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CHRYSOMYA BEZZIANA AS A BIOMARKER TO DETERMINE THE POST MORTEM INTERVAL IN DIAZEPAM CONSUMED CORPS

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ABSTRACT : *Chrysomya bezziana* is one of the fly of forensic importance useful for the determination of the post mortem interval. Many times the victims commit suicide or die by taking oversdose of a sedative drug, diazepam. The sedative drug in putrifying body can affect the life cycle stages of the fly and hence for the correct PMI interval determination, this work has been carried out. Newly hatched larvae of *Chrysomya bezziana* were allowed to feed on diazepam treated partially putrified meat and allowed to develop. In control, the larvae grow normally while at the lower concentration larvae go for pupation. As the quantity of diazepam increased in the food the growth of the larvae was retarded and finally the larvae died. The pupation time and the emergence of the adults were delayed in diazepam fed maggots. The posterior spiracles are used as identification marks. So if a person dies due to consumption of diazepam drug, it will affect the development of larval growth of *Chrysomya bezziana* found on the corpse and the present data may be helpful to determine the correct PMI.

Key words : *Post mortem interval, Life cycle, Chrysomya bezziana, Diazepam drug.*

INTRODUCTION

The insect larvae found on the cadvers are best indicators to calculate PMI and they can also contribute to the qualitative identification of substances or abuse drugs present in the corpse (1). The *Chrysomya bezziana* (Calliphoridae) is the Dipteran fly which arrives immediately after the death of animals and deposits the eggs on the openings. If the death of person took place by consumption of some drug, drug present in the tissue makes the effect on the developmental rate of flies and hence it is necessary to study the effect of drugs on the duration of life cycle stages of the flies of forensic importance.

Diazepam drugs are readily available in the medical stores. Diazepam is a drug of potential abuse and can cause serious problems of addiction. Diazepam, a benzodiazepine drug has sedative and tranquilizing effects. Urgent action by national governments has been recommended to improve prescribing patterns of benzodiazepines such as diazepam. They can be classified into short, intermediate and long acting compounds. Diazepam is included in the long duration group and it is widely prescribed as an ant anxiety, antispasmodic and antiepileptic drug (2,3). It is one of the tranquilizer drugs mostly associated with unexpected deaths caused by drug overdose (4,5). In addition diazepam has been shown to be able to substitute the behavioral effects of barbiturates in a primate study. The purpose of this study was to determine the effect of diazepam on development and growth of larvae as well as to verify the time of emergence of adults that can help to find the postmortem interval.

MATERIAL AND METHODS

The *Chrysomya bezziana* (Calliphoridae) flies were collected identified and were allowed to lay the eggs on the partially putrefied flesh. 50 gm each of grinded liver and meat of goat was placed in five petry dishes as a food of larvae. First dish was used as control which contained grinded tissue of goat. In remaining four petridishes, diazepam was mixed so as to make 4 ppm, 8 ppm and 12 ppm and 16 ppm diazepam concentrations. Eggs of *Chrysomya bezziana* were collected from the rearing cage. The fly lay near about 200 to 250 eggs at a time. Eggs were in bunches in adhesive condition. Eggs were seperated by brush. Near about 40 eggs were placed on each petry dish. All the dishes were placed in separate rearing chambers. On each day the observations were made. The larval stages were collected and stored in absolute alcohol every day. The maximum and minimum temperature and humidity were noted. The growth of the larvae was noted and their photographs were taken.

RESULTS AND DISCUSSION

The eggs of *Crysomya bezziana* were having length 0.28 cm, width 0.04 cm and weight 0.001gm. On second day eggs hatched and emerged larvae started feeding on the respective food provided. With the progress in the growth, it was observed that the rate of growth of the maggots was slower in the diazepam treated larvae and was in relation to the dose of diazepam. On 7 th day the maggots in control went for pupation and were dark brown in colour, in 4 ppm diazepam, maggots also went for pupation but were light brown in colour while and the maggots in 8 ppm,12 ppm,16 ppm were still growing slowly. On 8 th day in control and 4 ppm diazepam pupa were observed, in 8 ppm and 12 ppm live maggots were with stunted growth while in16 ppm

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maggots died. On 9 th day in control and 4 ppm pupal stages, in 8 ppm live maggots and in 12 ppm dead larvae were observed. On 10 th day maggots from 8 ppm died.

