



Full Length Article

Effect of Hot Alcoholic Extract of Algae, *Enteromorpha ralfsii* on the Mortality and Emergence Rate of Housefly *Musca domestica*

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Abstract

The present study was carried out to evaluate the effect of the hot extract of *Enteromorpha ralfsii* on *Musca domestica* under laboratory conditions. In this study, three concentrations (4, 8 and 16 mg/mL) of algal extract were used against the third instar of housefly larva using two application methods. The hot extract of algal had a biological effect, characterized by increased mortality of larvae when food media treated with the algal extract reached 5.33 ± 0.33 at a concentration of 16 mg/mL. The percentage of emerging natural insects was lowest (2.67 ± 0.88) in larvae fed with a diet containing algal extract at concentration of 16 mg/mL. In the direct spraying method, the average number of dead housefly larvae was highest (3.33 ± 0.67) with concentration of 16 mg/mL. The treatment of pupae at the age of 24 h with the algal extract showed numerous morphological malformations, including mature pupae and partial emergence. The chemical compounds present in the hot alcohol extract of alga were identified, which might be responsible for the lower survival and emergence rates of *M. domestica*. © 2024 Friends Science Publishers

Keywords: *Enteromorpha ralfsii*; Algae extract; Emergence rates; Mortality; Housefly; GC-Mass

Abbreviations: *E. ralfsii* *Enteromorpha ralfsii*; mg/mL Milligram per milliliter; ARASCO Arabian Agricultural Services Company; U.V Ultraviolet; GC-Mass Gas Chromatography-Mass; L S D Least Significant Difference; SPSS Statistical Product and Service Solutions; NS Non-Significant; SD Standard Deviation

Introduction

The housefly, *Musca domestica* L., is a widespread insect intrinsic to humans, medically important, and a mechanical transmitter of many pathogens such as viruses, bacteria, protozoans, and worms, in addition to its rapid multiplication and severe disturbance to humans (Khamesipour *et al.* 2018; Al-Khafagi and Mohammed 2022). Many synthetic chemical pesticides, such as organophosphates, pyrethroids, and organochlorines, have been used to control *M. domestica* (Palacios *et al.* 2009). The excessive, repeated, and incorrect use of these pesticides also led to the killing of parasites and predators (natural enemies), weakening their role in the process of natural control and causing an imbalance in the environment (Klakankhai 2022). Their incorrect use also led to the emergence of resistance to pesticides, leading to the predominance of new pests that did not exist previously (Fayyad *et al.* 2022). Therefore, interest has begun in developing alternative control methods for manufactured pesticides and devising new control methods. The scientists began using natural organic pesticides such as plant extracts,

vegetable oils, and solutions of mineral metals, which do not have any harm to human health and the environment and are environmentally friendly. Research is still ongoing that supports the use of some algae by extracting some effective compounds. Preliminary experiments were conducted to support the toxicological effects of algae (Mohammed 2018).

Algae are known as autotrophic primitive plants that convert inorganic matter into organic matter through the process of photosynthesis (Lee 2019). These are simple in structure, lack vascular tissue, contain chlorophyll, have simple reproductive structures, are not surrounded by a sterile wall, and do not rise to the level in contrast to higher plants (Fayyad *et al.* 2022). The effect of algae extracts is due to the presence of numerous compounds such as alkaloids and phenols that act as anti-insects leading to the destruction of insects and affecting the hormones to decline the egg rate (Mohammed *et al.* 2018).

The present study aims to know the effect of the hot alcoholic extract of *E. ralfsii* algae on some stages of houseflies as an alternative to chemical insecticides.

Materials and Methods

General description

Rearing of housefly: The adult insects (*Musca domestica*) were raised in the laboratory in wooden cages (30 × 30 × 30 cm: L x W x H), covered with a muslin cloth, with one side in a sleeve shape to deal with insects. A plastic container of 500 mL containing powdered sugar and dried milk in a ratio of (1:1) (weight: weight) and glass bottles containing cotton moistened with water were used to feed adults.

