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# Biological and biochemical studies on the veterinary important blowfly, *Chrysomya albiceps* (Diptera: Calliphoridae)

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**Abstract:** Blowflies have a double-edged relationship with animals. On one hand, blowfly maggots cause serious myasis in animals, On the other hand their ability to cause myasis may be used to cure chronic wounds in precious animals. Life cycle of *Chrysomya albiceps* (*Ch. albiceps*) was investigated under controlled conditions of 26 ± 2 °C, 55 ± 10% relative humidity (RH), and 12L:12D photoperiod, and the larvae were fed on beef meat. Whole body maggot extracts were prepared by different methods and their protein content and electrophoretic protein patterns were compared. It was found that the post-embryonic development of *Ch. albiceps* took about 8.97 days with a significantly higher females ratio ($P < 0.0001$) which also had a longer life span than males ($P = 0.0024$). Maggot extract prepared by snap freezing in liquid nitrogen yielded the highest protein content and its amino acids were separated. So, the extraction technique has a great effect on the protein content which is essential in the future uses of maggot extract. Further investigations on the bioactive components of maggot extract are recommended.

**Keywords:** *Chrysomya albiceps*, maggot extract, myasis, maggot therapy, proteins, amino acids

## Introduction:

Blowflies are known for their affinity to breed in decaying organic matter especially carrions **(Harvey, 2022)**. Carrions are more favorable for blowflies than living tissues, but under suitable conditions living animal tissues may be infested by blowflies larvae causing myiasis **(Sotiraki and Hall, 2012)**. Most of the calliphorid species may cause myiasis, such as *Lucilia sericata* and *Ch. albiceps* which are facultative parasites. Gravid female blowflies are attracted to lay eggs in the fouled fleece of animals **(Erzinçlioglu, 1996)**, open wounds, and natural body openings with smelly discharges . On hatching, larvae may feed on the superficial necrotic tissues or burrow into the skin feeding on the subcutaneous living tissues causing inflammation. Infested animals lose their appetite, suffer from blood deficiency,

become susceptible to secondary invasion by other blowflies species, and in case of severe injury animals die from septicemia and toxemia if left untreated **(Singh and Singh, 2016)**. Myiasis in diary animals leads to reduction in their productivity and even death causing great economic loss **(Rahman *et al.*, 2009)**.

The myiasis causing nature of blowflies maggots that is responsible for diseases and even death in animals has a therapeutic use in treatment of chronic and hard to heal wounds. The controlled application of maggots to chronic wounds has a fascinating effect on debridement, disinfection, and proliferation of these hopeless wounds in a process called maggot therapy **(Nigam and Wilson, 2022a)**.

Maggot therapy may be the last straw the veterinarians clutch to save animals, especially precious and rare ones, which are susceptible to dangerous injuries due to their wild nature. When the wound did not respond to regular medical and surgical therapy, maggot therapy introduced to stimulate debridement and control infection avoiding amputations and euthanasia **(Sherman *et al.*, 2013)**.

Due to their magical effect in wound healing, researchers paid attention to the components of secretions, excretions, and even the whole body of calliphorid maggots in order to understand the mechanism and bioactive elements responsible for wound healing by medicinal maggots. These investigations may enable the researchers to replace the living maggot therapy by maggot-derived drugs that will overcome the obstacles of living maggot therapy techniques by extending shelf-life, avoiding discomfort related to the feeding activity of living maggots, and reducing the dressing precautions **(Barnes *et al.*, 2010)**. These active components may also be synthesized and applied to wounds in an appropriate mechanism with no need to use live larvae **(Nigam and Wilson, 2022b)**

Digestion in blowflies larvae is extracorporeal. Once the larvae access the food substrate, the food is externally digested by larval secretions which were found to contain proteolytic enzymes, antimicrobial substances, deoxyribonuclease, and ammonia **(Brown *et al.*, 2012)**. About half of all natural anti-microbial peptides (AMPs) are obtained from insects **(Ratcliffe *et al.*, 2011)**. Several peptides with antimicrobial properties, including diptericin and defensins, have been isolated from calliphorid maggots **(Rahimi *et al.*, 2023)**. Amino acids isolated from maggots ES are involved in accelerating wound healing **(Bexfield *et al.*, 2010)**.

