EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
ENTOMOLOGY

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ISSN
1687-8809

WWW.EAJBS.EG.NET

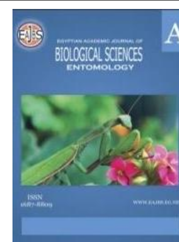
Vol. 18 No. 1 (2025)

Citation: *Egypt. Acad. J. Biolog. Sci. (A. Entomology) Vol. 18(1) pp.101-110 (2025)*
DOI: **10.21608/EAJBSA.2025.413369**

Egypt. Acad. J. Biolog. Sci., 18(1):101-110(2025)

Egyptian Academic Journal of Biological Sciences
A. Entomology

ISSN 1687- 8809
http://eajbsa.journals.ekb.eg/



Influence of Killing Methods and Preservative Solutions on Some Morphological Aspects of *Lucilia sericata* Maggots (Diptera: Calliphoridae)

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ARTICLE INFO
Article History
Received:21/1/2025
Accepted:21/2/2025
Available:25/2/2025

Keywords:
Boiling water killing (BWK), Hot water killing (HWK), Kahle's solution, Larval length, Coloration, Turgidity, Preservatives.

ABSTRACT

A key factor in forensic entomology is to determine the age of the maggots found on a body. This is achieved mainly by measuring their length and using established growth models. However, for accurate measurements, the way maggots are collected, killed, and preserved needs to be consistent. This study examines how different killing methods and preservatives affect the length, coloration, and turgidity of *Lucilia sericata* larvae. A colony of *L. sericata* was reared under controlled laboratory conditions and larvae were subjected to various killing methods: live preservation, hot water killing (HWK), and boiling water killing (BWK) before immersion in five different preservatives. Larval lengths were measured over a 15-day period; color changes and turgidity were also observed. Killing methods significantly influenced larval length, with HWK and BWK yielding longer larval length compared to live preservation. The study also found that certain preservatives, particularly 10% formalin and Kahle's solution, maintained larval length effectively, while others caused significant increases over time. Coloration changes were noted based on preservation techniques and turgidity assessments indicated varying physical states among groups. These findings underscore the importance of standardized methods in forensic entomology to enhance the reliability of minimum post mortem interval (PMImin) estimations and contribute to legal investigations.

INTRODUCTION

Forensic entomology mainly aims to estimate the period since death or minimum post-mortem interval (PMImin) (Greenberg and Kunich, 2002; Amendt *et al.,* 2011; Siva Prasad and Aneesh, 2022). In a criminal inquiry, entomologists' estimates of PMImin were found to be potentially more precise than autopsies (Kashyap and Pillay, 1989). The assessment of PMImin relies upon a number of generally accepted assumptions; nevertheless, deviation from any of them may distort the estimate's accuracy (Catts, 1991; Matuszewski, 2021). One of these assumptions is the estimation of the age based on the accumulated degree hours (ADH) of the oldest maggots collected from the carcasses (Sharma *et al.,* 2015). The ADH linear growth model can be used to estimate the PMImin using different approaches, such as killing the collected larvae, measuring their size, and then utilizing the growth curves to calculate the total ADH required for the larvae to reach the largest size that was observed on the corpse. The length or weight of the larvae can be

used to express the larval size. Accordingly, an accurate measurement of larval length is required to calculate PMImin precisely (Adams and Hall, 2003).

Collection techniques, killing methods, and preservatives used during the larval collection can all affect the accurate PMImin estimation (Greenberge and Kunich, 2002; Bambaradeniya *et al.,* 2023). Many authors highlight how different preservatives and methods impact the length of maggots from various species. Day and Wallman (2008) studied *Calliphora augur* and *Lucilia cuprina*, finding that 10% formalin and Kahle's solution were most effective for preserving larval length, although formalin should be avoided for DNA recovery. Açıkgöz and Açıkgöz (2018) suggested that killing *Lucilia sericata* larvae in hot water preserved their length better than ethanol or cold vinegar, recommending subsequent storage in 96% ethyl alcohol. Matthes *et al.,* (2021) investigated ethanol concentrations for *L. sericata* larvae killed in hot water, ultimately advising storage in at least 90% ethanol. Overall, responses to preservatives vary by species. Accordingly, this study specifically investigated the impact of employing hot and boiling water before preservation or directly introducing *L. sericata* post-feeding third instar larvae into preservatives. Additionally, the research examined the morphological changes exhibited by most prevalent and dominant fly species colonizing carcasses in El-Sharkia Province, *L. sericata*, larvae when preserved using five commonly used types of preservatives.