The experiments were carried out at Mustansiriyah University in Baghdad's animal house, which is equipped with specialized insect incubators. For egg laying and larval growth, the cage was also provided with another plastic container the same size as the previous one containing the culture medium of 200 g of floating fish broth (protein 20%, fat 4% and fiber 4%).

The culture medium was grinded and sterilized in an autoclave at 121°C and 1 bar pressure for 3 min. 10 g of dry yeast and sterile distilled water was added to get the final volume of 100 mL (Hermize *et al.* 2016). The colony was perpetuated for ten generations to avoid the presence of any residual effect of pesticides to obtain a specific sensitive strain in constant conditions (28 ± 2°C and relative humidity 65 ± 5%) before conducting life experiments (Martins 2013).

Collection of algal sample: Algae samples were collected using plastic containers and shard-edged tools on March 01, 2022, from the University of Baghdad, Al-Jadriya, Iraq. The geographical coordinates of the location were longitude 33°01'.94"E and latitude 44°20'.41"N, from the bottom, with a depth of 10–30 cm.

Preparation of the algae powder: The isolated algae were washed well with tap water to remove the mud and dirt and let dry at room temperature with continuous stirring. Later, these were ground with an electric grinder, and the algae powder was kept in dry packages in the refrigerator at 4°C until used. The method of Swain (1966) was followed to prepare the Soxhlet alcoholic extracts of the green algae. The extract was taken and filtered with (Whatman No.1) filter paper, and the remaining filtrate was dried in an incubator at 37°C for 48 h.

To obtain the dry algae powder, it is stored in the refrigerator until use. Different concentrations of algae powder (4, 8 and 16 mg/mL) were prepared by taking one gram of the extract and dissolving it into 5 mL of solvent (96% ethanol alcohol). The final volume of 10 mL was prepared to obtain a concentration of 1 gm/10 mL which is equal to 100 mg/mL (stock solution).

Chemical detection of algae: Preliminary detection of the active compounds in the hot alcoholic extract of *E. ralfsii* (as seen in Table 1) was based on the method of Harbone (1984). The initial detection of the active compounds in the hot alcoholic extract of algae, *Enteromorpha ralfsii* was done using specific reactions and reagents according to previous protocols (Al-Khafagi and Mohammed 2022;

Palaniyappan *et al.* 2023) as given in Table 1. GC-Mass technology was used to detect the active compounds present in the hot alcoholic extract of *E. ralfsii* by following a special thermal system (Sekaran *et al.* 2010).

Detailed description of laboratory experiments: The effect of different concentrations of the hot alcoholic extract of *E. ralfsii* on the larval and pupal stages of the housefly was investigated via the following laboratory experiments.

First: Effect of algal extract on third instar larvae of housefly

The effect of concentrations (4, 8 and 16 mg/mL) of the hot alcoholic extract of *E. ralfsii* algae on the third instar larvae of houseflies was investigated in two ways.

Feeding of algae extract through larval diet: The larval diet was treated with extract of *E. ralfsii* according to the method of Klakankhai (2022). To prepare 60 g of diet, 6 mL of 4 mg/mL concentration was added and mixed for two minutes. The treated larval food was divided into three plastic containers (diameter of 4.5 cm and height of 3.5 cm). Ten larvae (third instar) from the breeding colony were transferred to each container, which were then covered with muslin cloth to allow the larval breathing and to block their escape from the container. The study was repeated in a similar way using two other concentrations (8 and 16 mg/mL) of algal extract.

For control treatment, only sterile distilled water was added to the larval diet. The plastic containers were transferred to an incubator at 28 ± 2°C, RH 65 ± 5%, and duration of illumination 12:12 (light: dark). The experiment was monitored daily and the numbers of dead and distorted larvae, dead pupae, and emerging adults of the surviving pupae from treated larvae were recorded. The photographs of dead and distorted larvae were also recorded.