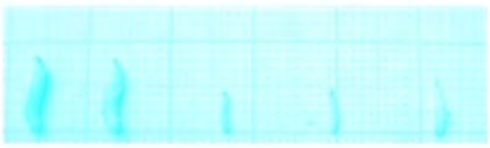
Temperature and humidity plays important role in the duration of the life cycle stages of the insects and hence along with the dimensions of all life cycle stages, temperature and humidity was recorded and mentioned in the Tables.1,2 and are also shown graphically in Fig.1, while photographs are given on Plate.1.

Control 4ppm 8ppm 12ppm 16ppm

2nd Day

Control 4ppm 8ppm 12ppm 16ppm

3rd Day

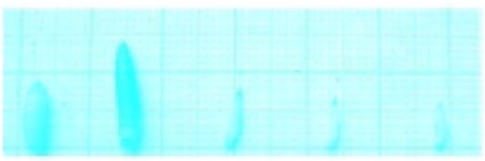


Control 4ppm 8ppm 12ppm 16ppm

4th Day

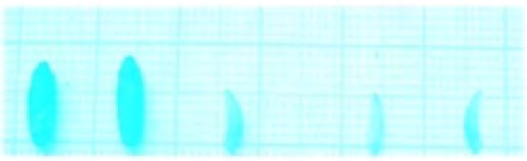
Control 4ppm 8ppm 12ppm 16ppm

5th Day



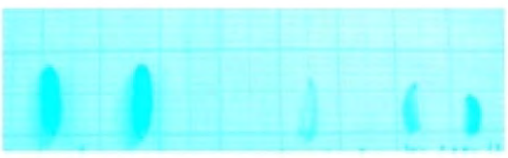
Control 4ppm 8ppm 12ppm 16ppm

6th Day



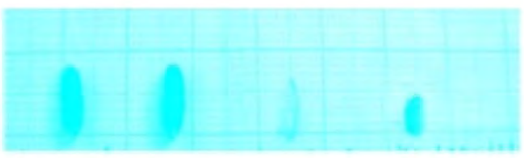
Control 4ppm 8ppm 12ppm 16ppm

7th Day



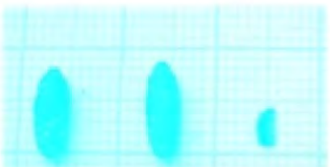
Control 4ppm 8ppm 12ppm 16ppm

8th Day



Control 4ppm 8ppm 12ppm

9th day



Control 4ppm 8ppm

10th Day

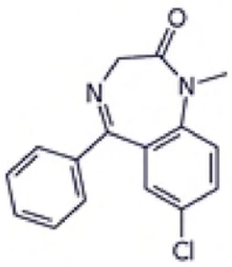


Plate 1 Structure of Diazepam and effect of diazepam on the life cycle stages of *Chrysomya bezziana*.

