions found at the crime place. Forensic entomology is used in investigation of death cause, to detect drugs and poisons, determine the time of the infliction of wounds and location of an incident. Blowflies are the species of greatest forensic importance that are generally the first organism to reach at the corpse, sometimes within few minutes of death [7, 3, 1]. The availability of accurate PMI can be helpful for the overall direction of an investigation and entomological evidence may be the deciding factor in determination of guilt or innocence in court of law [3].

Sedative drugs at higher doses may results in staggering giant, slurred speech, uncertain reflexes and poor judgement. Sedatives taken continuously for long period of time can cause psychological and physiological dependence [18, 5, 17]. When persons become psychologically dependent, they need the drug for body functioning.

Alprazolam is mostly used in treatment of anxiety disorders, moderate depression and panic attacks. Overdose of alprazolam is more toxic as compared to other benzodiazepines. Pawar [15] studied effect of diazepam on *Chrysomya megacephala*.

Forensic entomology-toxicology is recognized method of estimation of PMI, it includes the study of effects of toxicants on growth and development of carrion-feeder, but comparatively very little research work has done in this field in India. The sedative drugs present in the dead tissue of animal can also affect on the duration of life cycle stages and hence it is important to study the effect of the sedative drug alprazolam on development of blow flies.

2. Materials and Methods

The *Lucilia cuprina* flies were collected as the biomaterials by the procedure of Pawar [15]. The untreated food maintained as control, preparation of various concentrations of alprazolam treated food (0.4, 0.8, 1.2 and 1.6 ppm), collection of developing stages of flies, their length and weight is measured by the method of Pawar [16]. The mean, standard deviation and t-test is calculated for obtained results.

3. Results and Discussion

Eggs of *Lucilia cuprina* were collected on first day for the treatment of alprazolam. Thirty eggs were placed separately on 0.0 (Control), 0.4, 0.8, 1.2 and 1.6 ppm alprazolam containing food and daily observations were recorded (Table 1).

The obtained results showed that alprazolam treated food cause the effect on larval

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development. With increase in concentration of alprazolam in the treated food the larval and pupal development slows down. The flies emerged earlier from control then from

alprazolam treated food (Table 1). The temperature and humidity at the time of experiment in the room conditions are also mentioned in the table.