This study was the first step in studying life cycle of *Ch. albiceps*, a calliphorid fly, investigating the appropriate maggot extraction method, and separating proteins and amino acids present in the maggot extract.

## Materials and methods

### Insect collection and colonization

*Chrysomya albiceps* colony was established from a previously collected fly adults and larvae specimens from a rabbit cadaver, slaughtered and left exposed at the Entomology Laboratory, Zoology Department, Faculty of Science, Zagazig University. The collected specimens were reared at the laboratory to complete their life cycle according to **Byrd and Tomberlin (2019)**. From the collected species, adults of *Ch. albiceps* were taxonomically identified according to **Lutz *et al.* (2018)** and selected for our experiments.

Adults of *Ch. albiceps* were kept in wire mesh aerated wooden cages designed for rearing purposes and supplied with sugar crystals, water soaked cotton pieces, and beef meat as a protein source and oviposition medium and reared for one generation.

### Biological Studies

Biological experiments were conducted under controlled conditions of 26 ± 2 °C, relative humidity (RH) 55 ± 10% and a photoperiod of 12L:12D (Light: Dark) hours. Temperature and humidity were recorded from a laboratory set digital thermo/hygrometer twice a day. Beef meat was used as a protein source for adults, oviposition medium, and larval diet. To calculate adult emergence and sex ratio of *Ch. albiceps*, four groups each of 100 newly hatched larvae were reared separately on 100 gm of beef meat cubes. When the larvae entered the wandering stage, the larval rearing containers were placed uncovered in larger plastic containers with autoclaved sawdust (~ 5 cm deep) as a pupation medium and covered with lace fabric. After complete pupation, the pupae were sifted and transferred to the adult rearing cages. Number and sex of emerged adults of each group were recorded. Four groups each formed of a pair of newly emerged male and female were transferred separately to a rearing cage containing sugar crystals in Petri dish, water immersed cotton piece, and beef meat. Each group was observed at six hours interval and the time of first oviposition was recorded. Fecundity was determined by five groups each of a couple of recently eclosed male and female were kept separately in a rearing cage with sugar crystals, water saturated cotton piece, and beef meat. Each group was checked every six hours. The laid egg batch of each group was transferred by a wet fine brush to the center of previously wetted and sectioned filter paper and carefully detached to the paper sections and counted. Adult longevity was tracked in gregarious population of *Ch. albiceps*. Three groups each of 50 recently emerged adults were taken; the first group was only males, the second group was only females, and the third group was equally mixed males and females. Each group was maintained in a rearing cage and served with sugar crystals and water soaked cotton piece. The mortality of each group was recorded twice a day. To study the incubation period and hatchability, three groups each of a pair of newly emerged male and female were transferred separately to a rearing cage containing sugar crystals, water immersed cotton piece and beef meat as protein source and oviposition surface. Each group was observed at six hours intervals for oviposition. Once the egg batches were laid, the oviposition time was recorded and the three egg batches were transferred separately using a wet fine brush to a larval

rearing container with beef meat cube and covered with fabric lid. Each batch was examined every three hours to observe hatching. The time of hatching and the number of hatched larvae were recorded.

Three groups of 100 newly hatched larvae were transferred separately to the larval rearing containers on 100 gm beef meat cubes which were changed daily and covered with lace fabric. When the larvae reached the third instar, the larval rearing containers were placed uncovered in the pupation containers with sterilized sawdust (~ 5 cm deep) as a pupation substrate and covered with lace fabric lids till complete pupation. Time passed from egg hatching to complete pupation and the number of formed pupae were recorded. Three groups each of 100 wandering larvae were transferred separately to pupation containers with sterilized sawdust as a pupation substrate and covered with lace fabric lids. Each group was sifted hourly to detect the formation of white pupae. White pupae of each group were transferred to adult rearing cages. Time elapsed from the formation of white pupae to complete adult emergence was recorded.

