# EFFECTS OF VARYING VOLUME OF CYPERMETHRIN PESTICIDE ON *Chrysomya albiceps* (FABR.) (DIPTERA: CALLIPHORIDAE) REARED ON RABBIT CARRIONS

Supported by tetfund TERTIARY EDUCATION TRUST FUND

**T. Ekrakene[1]* and P. E. Odo[2]**

[1]Department of Basic Sciences, Benson Idahosa University, PMB 1100, Benin City, Nigeria
[2]Department of Animal & Environmental Biology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria
*Corresponding author: tekrakene@biu.edu.ng

**Received:** December 08, 2016 **Accepted:** February 27, 2017

**Abstract:** This research work assessed effects of varying volumes of cypermethrin pesticide on the larval body length, weight, and developmental time of blowfly *Chrysomya albiceps* (Diptera: Calliphoridae) reared on rabbit carrions. Sixteen rabbits (*Oryctolagus cuniculus*) with mean weight 2.14±0.18 kg (Mean ± S.D.) were sacrificed in groups of four with 3, 6 and 12 ml, respectively of cypermethrin pesticide. The control group (no cypermethrin pesticide) was sacrificed by cervical dislocation. These dosages are the same with those that are normally observed in homicide and abusive cases involving cypermethrin pesticide injection doses. Mettler Toledo weighing balance (with sensitivity of 0.001-1 g) was used to record the weights of larvae and a pair of compasses was used to measure the body length and read with a transparent meter rule. All stages of the insect were monitored and observations recorded. The mean minimum lengths and weights, i.e. 0.0045 g: 03.95 mm for 12 ml; 0.0053 g: 05.77 mm for 6 ml, and 0.0074 g: 06.56 mm for 3 ml were attained at 108 h after eggs were laid compared to the control group that recorded 0.0123 g: 11.13 mm at the same time. The mean total developmental periods were reached at 450.22 h (18.76 days), 401.05 h (16.71 days), 380.28 h (15.85 days) and 281.24 h (11.42 days) respectively for 12 ml, 6 ml, 3 ml groups, and the control. This result showed the possible post mortem interval estimation error of 7.34 days, 5.29 days and 4.43 days respectively in cases where 12, 6 and 3 ml dosages of cypermethrin pesticide are involved compared with the control group. Absolute care must be employed in the calculation, interpretation and usage of insects' data in forensic entomology, when and where toxins and drugs may be involved in the investigation.

**Keywords:** Carrions, *Chrysomya albiceps*, cypermethrin, forensic entomology, post mortem interval

## Introduction

Noone knows how many species of insects that inhabit the earth because so many are yet to be identified but more than 700,000 have already been described. This means that in terms of the numbers of species, 70 – 80 percent of the earth's creatures are insects (Carson, 2002). The vast majority of these insects are held in check by natural forces, without any intervention by man. Diverse as well as abundant, insects comprise approximately half of the earth's one and a half million known species (McGavin, 2007). These insects and their products are useful materials in the detection of toxins and drugs in the bodies of organisms especially in the cases where the normal toxicological specimens are not available or are not made available for the investigators. This aspect of forensic science is gaining more attention nowadays (Beyer *et al.*, 1980; Catts and Goff 1992; Goff and Lord 1994; Tracqui *et al.*, 2004) on the influence of these drugs and toxins on the development rate of the dipteran larvae (Carvalho 2010; River and Dahlem 2014). Medico-legal forensic entomologists apply larval growth and development in the calculation and estimation of the post-mortem interval (PMI) in the criminal investigation (River and Dahlem 2014). In the process of calculating the PMI it is very important to assume that during the first weeks of the decomposition process, these insects will develop at an expected pattern under a given climatic condition but the presence of toxins and drugs however could alter this predictable pattern of decomposition (Carvalho, 2010; Goff and Lord, 1994).

Entomotoxicology is a new branch of Forensic Entomology. It examines the application of analytical techniques to carrion feeding insects in order to identify the drugs and toxins present in the intoxicated tissue (Gautam *et al.*, 2013). These insects, as they feed on corpses, ingest and accumulate drugs and metabolites in the cadaver into their own bodies, they therefore are useful sources for toxicological analysis. This application of insects as alternative matrix for the detection of drugs and toxins is well documented and recommended when normal toxicological matrices such as blood, urine, and tissues are not available (Gautam *et al.*, 2013). The potential use of necrophagous insects for detecting drugs and other toxins in decomposing carrions have been illustrated by many authors including Kharbouche *et al*. (2008) and Introna *et al*. (2001). Besides, O'Brien and Turner (2004) among others have demonstrated that, drugs and toxins in putrefied tissues may have an influence on the development on the development of the necrophagous Diptera that can affect the estimation of the PMI. Apart from necrophagous species, bioaccumulations have also been observed in parasitoids, predators, and omnivorous species (Dayananda and Kiran, 2013).