Direct spray on the housefly larvae: The larvae were treated with direct spraying of algal extract following the method of Sharififard *et al.* (2011). The effect of spraying different concentrations (4, 8 and 16 mg/mL) of algae extract *E. ralfsii* on third instar larvae was evaluated. Thirty larvae (third instar) were transferred from the culture colony to a plastic container and 4 mL of 4 mg/mL of the algal extract was sprayed from a distance of 5 cm using a hand sprayer of 10 mL capacity. Afterwards, ten larvae were transferred to plastic containers (diameter of 4.5 cm and height of 3.5 cm) containing 20 g of larval culture medium. The experiment was also repeated using two other concentrations (8 and 16 mg/mL) of algae extract. Three replicates were carried out for the control treatment in which, the larvae were sprayed with 4 mL of sterile distilled water. The replicates of the experiment were preserved after covering them with a perforated muslin cloth (to prevent larvae from escaping) in the incubator at 30 ± 1°C, RH 65 ± 5%, and illumination duration of 12:12 (light: dark). The experiment was monitored daily and the numbers of dead and distorted larvae, dead pupae and emerging adults of the

surviving pupae were recorded for two weeks. The dead and distorted housefly larvae were photographed.

Second: Effect on housefly pupal mortality and emergence rates

The effect of the alcoholic extract of algae at tested concentrations (4, 8 and 16 mg/mL) was studied on housefly pupae (24 and 72 h old). Thirty pupae (24 h old) were taken with three replications with the control treatment, 4 mL of each tested concentration of the algal extract was sprayed using a hand sprayer of 10 mL capacity from a distance of 5 cm to ensure good coverage of the algal extract over all the pupae. Later, every 10 pupae were transferred to plastic containers (diameter of base 4.5 cm and height of 3.5 cm), covered with perforated tulle cloth, and transferred to the incubator ($28 \pm 2^\circ\text{C}$ and $65 \pm 5\%$ RH) and a duration of illumination 12:12 (light: dark). The experiment monitored the dead and distorted pupae and recorded the insects emerging from the treated pupae. Three replicates were carried out for the control treatment, three concentrations (4, 8 and 16 mg/mL) of algal extract were sprayed on pupae and larvae (24 and 72 h old), and photos of the experiment's deformities were taken. The same procedures were carried out on larvae and pupae that were sprayed with sterile distilled water as a control treatment.

Statistical analysis

The data was statistically analyzed using SPSS (2012) and the comparison of multiple means was done using least significant difference (LSD).

Results

Enteromorpha ralfsii is a species of macroalgae in the group green algae Chlorophyceae of the family Ulvaceae (Fig. 1). The preliminary detection of the active compounds from the hot alcoholic extract of algae showed the presence of alkaloids, tannins, flavonoids, and saponins, while glycosides, terpenes and phenols were absent (Table 2).

The data of gas chromatography (GC-Mass) showed the presence of seven major compounds in the hot alcoholic extract of *E. ralfsii* (Table 3 and Fig. 2). The comprehensive spectroscopic analysis of the compounds representing 86.32% of the total mass forming and the remaining 14% was not verified due to their low plenty from the hot alcoholic extract of *E. ralfsii*. The percentage of area was pentadecane (12.67%), nonadecane (4.60%), tetradecane (3.02%), octadecane (11.20%), hexadecane (6.7%) and salicylic acid (6.10%).

First: Effect on housefly larval mortality

Table 4 shows the effect of tested concentrations of the hot alcoholic extract of *E. ralfsii* alga fed to third-stage larvae of

housefly. The mean larval mortality was significantly different with values of 2.67 ± 0.33 , 3.00 ± 0.57 , and 5.33 ± 0.33 after feeding the algal extract at concentrations of 4, 8 and 16 mg/mL, respectively. Morphological abnormalities observed in the treated and dead larvae were represented as bursting of the digestive tract and excretion of digestive fluid with the blackening of the dead larvae (Fig. 3). A decrease in the mean natural larval emergence was observed with an increase in the concentration of algal extract in the larval diet, and natural emergence was least (2.67 ± 0.88) at higher algal concentration of 16 mg/mL (Table 4).

