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EFFECT OF KILLING METHODS AND PRESERVATIVE SOLUTIONS ON THE LARVAL BODY LENGTH OF *CHRYSOMYA MARGINALIS* (DIPTERA: CALLIPHORIDAE)

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Abstract

Estimation of the minimum post mortem interval (PMI) is the essential role of forensic entomology. The accurate determination of this interval depends on the correct larval identification and accurate estimation of larval age. One method of larval age estimation is the larval length measurement. Killing methods and the type of preservative solutions can greatly affect the larval length and therefore reduce the accuracy of PMI estimation. This study was conducted to determine which killing method and preservative can preserve the best larval length of *Chrysomya marginalis*.

Third instar *C. marginalis* larvae were either placed live in the preservatives, 10% formalin, 70% ethanol, XAA, Kahle´s solution and Pample´s fluid, or killed by boiling water (BWK) before being placed in the same preservatives. The larval length was recorded after day 1, 3, 5, 7 and 15 and for BWK larvae only; the length was recorded immediately after killing the larvae and before being placed in the preservatives.

The larval length of *C. marginalis* was significantly affected by killing method (live or BWK) and the type of the preservative used. The mean length of the larvae placed live in the preservatives decreases significantly than those killed by boiling water before being placed in the preservatives.It was observed that killing larvae by boiling water then preserved in 10% formalin and Kahle´s solution is the best method for preserving the larval length, but 10% formalin is not recommended if DNA recovery is desired.

Keywords: Blowflies, Forensic entomology, *C. marginalis*, Larval length, Killing and preservation methods, PMI.

Introduction

The estimation of the time since death which is known as post mortem interval is the major aim of forensic entomology. In criminal investigation, it was found that the estimation of PMI by entomologists could be more accurate than autopsy (Kashyap and Pillay, 1989). PMI can be estimated based on the age of the oldest larvae collected from the corpse. Erzinclioglu (1986) assured that at a crime scene, the samples collected by entomologists can give more accurate information than those collected by non-entomologists. The initial methods of collection and preservation of larvae can lead to errors in PMI estimation (O'Flynn, 1983; Greenberg and Kunich, 2002). Different methods of killing and preserving larvae had been reviewed. Lord and Rodriguez (1989) Anderson (1995) Benecke (1998) Benecke and Lessig, 2001; Sukontason *et al*, 2001) used ethanol as a preservative but they used different concentrations. Despite the hot water has been recommended as a killing agent, it is not always used (Hall *et al*, 1986; Anderson, 1995; Wells and LaMotte, 1995). Wells and Kurahashi (1994) used boiled 70% ethanol in the killing process of the larvae and used the same solution for preservation. Rodriguez and Bass (1983) preserved the collected insects without killing in a solution containing 10 ml 40% formalin, 85ml of 90% ethanol, and 5ml glycerin. Wells and LaMotte (1995) used Kahle's solution for preserving larvae (30ml 95% ethanol, 12ml formalhyde, 4ml glacial acetic acid and 60ml water). Adams and Hall (2003) used boiling water for killing blowfly larvae and 80% ethanol for presservation. Day and Wallman (2008) compared

the effect of 100% ethanol, 10% formalin and Kahle's solution on *Calliphora augur* and *Lucilia sericata* larvae (Diptera: Calliphoridae). They concluded that both Kahle's solution and 10% formalin were the best preservatives for the larval length, but they excluded 10 % formalin if DNA was wanted to be recovered.

Accordingly, this study aimed to evaluate influence of killing methods and different preservatives on length of *Chrysomya marginalis* larvae.