MATERIALS AND METHODS

Study Specimens:

A colony of *L. sericata* was created initially from flies gathered from rabbit carcasses maintained in front of the window of the Entomology Laboratory, Faculty of Science. Flies were identified using the morphological key of calliphorid adults (Lutz *et al.,* 2018) and confirmed by taxonomists of the Entomology Department, Faculty of Science, Ain Shams University. Rearing cages (35*35*35cm) were used to keep the collected flies under laboratory conditions at 27 ± 2 ºC and 60-65 % relative humidity and reared until the second generation (F2). Adults were provided with sugar, water, and fresh meat as an oviposition medium. Eggs were reared until the 3rd instar larvae roamed away from the meat. The post-feeding larvae were collected and categorized into three groups. In the 1stgroup, the larvae were immersed alive in the preservative without killing in hot/boiling water. The second group contained the larvae that were killed with hot –none boiling- water (~ 80 º C) (HWK) for 30sec. before immersion in the preservatives. The last group was treated with boiling water (~ 100 ºC) (BWK) for 30 sec. before preservation. Each group included 50 larvae divided into 5 subgroups(replicates), each contained 10 larvae and treated with 10 % formalin, 80 % ethanol, Kahle´s solution (30 parts 95% ethanol: 12 parts formaldehyde: 4 parts glacial acetic acid: 60 parts water), Pample´s fluid (5 parts 95% ethanol: 6 parts 34% formalin: 2 parts glacial acetic acid: 30 parts water) and acetic alcohol solution (1-part glacial acetic acid: 3 parts 70% ethanol) (Adams and Hall, 2003). Adults from the identical generation (F2) were utilized for up to three cycles (F5) to generate three sets of post-feeding third instar larvae for replication.

Larval Length Measurements:

The length of each larva was measured using a ruler, positioning it parallel to the ruler with the anterior end at the 0-point of the ruler scale (Fig. 1). The measurements were taken after storage at 1, 3, 5, 7, and 15 days in each preservative. In the case of using hot and boiling water killing, the measurement of the larval length was carried out immediately after death and before placing in the preservatives.

Fig.1. Measuring tool (ruler) of larval length.

Measurement of Discoloration and Turgidity:

Discoloration of the larval body from the normal creamy colour to any yellow, brown, or even black colour was observed by the naked eye after five days of preservation, while the turgidity of the body was tested by touching the larval body with fingers. The turgidity of larvae was classified into 4 classes; the first indicated the empty larval body texture, the second was expressed as soft body appearance, the third indicated the medium appearance, and the fourth indicated the rigid body appearance.

Statistical Analysis:

The data's normality was evaluated using the Shapiro-Wilk test prior to performing statistical analysis. A one-way analysis of variance (ANOVA) was applied to determine significant differences in the larval length across different preservatives and the mean value ± standard error (Mean ± SE) was computed. Post hoc comparisons were subsequently carried out using the least significant difference (LSD) test, with significance set at $P < 0.05$ to assess the impact of all preservatives on the length of larvae. SPSS (V. 14.00 for Windows; SPSS Inc.) was employed to perform the statistical analysis.

RESULTS

The method of killing, whether live or through killing in hot or boiling water (HWK or BWK), had a substantial impact on the length of *L. sericata* larvae, as shown in Table 1. A significant decrease ($P < 0.05$) in the average length of larvae preserved alive (11.66±0.31mm) was noticed compared to those subjected to hot or boiling water immediately after killing and prior to preservation (12.85±0.09 and 12.91±0.07mm, respectively). Additionally, both the type of preservative and the duration of preservation exerted an important influence on the length of the larval bodies. Following the placement of alive larvae in the preservatives and the examination of their lengths on days 1, 3, 5, 7, and 15, no statistically significant differences were found in larval length with 10% formalin and 80% ethanol ($P > 0.05$). Kahle's solution illustrated a non-significant rise between days 1 and 3 and from day 3 to 15. However, a significant increase was recorded between day 1 and the rest of the days ($P=0.014$, $df=5$, $F=3.167$). In the case of Pample's fluids and acetic acid solutions, a significant increase was observed between day 1 and the subsequent days.