**Biochemical studies**

**Preparation of maggots lysates**

The whole body late second/early third instar maggots lysate was intended to be prepared according to **Li *et al.* (2015)** with some modifications. So, the lysates were prepared by four different methods to compare the effect of different extraction methods on the total protein content and protein electrophoretic pattern of the lysate.

**The first method**

Maggots were collected from the larval food, washed three times with pure distilled water ($ddH_2O$) and one time with 70% ethanol, dried over filter paper, weighed to 20 gm in Falcon tube, and kept at -20 °C. Falcon tube containing maggots was brought out of the freezer and left to thaw on ice. After partial thawing, the maggots were ground with 15 mL PBS (1X) containing 0.02% sodium azide in a mortar. After complete homogenization, the extract was centrifuged for 15 minutes at a temperature of 4 °C at 5000 rpm. Supernatant was withdrawn and transferred to another Falcon tube from which the lysate was divided to µmL Eppendorf tubes in aliquots to avoid thaw and freeze cycles and kept at -20 °C. The lysate was labeled with letter (W).

**The second method**

Maggots were rinsed three times with $ddH_2O$, transferred to a fine mesh strainer to get rid of excess water, then maggots were placed in 3.5% formaldehyde normal saline solution for 5 minutes, and again on a fine mesh strainer to discard excess formaldehyde, then maggots were dropped in 2% $H_2O_2$ for 3 minutes, then in 1% hydrochloride acid for 5 minutes, followed by three washes with $ddH_2O$. Maggots were dried on filter paper, weighed to 20 gm, and preserved at -20 °C.

Maggots were partially thawed on ice, 15 mL PBS (1X) supplemented with 0.02% sodium azide was mixed with the maggots in a mortar. After complete homogenization, the extract

was centrifugated for 15 minutes at 4 °C and 5000 rpm. Supernatant was preserved in letter (S) labeled aliquots at -20 °C.

**The third method**

Maggots were washed three times with dd$H_2O$, dropped in 3.5% formaldehyde normal saline solution for 5 minutes, then maggots were put in 2% $H_2O_2$ for 3 minutes, then in 1% hydrochloride acid for 5 minutes, followed by three washes with dd$H_2O$. Maggots were dried on filter paper. The maggots were weighed to 20 gm and kept at -20 °C.

After partial thawing on ice, 15 mL PBS (1X) provided with 0.02% sodium azide was homogenized with the maggots in a mortar. After complete homogenization, the extract was centrifuged for 15 minutes at 4 °C and 5000 rpm. Supernatant was divided in aliquots labeled with letter (L) and kept at -20 °C.

**The fourth method**

Maggots were rinsed three times with dd$H_2O$, stood in 3.5% formaldehyde normal saline solution for 5 minutes, then dropped in 2% $H_2O_2$ for 3 minutes, and finally in 1% hydrochloride acid for 5 minutes, followed by three washes with dd$H_2O$. Maggots were dried up on filter paper.

The maggots were weighed to 20 gm and transferred immediately to 50 mL Falcon tube and directly snap frozen in liquid nitrogen ($LN_2$). After freezing, 15 mL PBS (1X) containing 0.02% sodium azide supplementation were added to the Falcon tube containing maggots, left for ~ 15 minutes, then milled with laboratory mortar and kept at -24 °C until laboratory process.

Falcon tube containing maggot extract was let to thaw on ice. After thawing, the extract was centrifuged at 4 °C and 5000 rpm for 15 minutes. Supernatant was collected and transferred to another Falcon tube. Fifteen µL (0.5mM) of Phenylmethylsulfonyl fluoride (PMSF) were added to the maggot lysate as protease inhibitor to prevent degradation of proteins, mixed well and centrifuged for 15 minutes at a temperature of 4 °C at 5000 rpm. Supernatant was transferred as aliquots to 1.5 mL Eppendorf tubes labelled with letter (N) and kept at -20 °C.