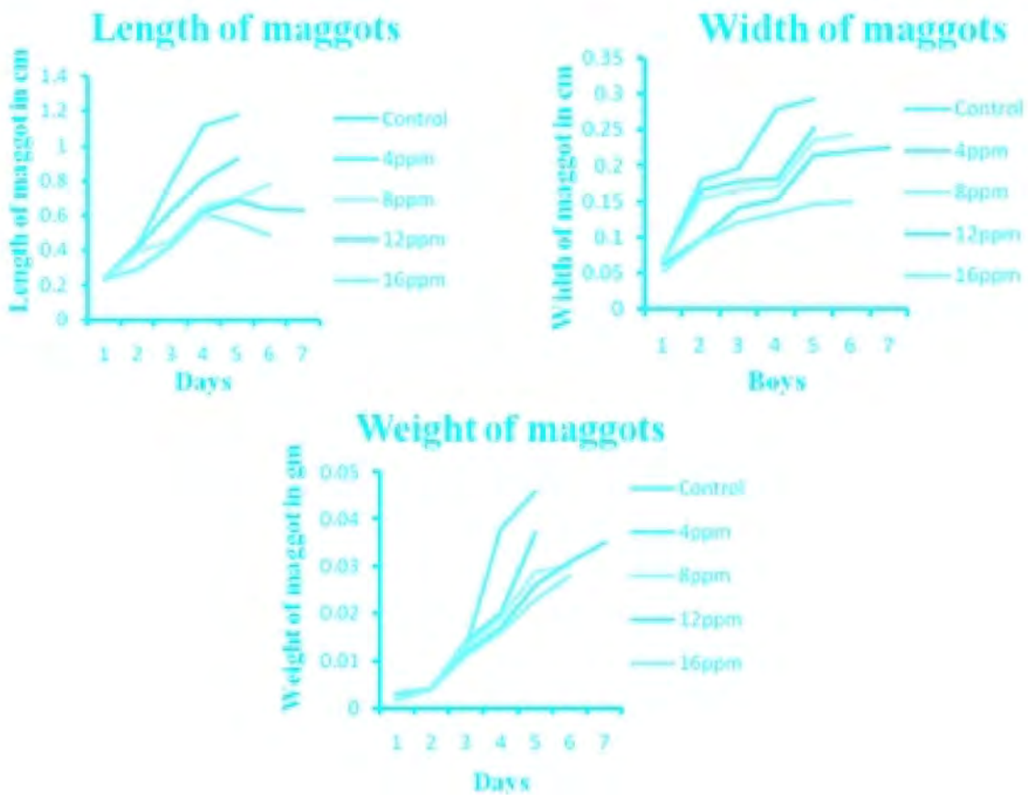


Fig. 1 Growth of Maggots of *Chrysomya bezziana* (Calliphoridae) in diazepam treated food.

Table. 1 Effect of diazepam on the morphometric parameters of life cycle stages of *Chrysomya bezziana.*

Date	Stage	Conc. of Diazepam	Length (cm)	Width (cm)	Weight (gm)	PMI Days	Temp.°C		Humidity %	
							Max.	Min.	Max.	Min.
1. day	Eggs		0.280± 0.008	0.040± 0.035	0.001± 0.0	-	35.3	21.0	61	40
	Maggot	Control	0.237± 0.019	0.068± 0.006	0.003± 0.00					
	Maggot	4 ppm	0.236± 0.020	0.067± 0.006	0.002± 0.00					
2. day	Maggot	8 ppm	0.236± 0.019	0.067± 0.0065	0.002± 0.00	1	35.3	21.0	61	40
	Maggot	12 ppm	0.235± 0.019	0.062± 0.006	0.002± 0.00					
	Maggot	16 ppm	0.232± 0.020	0.053± 0.005	0.002± 0.00					
	Maggot	Control	0.428± 0.039	0.179± 0.015	0.004± 0.001					
	Maggot	4 ppm	0.421± 0.039	0.167± 0.014	0.004± 0.001					
3. day	Maggot	8 ppm	0.398± 0.037	0.154± 0.013	0.004± 0.001	2	35.4	21.1	60	49
	Maggot	12 ppm	0.294± 0.027	0.098± 0.0095	0.004± 0.00					
	Maggot	16 ppm	0.289± 0.026	0.097± 0.0094	0.004± 0.00					