After killing larvae with hot or boiling water before storing in preservatives, 10% formalin after HWK or BWK and Kahle's solution after BWK showed a non-significant difference in the larval length among all days of preservation ($P > 0.05$). Storing larvae in 80% ethanol, Pample's fluid, and Kahle's solution after HWK illustrated a non-significant increase after day 1 immersion ($P > 0.05$), but a significant increase was recorded from day 3 to the subsequent days in comparison to day 1. BWK before using 80% ethanol led to a significant increase from immediately killing and after day 1 ($P = 0.014$, $df=5$, $F=3.154$) and

a considerably high significant increase from day 1 to the subsequent days (P < 0.01).

Preserving larvae in Pample's fluid after BWK resulted in a significant increase in larval length compared to that of immediately killing and to the rest of the days. However, a non-significant difference was recorded on days 3, 5, and 7. The use of acetic alcohol solution after HWK and BWK gave a significant (P < 0.05, df=5, F=9.435) and a highly significant rise (P< 0.001, df=5, F=12.044), respectively, from day 1 to all subsequent days.

Table 1: Effect of the tested preservatives on *Lucilia sericata* larval length after placing alive, after killing by hot (HWK) and boiling water (BWK) before storing in these preservatives

Killing method	Preservatives	Mean of larval length (mm) / Day Mean ± SE						Effect of days
		Immediately after killing	1	3	5	7	15	
Alive	10 % Formalin	------	10.72 ± 0.15a	10.78 ± 0.13a	10.80 ± 0.16a	10.84 ± 0.16a	10.98 ± 0.21a	*F*=0.357, P=0.876
	80 % Ethanol	------	12.47 ± 0.19b	12.60 ± 0.14bc	12.89 ± 0.13b	12.82 ± 0.19b	12.69 ± 0.24b	*F*=0.920, P=0.475
	Kahle´s	-------	11.34 ± 0.17c*	11.79 ± 0.12b	11.91 ± 0.15c*	11.96 ± 0.12c*	11.97 ± 0.12c*	*F*=3.167, P=0.014
	Pample´s	-------	11.53 ± 0.12c*	11.98 ± 0.17b*	12.27 ± 0.18c*	12.50 ± 0.17c*	12.50 ± 0.12c*	*F*=8.903, P=0.000
	Acetic alcohol	--------	12.22 ± 0.17b*	12.87 ± 0.17c*	13.37 ± 0.17b*	13.59 ± 0.20bd*	14.20 ± 0.24d*	*F*=8.588, P=0.000
Hot water	10 % Formalin	12.74 ± 0.18bc	12.77 ± 0.18be	12.78 ± 0.23c	12.79 ± 0.22b	12.79 ± 0.24b	12.79 ± 0.24b	*F*=0.029, P=1.000
	80 % Ethanol	12.70 ± 0.21c	13.02 ± 0.28bd*	13.94 ± 0.25e*	14.04 ± 0.18de*	14.08 ± 0.19d*	14.16 ± 0.16e*	*F*=8.156, P=0.000
	Kahle´s	13.17 ± 0.22a	13.27 ± 0.25df*	14.07 ± 0.22e*	14.07 ± 0.20de*	14.09 ± 0.16d*	14.09 ± 0.16ef*	*F*=4.688, P=0.001
	Pample´s	12.71 ± 0.20ab	13.16 ± 0.16bd*	14.10 ± 017e*	14. 12 ± 017de*	13.91 ± 0.21d*	13.91 ± 0.21de*	*F*=9.276, P=0.000
	Acetic alcohol	12.95 ± 0.19a*	13.65 ± 0.26efg*	14.23 ± 0.19ed*	14.40 ± 0.17df*	14.51 ± 0.18d*	14.54 ± 0.18de*	*F*=9.435, P=0.000
Boiling water	10 % Formalin	12.92 ± 0.09abc	13.02 ± 0.09d	13.03 ± 0.13c	13.03 ± 0.12be	13.00 ± 0.10d	13.00 ± 0.98df	*F*=0.146, P=0.980
	80 % Ethanol	13.06 ± 0.14a*	13.63 ± 0.12e*	13.80 ± 0.17ed	13.80 ± 0.17df	13.82 ± 0.19de	13.85 ± 0.17eg	*F*=3.154, P=0.014
	Kahle´s	13.04 ± 0.18abc	13.55 ± 0.27de	13.60 ± 0.26ce	13.60 ± 0.13df	13.60 ± 0.25d	13.60 ± 0.24de	*F*=0.639, P=0.671
	Pample´s	12.72 ± 0.12abc	13.20 ± 0.18dg*	13.70 ± 0.14ce*	13.78 ± 0.15e*	13.94 ± 0.18d*	13.94 ± 0.18de*	*F*=8.478, P=0.000
	Acetic alcohol	12.81 ± 0.1abc*	14.19 ± 0.20 e*	14.74 ± 0.30d	14.83 ± 0.27 f	14.99 ± 0.2e*	15.20 ± 0.24g*	*F*=12.044, P=0.000
Effect of preservatives			*F*= 20.775, P<0.001	*F*= 28.194, P<0.001	*F*= 32.545, P<0.001	*F*= 26.753, P<0.001	*F*= 27.604, P<0.001	