**Quantitation of total protein content**

Total protein content of each lysate was measured according to **Smith *et al.* (1985)** using Pierce™ BCA Protein Assay Kit (Thermo Scientific™, Cat. No. 23225) by mixing 200 µL of BCA (50A:1B) to 25 µL of each maggots lysate in 96-well plate, the plate was incubated at 37 °C for 30 minutes then the absorbance was measured at $\lambda_{490\ nm}$. The total protein concentration of each sample was calculated according to **Noble and Bailey (2009).**

$Y = mX + b$

**Equation1:** Y: absorbance of lysate, m: molar absorptivity, X: protein concentration, and b: absorbance of blank "zero" protein.

**Protein Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)**

SDS-PAGE was conducted according to the method described by **Laemmli (1970)**. Each lysate was mixed with sample buffer (6X) in a ratio of (5:1), water bathed at 95 °C for 30 seconds, then ice-cooled. Thirty micrograms of each sample and a prestained protein ladder,

as a molecular weight marker, (ACTGene™, Cat. No. ACT-IDMW26 for lysate N and PageRuler™, Thermo Scientific, Cat. No. 26616 for other lysates) were separated on SDS polyacrylamide gel (10% separating gel and 4% stacking gel) fixed in a vertical electrophoresis unit (BIO-RAD Mini-PROTEAN® Tetra Vertical Electrophoresis Cell, Cat. No. 1658005EDU) at 80 volt for 10 minutes, then at 150 volt for 30 minutes. The gel was stained with Coomassie Brilliant Blue R-250 dye (SDFCL, Cat. No. 42660) to visualize protein bands and analyzed by ImageJ 1.53K software (Wayne Rasband and contributors National Institutes of Health, USA).

**Separation of amino acids by thin layer chromatography (TLC)**

Amino acids present in lysate N were separated on Silica gel aluminum sheet (Supleco, Cat. No. 1.05553.0001) using standards of amino acid solutions as markers to estimate some of the amino acids present in the lysate according to **Sadasivam and Manickam (2008)**. The sample and each amino acid standard were blotted on a line representing the start line with 1 cm distance in between then the sheet was dropped standing in 1L glass beaker containing the mobile phase (solvent formed of butanol: glacial acetic acid: dd$H_2O$ (12: 3: 5)) and covered with aluminum foil. When the solvent reached the end line, the sheet was dried in oven then sprayed with the location reagent (ninhydrin 0.2 gm/100 mL acetone) and warmed in oven to develop the characterized color of each amino acid.

**Statistical analysis**

Data analyzed were obtained from three independent experiments and presented as mean ± SE. Data were analyzed using student's *t*-test to calculate and compare means and standard error of mean of the study groups. Multiple comparisons were conducted using two-way analysis of variance (ANOVA). Statistical significance was set at $P<0.05$ (GraphPad Prism, version 8.0.1; GraphPad Software, Inc., La Jolla, CA, USA).

**Results**

**Biological studies**

The mean percent of adult emergence of *Ch. albiceps* was found to be 99.7 ± 0.33% with significantly higher ratio of emerged females to the whole population ($P<0.0001$) than that of males as presented in

**Table 1**)**Figure 1**).

The mean pre-oviposition period of newly emerged females of *Ch. albiceps* was 4.22 ± 0.18 days ranging from 3.16 to 4.88 days while the mean number of eggs per female was 216 ± 21.4 eggs (

**Table 1**).

Studying the longevity of adult *Ch. albiceps* in both bisexual and unisexual populations showed that life span of both sexes in bisexual population is significantly longer ($P<0.05$)

than that of unisexual population but for both populations, females had significantly longer (P<0.05) life span than males (