Table 5 showed that after a direct spray of algal extract at different concentrations, a decrease in the mean natural emergence was observed with an increase in the concentration of hot alcoholic extract. The mean larval mortality (3.33 ± 0.67) of the third instar larvae treated with the concentration of 16 mg/mL was significantly different compared with the control (0.67 ± 0.33).

Second: Effect on housefly pupal mortality and emergence rates

The efficacy of the hot alcoholic extract of *E. ralfsii* on the treated housefly pupa (24 h old) can be seen in Table 6. The pupae mortality at concentrations of 8 and 16 mg/mL were the highest with values of 4.33 ± 0.67 and 5.00 ± 0.57 , respectively. These values were substantially higher than the pupae mortality in the control (0.67 ± 0.33). All concentrations gave equal partial emergence (2.00) of twisted pupae.

The natural emergence was significantly reduced with all tested concentrations compared to the control (9.33 ± 0.33). Among tested concentrations, least natural emergence (3.00 ± 0.57) was observed with highest concentration (16 mg/mL) followed by 8 mg/mL (3.67 ± 1.85).

Table 7 indicates a significant difference ($P < 0.00$) in the mortality for the treated pupae (72 h old) with algal extract concentrations. The pupal mortality was increased with an increase in concentrations (4, 8 and 16 mg/mL) of algal extract with values of 1.00 ± 0.57 , 4.33 ± 0.33 and 6.67 ± 0.33 , respectively. The natural emergence from the pupa was highest in the control treatment (10.00 ± 0.00), which differed significantly from the natural emergence of other tested concentrations. The natural emergence was decreased with an increase in the concentration of algal extract. The normal emergence of adults decreased from 9.00 ± 0.57 to 5.67 ± 0.33 and 3.33 ± 0.00 at concentrations of 4, 8 and 16 mg/mL, respectively (Table 7).

Discussion

Enteromorpha ralfsii is known to be a dominant species in saline coastal wetlands with high nitrogen levels (Gibson *et al.* 2001; Hayden *et al.* 2003). It floats or moves between the water's surface and the bottom, or it can be associated with rocks and plants (Fish and Fish 1989). The thallus

Table 1: Methods for detecting active compounds (Harbone 1984; Sekaran *et al.* 2010; Palaniyappan *et al.* 2023)

| Active compounds | Test/reagent | Method | Positive result |
|------------------|----------------------|--|---|
| Alkaloids | Dragendorff's test | Added 1 % Hydrochloric acid to the extract, then drops of Dragendorff reagent (potassium bismuth iodide solution) | Forming a red precipitate |
| Glycosides | Liebermann's test | Two mL of chloroform and a concentration acetic acid are added to the extract in an ice bath, then added two drops of concentrated sulfuric acid | Purple-green color is formed |
| Tannins | Gelatin test | Added 1% domain solution containing sodium chloride to the extract | Forming a white precipitate |
| Terpenoid | Salkowski test | a- Added a few drops of concentrated sulfuric acid and 2 mL of chloroform to the extract, shake and leave until set. b- 2 mL of chloroform and 3 mL of concentrated sulfuric acid are added to the extract. | a - Golden yellow color appears b - A yellow ring forms between the two layers that turn reddish-brown |
| Flavonoids | Wilson-tauboc | One mL of the extract is placed in a watch glass and heated in a water bath to dryness, then mixed with Wilson's reagent (5 mg boric acid + 5 mg acetic acid + 3 mL acetone) and dried again, then applied under U.V 365 nm. | Radiates green |
| Phenols | Ferric chloride test | Added 3-4 drops of iron chloride solution to the extract | Forming a dark blue or violet color |
| Saponins | Foam test | Add 1 mL of the extract in a test tube containing 5 mL of distilled water, and shake the tube well to form foam. | The foam stays for 10 minutes |