* Significance in each row in comparison to day 1
In the same column, means followed with different letters are significantly different (P<0.05), based on LSD test.

Coloration and turgidity of *L. sericata* maggots were found to be influenced by both preservative types and the method of killing (Figs. 2, 3 & 4). The creamy color of the maggots was found to be changed to pale brown, shiny brown, and black when they were preserved alive in (Kahle's, Pample's, and acetic alcohol), 10% formalin, and 80% ethanol, respectively (Fig. 2A, B, C, D & E).

The hot water used for killing larvae before preservation approximately maintained the color of the larval bodied except for a slight change to a yellowish color in the case of 10% formalin (Fig. 3 A) and faint brown after preserving in 80% ethanol (Fig. 3 B). Additionally, preserving the larvae after BWK kept the larval body creamy color, but a faint yellowish color was observed in the case of using Pample's fluid (Fig. 4 D)

Turgidity of the larval body was measured by touching them with fingers, and it was observed that the empty body appearance was recorded in the case of preserving larvae in 10% formalin after BWK; a soft appearance was observed when larvae preserved alive in Pample's fluid and acetic alcohol solution. Median body appearance was found in the case of preserving larvae alive in 10% formalin, 80% ethanol, Kahle's solution, and in 80% ethanol and acetic alcohol solution after BWK. All larvae stored in all tested preservatives after HWK and those preserved in Kahle's solution and Pample's fluid after BWK showed a rigid body appearance.

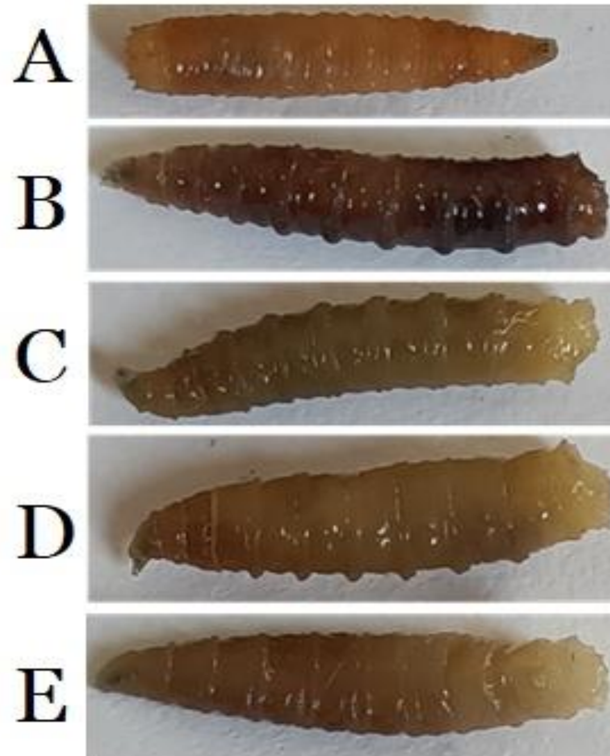

Fig.2. Preserved *L. sericata* larvae alive after 5 days in A: 10% formalin, B: 80% ethanol, C: Kahle's solution, D: Pample's fluid, and E: acetic alcohol solution.