**Table 1 and Figure 1**).

**Table 1**: Biological aspects of the adult stage of *Ch. albiceps* under laboratory conditions of 26 ± 2 °C, 55 ± 10% RH, and 12L:12D photoperiod and beef meat as a larval diet

| **Biological aspects** | | **Mean ± SE** | | | |
|---|---|---|---|---|---|
| **Adult stage** | **Percent of adult emergence (%)** | *99*.7 ± 0.33 | | | |
| | **Sex ratio** | **Females** | **Males** | **P-value** | |
| | | 80.7 ± 0.75[a] | 19.3 ± 0.75[b] | < 0.0001 | |
| | **Pre-oviposition period (Days)** | 4.22 ± 0.18 | | | |
| | **Fecundity (Eggs)** | 216 ± 21.4 | | | |
| | **Longevity (Days)** | **Sex** / **Population** | **Females** | **Males** | **P-value** |
| | | **Bisexual** | 16.62±0.29[a] | 14.39±0.07[b] | 0.0024 |
| | | **Unisexual** | 14.63±0.37[b] | 11.71±0.43[d] | 0.0004 |
| | | **P-value** | 0.004 | 0.0008 | |

**SE:** Standard error, and **P:** Probability value.

Means with similar letters are not significantly different P≥0.05.

Means with different letters are significantly different P<0.05.

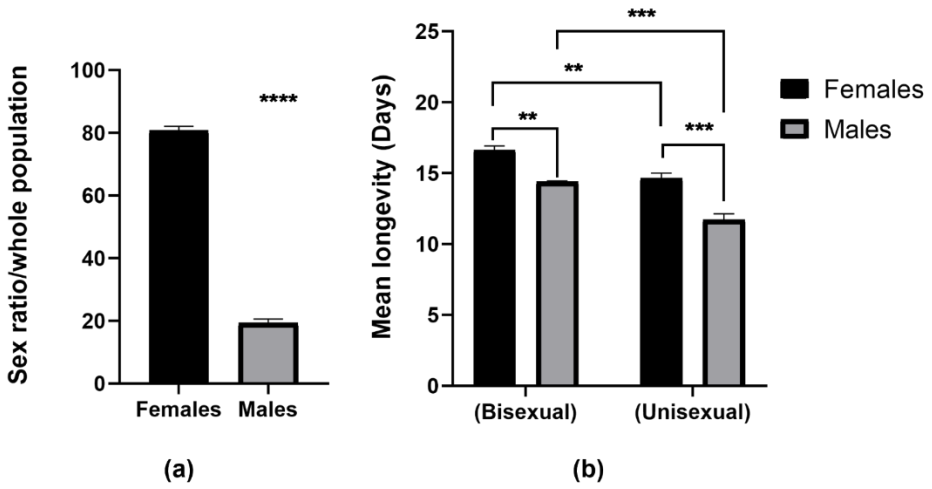


**Figure 1:** Sex ratio and longevity of adult *Ch. albiceps* under laboratory conditions of 26 ± 2 °C, 55 ± 10% RH, and 12L:12D photoperiod (**: *P*<0.01, ***: *P*<0.001, and ****: *P*<0.0001). (a) Sex ratio. (b) Longevity of adult *Ch. albiceps* in bisexual and unisexual population.

**Table 2**) showed that the mean incubation period of *Ch. albiceps* eggs was found to be 0.85 ± 0.09 day and the mean percentage of egg hatchability was 83.8 ± 2.5%. The mean larval and pupal durations were 3.45 ± 0.05 and 4.67 ± 0.042 days, respectively while the percentage of pupation was 98.7 ± 0.88%.