Materials and methods

Colony of *C. marginalis* was established from flies initially collected during June & July 2017 from El-Mansuryia, Giza Governorate and Dayrout, Egypt. Adults were kept in rearing cages (35×35×35cm) under laboratory conditions where the mean ambient temperature of 28±2ºC and relative humidity 50-60%. Adults were supplied with sugar and water and offered fresh beef meat as a protein source and egg deposition medium. Each deposited egg batch was transferred to a plastic jar (10.5×7cm) contained fresh beef meat. Newly hatching larvae were transferred to new jar containing fresh meat, covered with muslin and fastened with rubber band. Small number of larvae was kept in each jar to avoid the intraspecific competetion that might stunt growth, and the effects of maggot-generated heat to stimulate growth (Goodbrod and Goff, 1990). Proper ventilation of jars was considered to prevent death of the larvae.

The post feeding third instar larvae were collected and divided into 2 groups. The first one was placed live in the preservative solutions, while the other group was killed by boiling water before being placed in the preservatives. The preservative solutions were 10% formalin, 70% ethanol, XAA solution, Kahle´s solution and Pample´s fluid (Tab. 1). The larval length for both groups was recorded after 1, 3, 5,7and 15 days and for the 2nd group only, the length was measured immediately after killing and before placing in the preservatives.

The length measurement was taken by using a ruler. Each larva was put parallel to ruler with its anterior end at 0-point of the scale.In general, data obtained during the present study were statistically tested and mean value ± standard error (Mean ±SE) was calculated. Computer program SPSS (Statistical Package for Social Sciences) software (Version 14.0 for windows, SPSS Inc.) was used for this purpose.

Table 1: Chemical composition of the tested preservatives.

Preservative	Chemical composition
XAA	4 part xylene: 6 parts isopropanol: 5 parts glacial acetic acid
Kahle's	12 parts formaldhyde: 4 parts glacial acetic acid: 60 parts water: 30 part 95% ethanol
Pample's	6 parts 34% formalin: 2 part glacial acetic acid: 30 parts water: 15 part 95% ethanol

Results

The killing method (live or BWK) significantly affected the larval length of *C. marginalis* (Tabs. 2 & 3). There was a highly significant decrease ($P < 0.001$) in the mean length of the larvae placed live in the preservatives (16.58±0.24mm) than those killed by boiling water before being placed in the preservatives (18.61±0.18mm). Also, the preservative type and the period had a great effect on the larval body length.

After placing the larvae live in the presservatives and comparing the larval length after day 1, 3, 5, 7 & 15, 10% formalin revealed a non-significant difference in larval length ($P > 0.05$). But, 70% ethanol showed a non-significant decrease from day 1 (16.26± 0.23) to day 3 (15.78±0.25) which still will be constant during the next tested days ($P > 0.05$). A significant decrease from the day 1 (18.37±0.19mm) till day 3 (17.67±0.19mm), ($P < 0.05$), and a highly significant decrease after day 5, 7 & 15 (16.90±0.17mm) ($P < 0.001$) was recorded when larvae were preserved live in XAA solution.

Kahle´s solution showed a non-significant increase from day 1 (16.05±0.25mm.) to day 3, 5 & 7 (16.40±0.21, 16.52±0.20 & 16.53±

0.20 mm, respectively) (P>0.05) and then a highly significant increase (17.01±0.19mm) after day 15 (P<0.001), Pample´s fluid caused a highly significant increased from day 1 to last preservation days (P<0.001).

After killing larvae in boiling water and placing them in preservatives, both 10% formalin & Kahle´s solution showed a non-significant difference between length measured immediately after BWK (17.66±0.14 &17.80±0.17mm, respectively) and day 1, 3, 5, 7 & 15 (17.66±0.14, 17.66±0.14, 17.56± 0.13, 17.56±0.13 & 17.56±0.13mm, respectively for formalin10%) and (18.00±0.18 mm. for Kahle´s) (P > 0.05).

When 70% ethanol was used, the mean length recorded immediately after BWK (17.35±0.22mm) was highly significantly small than those recorded for the next days: day 1 (19.30±0.23mm), and constant during next days in this preservative (P < 0.001).

Application of XAA solution and Pample´s fluid showed a significant increase (P < 0.001& P < 0.05) in larval length. The length measured immediately after BWK was very significantly smaller (18.05±0.13 & 17.72±0.15mm, respectively) than on subsequent days (19.05±0.12 & 18.26±0.13mm, respectively).