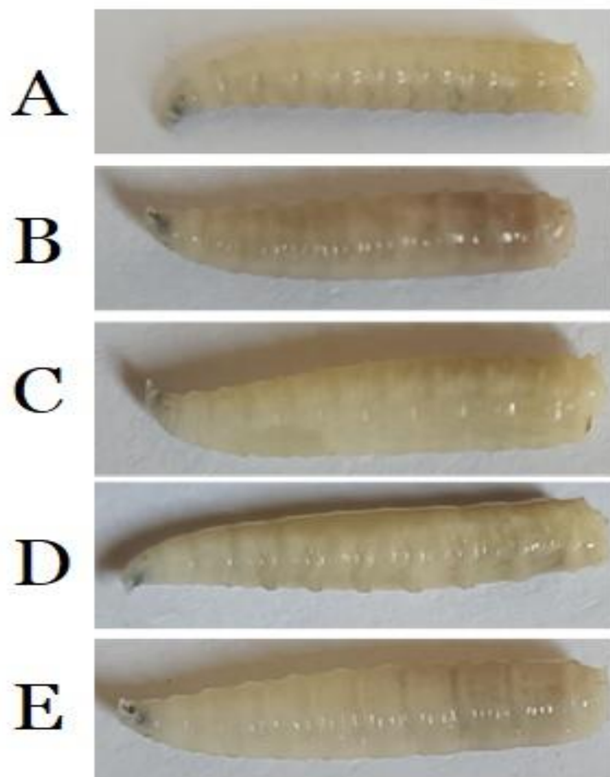

Fig.3. Preserved *L. sericata* larvae after killing in hot water for 5 days in A: 10% formalin, B: 80% ethanol, C: Kahle's solution, D: Pample's fluid, and E: acetic alcohol solution.

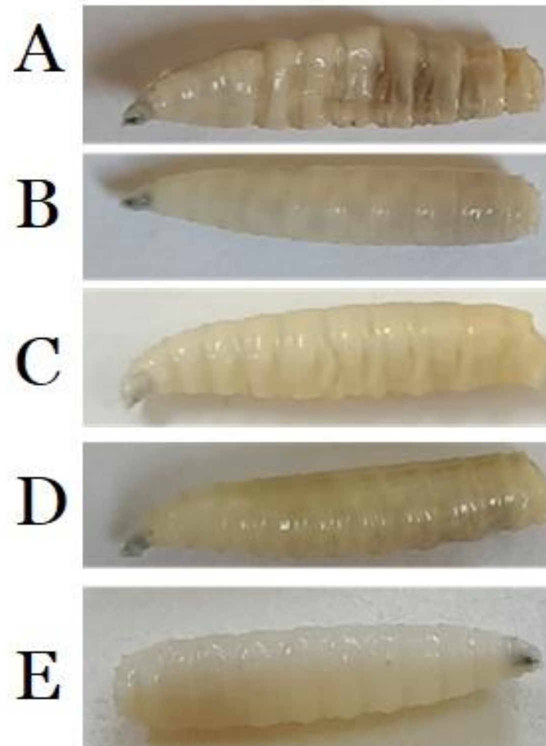

Fig.4. Preserved *L. sericata* larvae after boiling water killing after 5 days in A: 10% formalin, B: 80% ethanol, C: Kahle's solution, D: Pample's fluid, and E: acetic alcohol solution.

DISCUSSION

The preservation of maggots serves the purpose of halting their growth, enabling identification, and facilitating the measurement of body length for estimating the minimum post-mortem interval (PMImin). Maggot body length proves to be more effective in determining PMImin compared to body weight and width, as indicated by Richards *et al.* (2013).

Our findings revealed that maggots exhibited shrinkage with direct preservation in all tested preservatives compared with those killed previously in hot or boiling water. This observation aligns with the studies of many authors who observed that larvae immersed in preservatives underwent shrinkage attributed to dehydration (Tantawi and Greenberg 1993; Adams and Hall 2003; Niederegger 2021). The internal fluids were expelled, moving into areas of higher solute concentration, leading to the observed shrinkage. HWK of *L. sericata* larvae near boiling point (80ºC) proved to be very effective method for fixation as observed in many blow fly maggots (Amendt *et al.,* 2007, Bugelli *et al.,* 2017, Matthes *et al.,* 2021, Bambaradeniya *et al.,* 2023), enabling accurate measurement of body length as well as identification of species through analysis of physical characteristics. This suggests that the immediate post-mortem physiological state of the larvae, as influenced by the killing method, can impact their subsequent preservation.