Table 1: Effect of alprazolam on life cycle stages of *Lucilia cuprina*

PMI Days	Stages	Conc of Alprazolam (ppm)	Length (mm)	Width (mm)	Weight (mg)	Temperature °C			Humidity %		
						Max	Min.	Recorded	Max	Min.	Recorded
1.	I st Instar	Control	3.9±0.29	0.9±0.009	04±0.23	38.5	31.1	34.4	37	14	26
	I st Instar	0.4	3.8 ^{NS} ±0.26	0.8 ^{NS} ±0.007	04 ^{NS} ±0.21						
	I st Instar	0.8	3.7*±0.25	0.8 ^{NS} ±0.008	03 ^{NS} ±0.19						
	I st Instar	1.2	3.6*±0.23	0.7*±0.008	02*±0.11						
	I st Instar	1.6	3.5**±0.22	0.6*±0.007	02*±0.10						
2.	II nd Instar	Control	7.5±0.58	1.6±0.05	13±0.79	38.1	31	34.3	38	14	26
	II nd Instar	0.4	7.4 ^{NS} ±0.55	1.5 ^{NS} ±0.04	12 ^{NS} ±0.76						
	II nd Instar	0.8	7.4 ^{NS} ±0.53	1.4 ^{NS} ±0.041	11 ^{NS} ±0.68						
	II nd Instar	1.2	7.3*±0.52	1.3*±0.037	11 ^{NS} ±0.71						
	II nd Instar	1.6	7.2*±0.51	1.2*±0.028	10*±0.67						
3.	III rd Instar	Control	10±0.68	2.2±0.047	36±1.12	38	30.5	34.1	39	15	27
	III rd Instar	0.4	9.9 ^{NS} ±0.65	2.2 ^{NS} ±0.046	35 ^{NS} ±1.19						
	III rd Instar	0.8	9.8*±0.67	2.1 ^{NS} ±0.040	34 ^{NS} ±1.14						
	III rd Instar	1.2	9.7*±0.63	2.0*±0.031	33*±1.15						
	III rd Instar	1.6	9.6**±0.61	1.9*±0.030	32*±1.13						
4.	Pre-pupa	Control	9.8±0.68	2.5±0.047	37±1.38	37.5	30	33.8	40	16	28
	Pre-pupa	0.4	9.7 ^{NS} ±0.64	2.4 ^{NS} ±0.035	36 ^{NS} ±1.34						
	Pre-pupa	0.8	9.6*±0.59	2.3*±0.038	35 ^{NS} ±1.30						
	III rd Instar	1.2	9.8 ^{NS} ±0.60	2.2*±0.033	38 ^{NS} ±1.46						
	III rd Instar	1.6	9.7 ^{NS} ±0.57	2.1**±0.032	37 ^{NS} ±1.37						
5.	Pre-pupa	Control	9.5±0.59	2.6±0.043	38±1.74	37.2	29.4	33.6	41	17	30
	Pre-pupa	0.4	9.4 ^{NS} ±0.58	2.5 ^{NS} ±0.041	36 ^{NS} ±1.63						
	Pre-pupa	0.8	9.4 ^{NS} ±0.56	2.5 ^{NS} ±0.038	36 ^{NS} ±1.73						
	Pre-pupa	1.2	9.3*±0.57	2.4*±0.034	37 ^{NS} ±1.68						
	Pre-pupa	1.6	9.1*±0.63	2.3*±0.031	36 ^{NS} ±1.63						
6.	Pupa	Control	8.7±0.61	2.9±0.049	38±1.71	36.6	28	32.8	43	18	31
	Pupa	0.4	8.5*±0.58	2.7*±0.045	37 ^{NS} ±1.53						
	Pre-pupa	0.8	9.1**±0.62	2.6*±0.043	37 ^{NS} ±1.55						
	Pre-pupa	1.2	9.0*±0.60	2.5**±0.044	38 ^{NS} ±1.61						
	Pre-pupa	1.6	8.9*±0.57	2.4**±0.041	36 ^{NS} ±1.51						
7.	Pupa	Control	8.4±0.57	3.0±0.041	37±1.58	36.4	28	32.6	43	18	32
	Pupa	0.4	8.5 ^{NS} ±0.60	2.9 ^{NS} ±0.039	36 ^{NS} ±1.62						
	Pupa	0.8	8.7*±0.61	2.7*±0.034	35 ^{NS} ±1.65						
	Pre-pupa	1.2	8.6*±0.63	2.6**±0.032	39 ^{NS} ±1.78						
	Pre-pupa	1.6	8.5 ^{NS} ±0.62	2.5**±0.030	38 ^{NS} ±1.73						
8.	Adult	Control	8.2±0.59	4.0±0.048	46±1.98	38.4	26	34.5	38	12	29
	Pupa	0.4	7.9**±0.51	2.8**±0.038	36**±1.43						
	Pupa	0.8	8.4*±0.51	2.9**±0.039	35**±1.48						
	Pupa	1.2	8.0*±0.42	2.8**±0.037	38**±1.64						
	Pupa	1.6	7.9*±0.53	2.7**±0.035	37**±1.37						
9.	Adult	0.4	8.0*±0.57	4.0 ^{NS} ±0.046	45 ^{NS} ±1.94	37.6	31	34.3	36	13	25
	Pupa	0.8	8.1 ^{NS} ±0.59	3.0**±0.042	35**±1.28						
	Pupa	1.2	7.7**±0.64	2.9**±0.041	37**±1.33						
	Pupa	1.6	7.6**±0.65	2.8**±0.039	36**±1.31						
10.	Adult	0.8	7.9*±0.69	4.0 ^{NS} ±0.47	45 ^{NS} ±1.95	38.1	32	35	35	12	24
	Pupa	1.2	7.7**±0.63	3.0**±0.041	37**±1.29						
	Pupa	1.6	7.5**±0.67	2.9**±0.043	35**±1.24						
11.	Adult	1.2	8.0*±0.58	3.9 ^{NS} ±0.049	44 ^{NS} ±1.87	37.5	30	34.8	37	13	25
	Pupa	1.6	7.5**±0.65	3.0**±0.047	35**±1.27						
	Pupa	2.0	7.4**±0.63	2.8**±0.042	33**±1.21						
12.	Adult	1.6	8.0*±0.59	4.0 ^{NS} ±0.48	45 ^{NS} ±1.91	36.8	29.6	33.7	38	14	27

Where, *p<0.05 (Significant t test), NS- Not significant

Lucilia cuprina arrive early on dead body within few hours or minutes after death. Like all other flies *Lucilia cuprina* are holometabolous, meaning they show complete metamorphosis [3]. The eggs of blowfly have been studied in many parts of the world [8, 12, 9, 10]. Blowflies feeds on decaying organic matter and may show how much time has passed between the discovery of the corpse and time of death [14].

It is assumed that the blow flies are the first individuals that reaches at corpse and lay eggs within few hours after death [4]. According to Greenberg [11] developmental data of blow flies provide most accurate information for estimating the PMI. Pawar [15] observed effects of diazepam on development rates of *Chrysomya megacephala* maggots feeding on treated meat were different from development rates of maggots feeding on

untreated meat. Mali ^[13] reported same results in the study of effect of endosulfan on growth of *Chrysomya rufifacies*.

Several studies in different parts of world showed that various toxins and drugs can affect on larval development of blow flies, resulting in inaccurate assessments of postmortem intervals ^[6, 2, 15]. Pawar ^[16] studied the effects of various concentrations of diazepam on growth and development rates of *Lucilia cuprina* (Diptera: Calliphoridae).

4. Conclusion

Sometimes victims suicide by taking sedative drugs or murdered by giving the drugs in that situations the body tissue has large quantity of the sedative drugs. The drug can affect the development of blow flies and hence in that condition it is important to find out the correct PMI. In the present study commonly used sedative drug, alprazolam were used to find out their effect on different life cycle stages of *Lucilia cuprina* and the data helps in the investigation of crime.

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