Table 2: Effect of different preservatives when larvae of *Ch. Marginalis* placed live in preservative.

Preservative	Mean of larval length (mm) / Day				
	1	3	5	7	15
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
10 % Formalin	15.80± 0.77	15.80± 0.77	15.80± 0.77	15.80 ± 0.77	15.80 ± 0.77
70 % Ethanol	16.26± 0.23	15.78± 0.25	15.78± 0.25	15.78 ± 0.25	15.78 ± 0.25
XAA	18.37± 0.19	17.67± 0.19	16.90± 0.17	16.90 ± 0.17	16.90 ± 0.17
Kahle´s	16.05± 0.25	16.40± 0.21	16.52± 0.20	16.53 ± 0.20	17.01 ± 0.19
Pample´s	16.05± 0.22	17.00± 0.18	17.00± 0.18	17.00 ± 0.18	17. 53 ± 0.15

Table 3: Effect of different preservatives when larvae of *Ch. Marginalis* killed in water and put in preservative.

Preservative	Mean of larval length (mm) / Day					
	Immediately after BWK	1	3	5	7	15
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ±SE
10% Formalin	17.66±0.14	17.66±0.14	17.66± 0.14	17.56± 0.13	17.56±0.13	17.56 ±0.13
70 % Ethanol	17.35±0.22	19.30± 0.23	19.30± 0.23	19.30± 0.23	19.30± 0.23	19.30± 0.23
XAA	18.05±0.13	19.05± 0.12	19.05± 0.12	19.05± 0.12	19.05± 0.12	19.05± 0.12
Kahle´s	17.80± 0.17	18.00± 0.18	18.00± 0.18	18.00± 0.18	18.00± 0.18	18.00± 0.18
Pample´s	17.72±0.15	18.26± 0.13	18.26± 0.13	18.6 ± 0.13	18.26± 0.13	18.26± 0.13

Discussion

The larval length of *Ch. marginalis* was significantly affected by killing method and the type of the preservative used. The mean length of the larvae placed live in the preservatives decreases significantly than those killed by boiling water before being placed in the preservatives. No significant difference was observed in the larval length when the larvae were placed live in 10% formalin or killed by boiling water before preserved in 10% formalin and Kahle´s solution. This agreed with Day and Wallman (2008) who applied the same preservatives on the larvae of *C. augur* and *L. sericata*. They concluded that Kahle's solution and formalin10% were the best preservatives for the larval length. There was non-significant decrease in larval length put alive in 70%, but a significant increase when they were killed by boiling water before placed in 70% ethanol, XAA solution and Pample´s fluid, revealed a significant decrease in length when the larvae were placed live in this preservative. This some-how agreed with Tantawi and Greenberg (1993) who found that the larvae of different species shrink when placed live in preservatives. Adams and Hall (2003) observed that larvae of *L. sericata* became shorter when placed live in 80% ethanol, whereas *Ch. vomitoria* larvae showed expansion when put in same preservative after hot water killing. Amendt *et al.* (2007) reported shrinkage in larval length when were exposed live to ethanol. Richards *et al.* (2013) showed that the size of *C.*

vicina 3rd instar larvae increased during storage. But, some observations do not agree with Tantawi and Greenberg (1993) who found that *C. vicina* larvae length killed by boiling water neither changes in measure nor after five days storage in 70% ethanol.

Rosilawati *et al*. (2014) stated that killing larvae in hot water (80 ºC or 90 ºC) reduced the length and protected their morphological appearance. Açıkgöz and Açıkgöz (2018) found that killing *L. sericata* larvae by hot water well preserved their length.

Recommendations

1- Killing *Ch. marginalis* larvae in boiling water for about 30 seconds before storage in preservative, made the body rigid and fully extended.

2- Using Kahle's solution as preservation was the best for the larval length.

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