The type of preservative and the duration of preservation play a critical role in larval length. In this experiment, Kahle's solution after BWK, as well as 10% formalin after HWK and BWK, gave a non-significant difference in larval length, although10% formalin, when applied after BWK, led to the empty body appearance of the larvae. This result is in line with the study of Day and Wallman (2008), who concluded that the best preservatives for the larval length of both *Lucilia cuprina* and *Calliphora augur* were 10% formalin and Kahle's solution. The concentration of 10% formalin is commonly employed for preserving

substantial tissue specimens for microscopic examination in histopathology (Melissa *et al.,* 2006). Despite its effective preservation of maggots for a period of 6 months (Linville *et al.,* 2004), it is not recommended to utilize this solution for preservation as it was recognized to induce DNA degradation (Nakamura *et al.,* 1990).

Using 80% ethanol, Pample's fluid, and acetic alcohol solution after BWK resulted in a highly significant elongation in the larval length but using the same preservatives after HWK did not significantly increase the larval length. In this study, the only case that may recommend using BWK is to preserve the larvae in Kahle's solution after BWK. Accordingly, based on these observations, we recommended killing larvae in hot water (~ 80 ºC) before storing them in the preservatives. This finding agrees with Adams and Hall (2003), who recommended immersing larvae in hot water ≥ 80 ºC for 30 sec. minimum time for killing before preservation.

Several commonly utilized preservative solutions for storing organisms including insect samples have been shown to induce substantial morphological changes, hence causing potential repercussions on the outcomes of drug and DNA analyses if these organisms are employed as a source for molecular and toxicological analysis (Carter, 2003; Day and Wallman 2008; Byrd and Tomberlin, 2019). Our observations showed that, the coloration of larvae varied significantly based on the physiological state of larvae at the time of preservation and the preservation method. Larvae preserved alive in tested solutions changed from a creamy color to shades of brown or even black suggesting chemical reaction between those preservatives and larval tissues. HWK maintained a more consistent coloration of larvae indicating that thermal stress may mitigate some adverse effects of preservatives. Same observations were recorded by Matthes *et al.,* (2021) and Siva Prasad and Aneesh, (2022), who reported that biochemical responses to preservatives depend on whether the larvae are alive or dead at the time of preservation, impacting the forensic interpretation of color changes in relation to larval life stages.

Notably, killing maggots in hot water at 80ºC resulted in the least alterations in larval turgidity. When maggots were subsequently immersed in Pample's fluid and Kahle’s solution, their morphological characteristics were preserved better compared to those killed with boiling water or placed alive in all other preservatives. This was noticed by Rosilawati et al., (2014), who mentioned that Kahle’s solution proved more effective in preserving maggots of *Chrysomya megacephala* compared to 70% ethanol and 10% formalin. On the contrast, López-García *et al.* (2024) found that killing puparia of *Calliphora vicina* in boiling water for 30 sec., then preserving them in 80% ethanol revealed the highest preservation scores for the intra-puparial forms of this species. Turgidity may influence the assessment of larval conditions which in turn may impact PMImin estimation. For example, larvae that look empty or overly soft bodied might not accurately reflect their developmental stage or the environmental conditions post mortem (Rosilawati *et al.,* 2014; Matthes *et al.,* 2021).

Conclusions

The techniques employed in the killing and preservation of entomological samples have the potential to impact the accurate estimation of larval age; consequently, determining the minimum post-mortem interval (PMImin). Therefore, selecting an effective preservative solution and a universally recognized method for measuring larval length becomes crucial. We concluded that immersion of *L. sericata* larvae in hot water (~ 80 ºC) is the best killing method before storing them in preservatives as 10% formalin & Pample's fluid and led to minimal morphological changes for all preservatives. Also, using boiling water to kill *L. sericata* larvae before preserving them in Kahle's solution is recommended. Our findings highlight the necessity for further investigations to employ a standardized preservation technique to maintain the integrity of specimens for subsequent analysis.

Declarations:

Ethical Approval: Not applicable.

Authors Contributions: A.M.S and E.E.Z. conceived, designed, analyzed and wrote the paper. S.S.R analyzed and wrote the paper. The manuscript was read and approved by all authors.

Competing Interests: The authors declare that the research was carried out without any commercial or financial relationships that could be interpreted as a potential conflict of interest.

Availability of Data and Materials: All data generated or analyzed during this study are included in this manuscript.

Funding: Not applicable**.**

Acknowledgements: The authors would like to express their gratitude to their colleagues at Faculty of science, Zagazig University and Cairo University for their support and technical help.