**Table 2:** Some biological aspects of immature stages of *Ch. albiceps* under laboratory conditions of 26 ± 2 °C, 55 ± 10% RH, and 12L:12D photoperiod and beef meat as a larval diet

| **Biological aspects** | | **Mean ± SE** |
|---|---|---|
| **Egg stage** | **Incubation period (Days)** | 0.85 ± 0.09 |
| | **Hatchability (%)** | 83.8 ± 2.5 |
| **Larval stage** | **Larval duration (Days)** | 3.45 ± 0.05 |
| **Pupal stage** | **Pupal duration (Days)** | 4.67 ± 0.042 |
| | **Percent of pupation (%)** | 98.7 ± 0.88 |

**Biochemical studies**

**Total protein content**

A significantly higher ($P$<0.0001) total protein concentration was found in lysate N than that found in lysates S, W, and L which did not significantly differ ($P$>0.05) from each other as mentioned in **Table 3**).

**Table 3:** Absorbance and total protein concentration of whole body late second /early third instar *Ch. albiceps* maggot lysates N, L, S, and W

| Lysates / Chemical parameters | Lysate N | Lysate L | Lysate S | Lysate W |
|---|---|---|---|---|
| **Absorbance** | 1.37 | 1.44 | 1.68 | 1.57 |
| **Protein concentration (µg/µL)** | $27^{a}$ | $5.7^{b}$ | $6.6^{b}$ | $6.2^{b}$ |

Values with similar letters are not significantly different $P \geq 0.05$.

Values with different letters are significantly different $P$<0.0001.

**Protein electrophoresis**

The electrophoretic protein profiles of the four maggot lysates were slightly different. Seven protein bands were separated in lysate N with molecular weights (MW) ranging from 10 to 72 kilodalton (kDa). While the remaining lysates were separated into ten protein bands with MW ranging from 22 to 82 kDa, 21 to 102 kDa, and 22 to 83 kDa for lysates L, S, and W, respectively. One distinctive band was recorded in lysate N with a MW of 10 kDa and intensity of 70.5 $\times 10^{2}$ and another one was detected in lysate S with a MW of 102 kDa. In general, lysate N had the lowest MW bands while the remaining lysates had approximately the same range of molecular weights as presented in **Table 4**) and **Figure 2**).

**Table 4:** Molecular weight (MW) and intensity (Int.) of bands found in electrophoretic protein profiles of whole body late second /early third instar *Ch. albiceps* maggot lysates N, L, S, and W

| Lysates / Bands | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Lysate N** | **MW (kDa)** | 72 | 60 | 55 | 46 | 42 | 26 | 10 | | | |
| | **Int. ($\times 10^{-2}$)** | 22.2 | 2.4 | 1.8 | 1.2 | 2.7 | 1.7 | 70.5 | | | |
| **Lysate L** | **MW (kDa)** | 82 | 72 | 54 | 49 | 46 | 44 | 36 | 32 | 28 | 22 |
| | **Int. ($\times 10^{-2}$)** | 12.7 | 3 | 4 | 7 | 3.3 | 1.8 | 1.9 | 0.8 | 7.6 | 2 |
| **Lysate S** | **MW (kDa)** | 102 | 84 | 77 | 53 | 49 | 45 | 35 | 30 | 26 | 21 |
| | **Int. ($\times 10^{-2}$)** | 1.2 | 7.6 | 1.6 | 0.8 | 5.2 | 8.1 | 6.1 | 0.9 | 2.5 | 4.6 |
| **Lysate W** | **MW (kDa)** | 83 | 50 | 45 | 39 | 36 | 32 | 28 | 26 | 25 | 22 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Int. ($\times 10^{-2}$)** | 11.5 | 6.2 | 20.9 | 0.6 | 8.1 | 1.3 | 2.1 | 1.5 | 1.1 | 4.3 |