Life in general does not only have to adjust to calcium, silica, copper and all other minerals from rocks; they are also to adjust to synthetic creations of man's inventive mind. Adjusting to these chemicals would require time on the scale that is natural. Pesticides are mixture of substances, of chemical or biological origin, used by human society to mitigate or repel pests such as bacteria, nematodes, insects, mites, mollusks, birds, rodents, and other organisms that affect food production or human health. They usually act by disrupting some component of the pest's life processes to kill or inactivate it. In a legal context, insecticides also include substances such as insect attractants, herbicides, plant defoliants, desiccants, and plant growth regulators. These insecticides disrupt the balance between a host and its natural enemy (Xu *et al*., 2001). Studies have shown that pesticides applied to insects' pests cause various sub-lethal effects on the parasitoids, such as changes in development and emergence rates, sex ratios (Kreipi *et al*., 1991; Willrich and Boethel 2001).

Pyrethroids are among the most commonly used pesticides globally, accounting for more than thirty percent of the world use (Shukla *et al*., 2002). Cypermethrin is a synthetic pyrethroid (Shukla *et al*., 2002) which is used widely in the control of various agricultural insects belonging to Diptera, Lepidoptera and Hemiptera (Cox 1996; Liuet *al.*, 1998; Suh *et al.*, 2000). Cypermethrin, a pyrethroid compound is widely used due to its high insecticidal potential and slow resistance in pest (Aggarwal, *et al*., 2015). Like all pyrethroids, cypermethrin kills insects by disrupting normal functioning of

the nervous system, even sub lethal doses of this insecticide can have profound effects on parasitoids (Smilarick *et al*., 1996).

This work aimed at establishing the effect of cypermethrin pyrethroid pesticide, on the body mass index of *Chrysomya albiceps* through the assessment of the larvae body weight, length and the developmental period.

## Materials and Methods

### *The experimental site*

The experimental site was the Biology Departmental farm of the College of Education Warri, Warri South Local Government Area, Delta State, Nigeria. The study was carried out between March, 7th and 25th, 2016 in an open fallow plot of the farm. The college is located in Warri on Latitude 5.5432 and Longitude 5.7382, accuracy of 7.1 m. It has a tropical climate characterized by two distinct seasons; the wet season occurs between April and October with a break in August while the dry season is from November to April with a cold harmattan between December and January. Temperatures in Warri range from 32°C to 37°C at an altitude of 21 m, with mean annual rainfall of 2673.8 mm. The natural vegetation is rainforest. The forest is rich in timber tree, and other flowering plants (Egborge, 1994).

The farm lies east of a botanical garden and southeast by a plantation orchard and surrounded by other research crop plots. Grasses, wildflowers, herbs, and weeds cover the field. Measurement of the Experimental area approximate 50 m × 150 m. This size of land is to reduce overlapping olfactory.

### *Experimental animals*

Rabbit has been and continues to be used in laboratory works such as in toxicological and forensic sciences (Goff *et al.*, 1989; 1991); production of antibodies for vaccines and research for human male reproductive system toxicology (Jodie *et al.*, 2006). Sixteen healthy rabbits with mean weight of 2.14±0.18 kg were bought from Ogbuwangue market, along Nigerian Port Authority (N.P.A) express road, Warri in Warri South Local Government Area of Delta State.

### *The killing method and the experimental layout*

Sixteen rabbits used for the experiment were divided into four groups. Each group had four rabbits each. Four rabbits in in the Group 1 each received 3 ml cypermethrin intravenously. Group 2 four rabbits each received 6 ml while Group 3 four rabbits were injected with 12 ml each. These dosages are similar to those normally witnessed in suicidal and abuse cases involving cypermethrin pyrethroid pesticide. Group 4 four rabbits were used as control. No cypermethrin was injected. Injected rabbits died after 18-24 h, while the control group was killed by cervical dislocation. Rabbit carrions were labeled accordingly, placed in thrash bags and carried from the killing centre to the study site. The rabbit carrions were guarded against vertebrate scavengers with wire mesh that permits entrance of all range of insects and other arthropods. The wire mesh was used to form cylindrical cages of height and width of 30 x 20 cm and supported by iron rods. Inter carrion distance of at least 20 m was maintained to minimize interruption of flies from adjacent colonies.