	Maggot	Control	0.799± 0.075	0.197± 0.016	0.013± 0.001					
	Maggot	4 ppm	0.628± 0.060	0.179± 0.015	0.014± 0.001					
4. day	Maggot	8 ppm	0.449± 0.041	0.167± 0.013	0.013± 0.000	3	34.4	21.1	49	38
	Maggot	12 ppm	0.428± 0.039	0.142± 0.011	0.012± 0.000					
	Maggot	16 ppm	0.421± 0.039	0.121± 0.009	0.011± 0.001					
	Maggot	Control	1.116± 0.10	0.28± 0.026	0.038± 0.003					
	Maggot	4 ppm	0.81± 0.080	0.181± 0.016	0.020± 0.001					
5. day	Maggot	8 ppm	0.655± 0.062	0.171± 0.014	0.019± 0.001	4	35.3	21.0	48	39
	Maggot	12 ppm	0.625± 0.059	0.153± 0.012	0.017± 0.001					
	Maggot	16 ppm	0.611± 0.059	0.132± 0.011	0.016± 0.001					
	Maggot	Control	1.175± 0.10	0.293± 0.027	0.046± 0.004					
	Maggot	4 ppm	0.922± 0.090	0.282± 0.026	0.037± 0.003					
6. day	Maggot	8 ppm	0.699± 0.065	0.235± 0.020	0.029± 0.002	5	35.4	21.1	61	41
	Maggot	12 ppm	0.687± 0.066	0.215± 0.019	0.026± 0.001					
	Maggot	16 ppm	0.555± 0.053	0.146± 0.011	0.023± 0.002					
	Pupa	Control	0.866± 0.082	0.360± 0.034	0.047± 0.004					
	Pupa	4 ppm	0.851± 0.082	0.359± 0.034	0.043± 0.004					
7. day	Maggot	8 ppm	0.780± 0.065	0.243± 0.020	0.030± 0.002	6	35.4	21.1	60	45
	Maggot	12 ppm	0.639± 0.060	0.178± 0.015	0.031± 0.002					
	Maggot	16 ppm	0.487± 0.066	0.125± 0.010	0.028± 0.001					
	Pupa	Control	0.862± 0.083	0.353± 0.031	0.046± 0.004					
	Pupa	4 ppm	0.801± 0.080	0.353± 0.031	0.045± 0.004					
8. day	Maggot	8 ppm	0.859± 0.082	0.351± 0.032	0.043± 0.004	7	35.4	21.1	57	38
	Maggot	12 ppm	0.635± 0.060	0.176± 0.014	0.020± 0.002					
	Maggot	16 ppm	Maggots die							
	Pupa	Control	0.861± 0.084	0.347± 0.031	0.049± 0.004					
9. day	Pupa	4 ppm	0.848± 0.082	0.345± 0.030	0.048± 0.004	8	35.4	21.1	57	39
	Maggot	8 ppm	0.847± 0.081	0.345± 0.031	0.048± 0.004					
	Maggot	12 ppm	Maggots die							
10. day	Pupa	Control	0.860± 0.084	0.343± 0.030	0.050± 0.004					
	Pupa	4 ppm	0.859± 0.082	0.340± 0.030	0.047± 0.004					
	Maggot	8 ppm	Maggots die							

From the above results diazepam drug affect the growth of the larvae (6). As the quantity of the diazepam increases in the food the growth of the larvae ceases and larvae also die in proportion to the dose of the diazepam. It also affect the total period required for the emergence of adult. The emergence of the adults was delayed in 4 ppm diazepam fed maggots.

Goff & Lord (1994), Miller *et al.* (1994) and Bourel *et al.* (1999) using in their experiments with Methamphetamine, cocaine, morphine and amitriptyline drugs alter the rate of development of some dipteran flies (7,8,9). Similar results were found in the development of *Crysomya bezziana*, however due to variations in the climatic conditions the time durations are found to be variable and present data can be used to determine the PMI in this region under such circumstances.

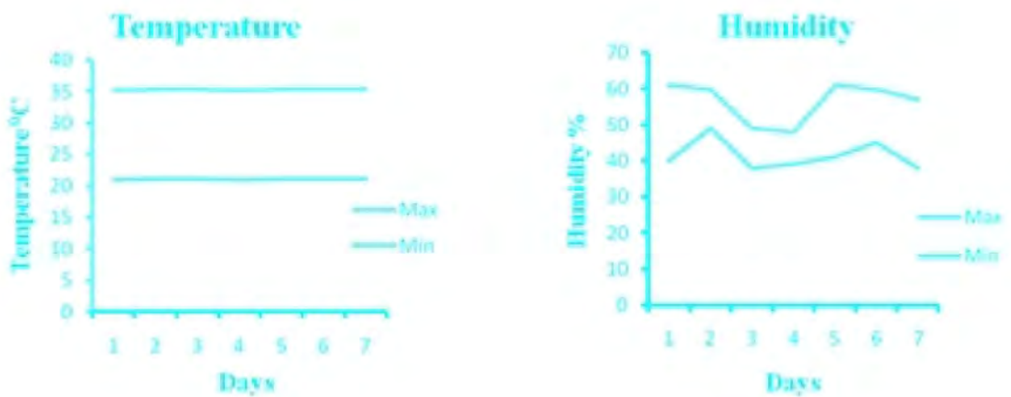


Fig. 2 Growth of Maggots of *Chrysomya bezziana* (Calliphoridae) in diazepam treated food.

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