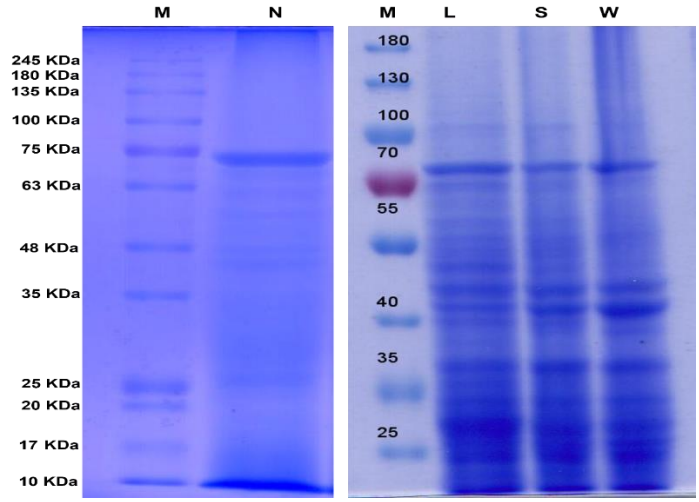


**Figure 2:** Electrophoretic protein patterns of whole body late second /early third instar *Ch. albiceps* maggot lysates. (M: Protein marker, N: Lysate N, L: Lysate L, S: Lysate S, W: Lysate W, and kDa: Kilodalton).

**Thin layer Chromatography (TLC)**

**Table 5**) and **Figure 3**) illustrated the six amino acids separated from lysate N, four of them have the same $R_F$ values as lysine, arginine or histidine or both of them (their $R_F$ values are very close to each other and to that of the lysate spot), glycine or alanine or both of them ( have very close $R_F$ values), and tyrosine. The last two spots were unknown because no corresponding standard amino acids were employed. Proline with its unique yellow color, leucine, phenylalanine, and tryptophane were not detected in the lysate.

**Table 5:** Retintion factor ($R_F$) values of spots of whole body late second /early third instar *Ch. albiceps* maggot lysate N and standard amino acids

| **Spots** | | **$R_F$ values** |
|---|---|---|
| **Lysate** | **1** | **0.07** |
| | **2** | **0.11** |
| | **3** | **0.18** |
| | **4** | **0.27** |
| | **5** | **0.34** |
| | **6** | **0.48** |
| **Standard amino acids** | **Leucine** | **0.52** |
| | **Tyrosine** | **0.48** |
| | **Lysine** | **0.07** |
| | **Alanine** | **0.22** |
| | **Phenylalanine** | **0.5** |
| | **Proline** | **0.2** |
| | **Arginine** | **0.14** |
| | **Histidine** | **0.1** |
| | **Tryptophane** | **0.56** |
| | **Glycine** | **0.16** |

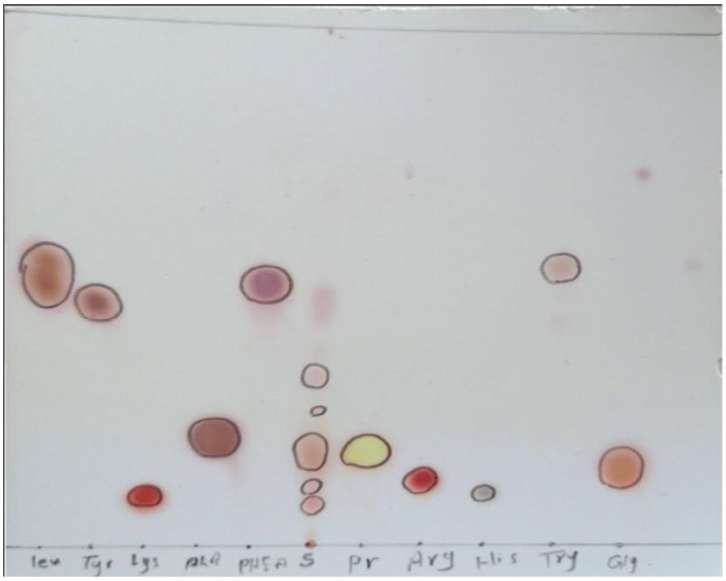


**Figure 3:** Thin layer chromatogram of whole body late second /early third instar *Ch. albiceps* maggot lysate N (1: Leucine, 2: Tyrosine, 3: Lysine, 4: Alanine, 5: Phenylalanine, S: Lysate N, 6: Proline, 7: Arginine, 8: Histidine, 9: Tryptophane, and 10: Glycine).