### *Arthropod sampling and data collection*

Sampling of *Chrysomya albiceps* for the entomotoxicological studies was carried out in accordance with the morphological aspects of the larval instars of *Chrysomyaalbiceps* as described by Queiroz*et al*., (1997), Abdalla and Mohamed (2014). Second instar larvae of the *C. albiceps* were collected from each decaying group of carrions and the larvae from each carrion were bred in the transparent plastic containers with 15 cm depth and width diameter of 11.5 cm at 28.0±3.0°C. Each breeding container was a combination of saw-dust and portion of the decaying carrion-remains to feed the immature insects. Muslin cloth was use to cover the container with rubber bands to permit ventilation and to hinder the escape of the insects. The second instar larvae were reared till adult stage. Developmental period of all the stages of the insects were recorded.

### *Measurement of larval body length and weight*

Measurement of larvae body length and weight was carried out at regular intervals of 6-12 h. Five larvae were randomly sampled from each carrion group in the rearing unit and demobilized in the boiled water according to the method of Adams and Hall (2003). The lengths and weights were measured and mean values recorded for each carrion group at each recording time. Mean values were used for statistical analysis. Portions of sampled larvae from each carrion in the rearing unit were allowed to complete their development cycle and this ensured time estimation for every of the insect stage in all carrion groups investigated. Times for pupation and adult emergence were recorded for each carrion group. This was followed by group mean developmental period. Mettle Toledo electrical weighing balance with sensitivity (of readability) of 0.001 g -1 g was used to measure the weight of the larvae while the length of the fly larvae from the second instar stage were obtained by a pair of compass and read on a transparent meter rule.

## Results and Discussion

### Effects of varying volume of Cypermethrin on the body mass index of *C. albiceps*larvae

The mean larval body length and mean larval body weight obtained are presented in Fig. 1 and 2, respectively; representing the carrions from varying cypermethrin volume (3, 6, and 12 ml) and the control group. The result showed that the body massindices (body length and weight)of the *C. albiceps* larvae is volume (dosage) dependent; as applied dosage increased from 3 to 12 ml, larval length and weight decreased; growing from 2 to 6.56 mm and 0.003 to 0.0074 g at 3 ml dose; 2 to 5.77 mm and 0.003 g to 0.0053 g for 6 ml; 2 to 3.95 mm and 0.003 to 0.0045 g at 12 ml doses of cypermethrin pesticide respectively, compared to the control group that grew from 2 to 11.33 mm and 0.003 to 0.0123 g at the same growth period of 110 hours. The body length decreased to 5.76 mm, 6.26 mm and 6.01 mm, respectively for 12 ml, 6 ml and 3 ml doses compared to control group that decreased to 07.76 mm at 144 h.

This research observed that the presence of cypermethrin at varying doses in the rabbit tissues resulted in the decrease in body indices (weight and length) of *C. albiceps* larvae contrary to increase in the same body indices in the control group. These results are at variance with the effect of cocaine and heroin which were respectively observed to have increased similar body mass indices on *Boettcherisca peregrine* (Diptera: Calliphoridae) (Goff *et al.*, 1989 and Goff *et al.*, 1991). This present work revealed that, the body mass indices (larval body length and weight) of the *C. albiceps* were affected by the cypermethrin pesticide and is dependent on the volume of the pesticide used. Larvae from the pesticide killed carrions were all smaller in length and body weight compared to the control group. This observation supports Ekrem *et al.* (2007) who opined that the sub–lethal doses of cypermethrin applied to *Achoria grisella* Fab. (Lepidoptera: Pyralidae) increased overall time to adult by more than 50% and the development time of the wasp increased in a dose dependent manner. Bourel *et al*. (1999) also noted that, the overall effects of morphine appear to be dose dependent as the larvae feeding on the rabbit that received the greatest dosages were the slowest to develop. Based on this study, they counsel that between the hours of 91 and 165 estimation of larval age based on the total length can be significantly be in error, if the presence of morphine in tissue is not considered. The error could be as much as 24 h for *Lucilia sericata* larvae measuring, from 8 to 14 mm total length.