REFERENCES

Adams, Z. J. O., Hall, M. J. R. (2003). Methods used for killing and preservation of blowfly larvae, and their effect on post-mortem larval length. *Forensic science international, 138*: 50- 61. https://doi.org/ 10.1016/j.forsciint.2003.08.010

Amendt, J., Campobasso, C. P., Gaudry, E., Reiter, C., LeBlanc, H. N., Hall, J. R. M. (2007). Best practice in forensic entomology Standards and guidelines. *International Journal of Legal Medicine, 121*(2):90–104. https://doi. org/10.1007/s00414-006-0086-x

Amendt, J., Richards, C. S., Campobasso, C. P., Zehner, R., Hall, M. J. R. (2011). Forensic entomology: Applications and limitations. *Forensic Science, Medicine, and Pathology*, *7*(4):379–392. https://doi.org/10.1007/ s12024-010-9209-2

Açıkgöz, H. N., Açıkgöz, A. (2018). Effect of killing liquids on the larval length of forensically important *Lucilia sericata*. *Adli.Tıp. Bülteni*, *23*:36-38.

Bambaradeniya, T. B., Magni, P. A., Dadour, I. R. (2023). A Summary of Concepts, Procedures and Techniques Used by Forensic Entomologists and Proxies. *Insects*, *14*(6): 536. https://doi.org/10.3390/insects14060536

Bugelli, V, Campobasso, C. P., Verhof, M. A., Amendt, J. (2017). Effects of different storage and measuring methods on larval length values for the blow flies (Diptera: Calliphoridae) *Lucilia sericata* and *Calliphora vicina*. *Science & Justice, 57*:159–164. https:// doi.org/10.1016/j.scijus.2016.10.008

Byrd, J. H., Tomberlin, J. (2019). Forensic Entomology: The Utility of Arthropods in Legal Investigations, 3rd ed.; *CRC Press*: Boca Raton, FL, USA; Taylor & Francis Group: Boca Raton, FL, USA, p. 620.

Carter, J. D. (2003). The effects of preservation and conservation treatments on the DNA of museum invertebrate fluid preserved collections. Master The Wales National Museum (Cardiff, UK), 120 pp.

Catts, E. P. (1991). Analyzing entomological data, in: Catts EP, Haskell NH (Eds.), Entomology and Death: A Procedural Guide. *Joyce's Print Shop Inc*., Clemson, pp. 124–137.

Day, D. M., Wallman, J. F. (2008). Effect of preservative solutions on preservation of *Calliphora augur* and *Lucilia cuprina* (Diptera: Calliphoridae) with implications for post- mortem interval estimates. *Forensic science international*, *179*:1-10.

Greenberg, B., Kunich, J. C. (2002). Entomology and the Law. Flies as Forensic Indicators. *Cambridge University Press*, Cambridge 306pp. https://doi.org/10. 1016/j. scijus.

2021.06.005

Kashyap, V. K., Pillay, V. V. (1989). Efficacy of entomological method in estimation of postmortem interval: a comparative analysis. *Forensic science international, 40*: 245-250.

Linville, J. J., Hayes, J., Jeffrey, D. W. (2004). Mitochondrial DNA and STR analyses of maggot crop contents: effects of specimen preservation technique. *Journal of Forensic Sci*ence. *49*: 2.

López-García, J., Piña, M. A., Clark, B., Hall, M. J. R., Martín-Vega, D. (2024). Methods for the optimal preservation of blow fly intra-puparial forms for morphological analysis in forensic casework. *International Journal of Legal Medicine* Vol.138 Pages 1769-1779 .https://doi: 10.1007/s00414-024-03172-9. Epub ahead of print. PMID: 38326653.

Lutz, L., Williams, K. A., Villet, M. H., Ekanem, M., Szpila, K. (2018). Species identification of adult African blowflies (Diptera: Calliphoridae) of forensic importance. *International Journal of Legal Medicine*, *132*: 831-842.

Matthes, K., Zehner, R., Amendt, J. (2021). Influence of storage on larval length and age determination of the forensically important blow fly *Lucilia sericata* (Diptera: Calliphoridae). *Science & Justice*, *61*: 579- 585.