## Discussion

In the present study, the whole life cycle of *Ch. albiceps,* from oviposition to adult emergence, was studied under laboratory conditions of 26 ± 2 °C, relative humidity (RH) 55 ± 10%, a photoperiod of 12L:12D (Light: Dark) hours, and beef meat as a protein source for adults, oviposition substrate, and larval food. It was found that the development of the immature stages of *Ch. albiceps* completed in about 8.97 days with a significantly longer pupal duration than larval duration ($P = 0.000059$). Females represented the highest ratio of newly emerged adults than males ($P < 0.0001$) and took about 4.22 ± 0.18 to develop its reproductive ability and lay eggs. Females had the longest life span than males in both unisexual and bisexual populations ($P = 0.0004$ and 0.0024), respectively. Significantly, both males and females had longer life span in bisexual population than in unisexual population ($P = 0.0008$ and 0.004), respectively.

Our results concerning development duration were consistent with **Bosly (2021)** who stated that pupal duration was longer than larval duration and **Rashed *et al.* (2015)** who also approved that females had longer life span than males. In contrast to our findings, **Al-Shareef and Al-Qurashi (2016) and Salazar-Souza *et al.* (2019)** contends that larval period of *Ch. albiceps* extended for longer time than pupal period.

Except in lysate N, when the whole body late second/early third instar maggots lysate was prepared by different methods, there was no significant difference in the crude protein concentration. Protein concentration of lysate N was significantly higher ($P < 0.0001$) than other lysates which may be due to the snap freezing of living maggots by ($LN_2$). Agreeing

with the fact that snap freezing in ($LN_2$) preserves the constituents of biological samples **(Mori *et al.*, 2023)** and prevents formation of ice crystals related to slow rate freezing which may result in protein denaturation **(Tan *et al.*, 2021)**.

It was noticed that electrophoretic protein patterns of lysates W, S, and L showed partial lanes smearing which may be due to protein degradation related to preparation techniques **(Gallagher, 2012)**. So, to avoid protein degradation PMSF was added during the preparation of lysate N as a protease inhibitor **(García-Carreño, 1992)**.

While electrophoretic protein profiles of all lysates were slightly different, a distinctive low molecular weight protein band (about 10 kDa) was noted in lysate N. **Ge *et al.* (2015)** stated that low molecular weight proteins (about 15 kDa) had antibacterial activity against *Escherichia coli* aligning with the findings of **Zhang *et al.* (2013)** who found that proteins with about 10 kDa MW had antibacterial activity against *Staphylococcus aureus*.

Proteins with molecular weights of 20 to 25 kDa may be related to the anti-microbial proteins (AMPs) family called attacins which mainly has antimicrobial activity against Gram-negative bacteria **(Pöppel *et al.*, 2015 and Buonocore *et al.*, 2021).**

Proteins with about 56 kDa MW may be related to the protein named blowfly larval immunosuppressive protein (BLIP) belonging to the SERPIN protein family, isolated from larval ES of *Lucilia cuprina,* which holds back immune system by partial inhibition of lymphocytes preventing further inflammation **(Elkington *et al.*, 2009)**.

Comparing the $R_F$ values of lysate N with those of standard amino acids suggested the presence of lysine, arginine, histidine, glycine, alanine, and tyrosine which were confirmed to be present in maggot extracts of blowflies by **Amer *et al.* (2019); Ahmad *et al.* (2022) and Mafimidiwo and Williams (2024)**. **Bexfield *et al.* (2010)** assumed that the amino acid-like components present in blowfly maggot ES may accelerate wound healing by promoting angiogenesis and histidine was proved to enhance the proliferation of human endothelial cells **(Nigam *et al.*, 2010)**.

## Conclusion

The extraction method of whole body late second /early third instar *Ch. albiceps* maggot lysates has a great effect on the composition of the extract and so its role in wound healing.

Further investigations and identification of the bioactive constituents of the maggot extracts and their role in wound healing are recommended.

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