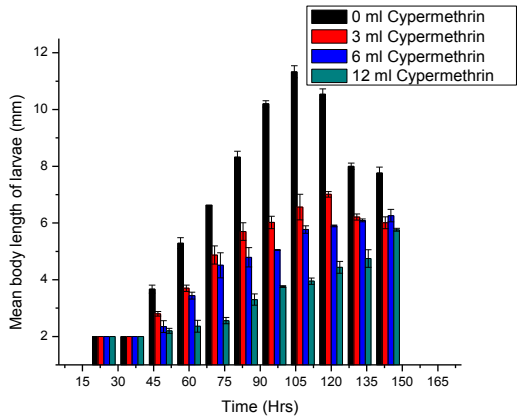


**Fig. 1:** Effects of different concentrations of Cypermethrin on the body length of the larvae of *C. albiceps*

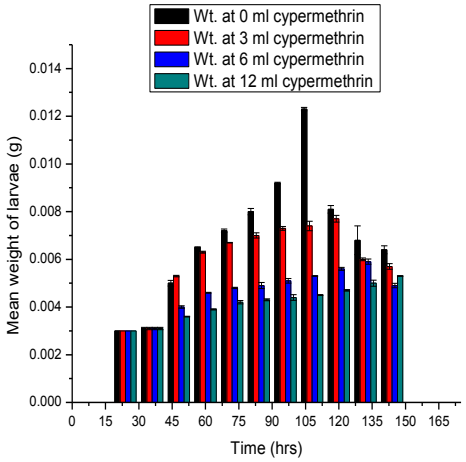


**Fig. 2:** Effects of different concentrations of Cypermethrin pyrethroid pesticide on the mean larval body weight of the *C. albiceps*

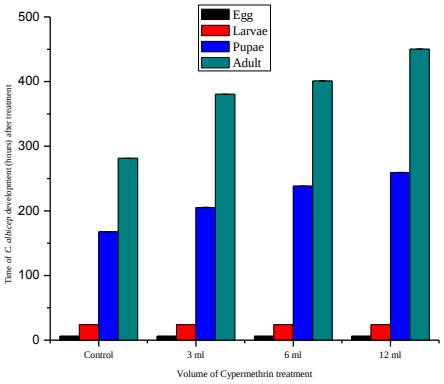


**Fig. 3:** Effects of Cypermethrin pyrethroid pesticide of different concentrations on the mean developmental period of *C. albiceps*

***Effects of varying doses of Cypermethrin on the developmental period of C. albiceps larvae***

The effects of different volume (12, 6, 3 and 0 ml) of the cypermethrin pyrethroid pesticide on the mean developmental period of *C. albiceps* are presented in the Fig. 3. On observationsof the development time from the eggs to larvae on the carrions, whereas the eggs and larvae were observed at the same period of 06.25 h and 24 h respectively across all the groups, the pupae and adult stages from the poisoned carrion groups recorded the longest period of emergence, and this was observed to be dose dependent. From the results, the pupae emerged at 259.41 h in the 12 ml group, 238.24 h in the 6 ml groups, and 205.20 h in the 3 ml group as compared to the control group whose pupae emerged at 167.50 h. Also on the emergence of the adult flies of the *C. albiceps*, the colony group killed with 12 ml of the pesticide emerged after 450.22 h (18.76 days), the 6 ml killed group emerged at 401.05 h (16.71 days) while 3 ml killed group emerged at 380.28 h (15.85 days) to compare to the control colony group (0 ml) that emerged at 281.24 h (11.42 days). This results in differences in emergence of 7, 5, and 4 days, respectively.

The outcome of the developmental period of the *C. albiceps* from the egg to adult under the same physical conditions revealed that the presence of the cypermethrin affected the developmental period and was dependent on the volume of the pesticides used. There were differences of 7.34 days in the 12 ml killed group, 5.29 days in the 6 ml pesticide killed group and 4.43 days in the 3 ml pesticide killed group when compared to the control group. This could possibly lead to PMI error of 7, 5, and 4 days, if any of the treated unit dosage was studied with the control alone. The implication of this result would be that, the estimation of Post Mortem Interval (PMI) without the full knowledge and consideration of the possible effects of toxins or drugs in a forensic entomology could lead to error, when insects' data are the only possible evidence in medicolegal investigation. This philosophy agrees with Goff and Lord (1994) who reviewed various studies in forensic entomology and concluded that, entomotoxicological testing was very important to the exact forensic entomology conclusion. Hence, data indicating the presence of drugs allow for corrections to the data in cases where drugs affect insects development.

**Conclusion**

The findings of this research revealed that cypermethrin ingestion retard the body mass indices and development period of *C. albiceps* larvae that colonize the carrions and could results in PMI error of 4 to 7 days depending on the volume of the cypermethrin used. It is important therefore, to be cautious in applying insect data as tenable evidence in medicolegal investigation.

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