Matuszewski, S. (2021) Post-Mortem Interval Estimation Based on Insect Evidence: Current Challenges. *Insects, 12*(4): 314. https://doi.org/10.3390%2Finsects1 2040314

Melissa, L. C, Carrie, L. S, Chandra, N. L., Zachary, S. S., Peter, J. K., Kanwar, N. M. K., Joseph, D. P., Robert, W. D. (2006). Assessment of fixatives, fixation, and tissue processing on morphology and RNA integrity. *Experimental and Molecular Pathology*, *80*: 183-191. https://doi.org/10.1016/j.yexmp.2005.10.002

Nakamura, T. Y., Satonaka, K., Maeda, S., Doi, K., Baba, S. (1990). Fundamental study on the mechanism of DNA degradation in tissues fixed in formaldehyde. *Journal of clinical pathology*, *43*: 748-751.

Niederegger, S. (2021). Technical Note: Effects of Makeshift Storage in Different Liquors on Larvae of the Blowflies *Calliphora vicina* and *Lucilia sericata* (Diptera: Calliphoridae). *Insects*, *12*(4):312. https://doi.org/10.3390/insects12040312

Richards, C. S., Rowlinson, C. C., Hall, M. J. R. (2013). Effects of storage temperature on the change in size of *Calliphora vicina* larvae during preservation in 80% ethanol. . *International Journal of Legal Medicine*, *127*: 231-241.

Rosilawati, R., Baharudin, O., Syamsa, R., Lee, H., Nazni, W. (2014). Effects of preservatives and killing methods on morphological features of a forensic fly *Chrysomya megacephala* (Fabricius, 1794) larva. *Tropical biomedicine, 31*:785-91.

Sharma, R., Garg, R. K., Gaur, J. R. (2015). Various methods for the estimation of the post mortem interval from Calliphoridae: a review. *Egyptian journal of forensic sciences, 5*: 1–12. https://doi.org/10.1016/j.ejfs.2013.04.002

Siva Prasad, M. S., Aneesh, E. M. (2022) Tools and techniques in forensic entomology- A critical review. *International journal of tropical insect science*, *42*: 2785-2794 https://doi.org/10.1007/s42690-022-00823-5

Tantawi, T. I., Greenberg, B. (1993). The effects of killing and preservation solutions on estimates of larval age in forensic cases. *Journal of forensic sciences, 38*: 702-707.

ARABIC SUMMARY

تأثير طرق القتل والمحاليل الحافظة على بعض الجوانب الشكلية ليرقات ذباب *ليوسيليا سيريكاتا* (ثنائية الأجنحة: كاليفوريدى)

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إن تحديد عمر اليرقات الموجودة على الجثة يعد أحد العوامل الرئيسية في علم الحشرات الجنائي. يتم تحقيق ذلك بشكل رئيسي من خلال قياس طول هذه اليرقات واستخدام نماذج النمو المعتمدة لكل نوع. ومع ذلك، للحصول على قياسات دقيقة، يجب أن تكون طريقة جمع اليرقات وقتلها وحفظها متسقة. تبحث هذه الدراسة في كيفية تأثير طرق القتل المختلفة ومواد الحفظ على طول اليرقات ولونها وصلابتها. تم تربية مستعمرة من ذباب لوسيليا سيريكاتا تحت ظروف معملية مضبوطة، وتم إخضاع اليرقات لطرق قتل مختلفة وهى: الحفظ الحي، القتل بالماء الساخن(HWK) ، والقتل بالماء المغلي (BWK) قبل غمرها في خمسة مواد حافظة مختلفة. تم قياس أطوال اليرقات على مدار فترة 15 يومًا، كما تم ملاحظة التغيرات في اللون وصلابة الجسم. أثرت طرق القتل بشكل كبير على طول اليرقات، حيث أسفرت طرق الغمر بالماء الساخن والماء المغلي قبل الحفظ عن يرقات أطول مقارنة بالحفظ الحي. كما وجدت الدراسة أن بعض المواد الحافظة، خاصة الفور مالين بنسبة 10% ومحلول كاهل (Kahle's solution) حافظت على طول اليرقات بشكل فعال، في حين أن مواد أخرى تسببت في زيادات ملحوظة بمرور الوقت. تم تسجيل تغيرات في اللون بناءً على تقنيات الحفظ، وأشارت تقييمات الصلابة إلى حالات جسدية متباينة بين المجموعات. تؤكد هذه النتائج على أهمية استخدام أساليب موحدة في علم الحشرات الجنائي لتحسين دقة تقدير الحد الأدنى للفترة الزمنية بعد الوفاة (PMImin) والمساهمة في التحقيقات القانونية.