Table 2: Presence and absence of active compound in a hot alcoholic extract of *E. ralfsii*

| No. | Active compounds | Hot alcoholic extract of <i>E. ralfsii</i> |
|-----|------------------|--|
| 1 | Alkaloids | + |
| 2 | Glycosides | - |
| 3 | Tannins | + |
| 4 | Terpenoid | - |
| 5 | Flavonoids | + |
| 6 | Phenols | - |
| 7 | Saponins | + |

+ = Present Active compound
- = Absence Active compound

Table 3: The main compounds detected by the gas chromatography (GC-Mass) technique of the hot alcoholic extract of *E. ralfsii*

| Compound | Retention time (min) | Area (%) |
|-------------------------|----------------------|----------|
| Pentadecanone | 12.72 | 12.67 |
| Nonadecane | 13.76 | 4.60 |
| Hexadecane-tetra methyl | 14.11 | 42.03 |
| Tetradecane-8-Methyl | 16.77 | 3.02 |
| Octadecane-8-Methyl | 17.07 | 11.20 |
| Hexadecane | 21.8 | 6.70 |
| Salicylic acid | 24.07 | 6.10 |
| Total | | 86.32 |

(body) algae is characterized by its smooth surface "silky", medium green, filamentous branching, extending to a length of 2–15 cm, consisting of several rows of cells (multi-seriate) (Lee 2019). Numerous active compounds were found in the algae extract mentioned by Sekaran *et al.* (2010) and Alghazeer *et al.* (2017). The secondary metabolites such as alkaloids, tannins, and saponins showed considerable effect against insects (Adesina and Rajashekar 2018). Alkaloids are organic substances that contain nitrogen in their composition and have strong physiological effects, even in very low concentrations (Zandavar and Babazad 2023). Tannins are polyphenolic compounds that are nitrogen-free, antiseptic, antimicrobial, and anti-inflammatory, tannins are antiseptic substances that protect against fungal and insect diseases. Saponins are natural compounds with chemical properties similar to glycosides, but they are distinguished by the fact that they produce soapy foam when shaken with water (Harbone 1984). Studies have shown that saponins have a physiological activity that is toxic to humans and animals, some of them are decomposing red blood cells Swain (1966).

In the present study, GC-mass analysis shows groups of hydrocarbons such as pentadecanone, octadecane-8-methyl, and hexadecane-tetra methyl that were the main and common compounds in the crude alcoholic extracts of *E. ralfsii*. These compounds are considered to have strong antimicrobial activity and reduce the effect of free radicals. This includes delaying the maturation of the eggs and increasing the thickness of the pupa to prevent the insect from emerging (Shaker 2019). Salicylic acid is a phenolic compound that was found in the algal extract in our study. It is widely used as a crystalline organic acid, colorless in organic synthesis and plant hormone functions derived from the metabolism of salicin present in the region (6.1%) from the analysis of GC Mass. One keratolytic is salicylic acid. It is a member of the same drug class (salicylates) as aspirin, which plays an important role in improving the plant's ability to withstand insects through acquired systemic resistance (ASR) and making their pesticides more effective (Hayat *et al.* 2010; Mohammed *et al.* 2018).

The mortality of larvae could be due to the toxic effect of compounds present in the hot alcoholic extract of *E.*

Table 4: Effect of different concentrations of the hot alcoholic extract of *E. ralfsii* on housefly larvae (treatment as larval diet)

| Concentration mg/mL | Dead larvae (Mean ± SD) | Partial emergence (Mean ± SD) | Natural emergence (Mean ± SD) |
|---------------------|-------------------------|-------------------------------|-------------------------------|
| 4 | 2.67 ± 0.33b | 0.667 ± 0.33a | 6.67 ± 0.33b |
| 8 | 3.00 ± 0.57b | 1.00 ± 0.50a | 6.0 ± 0.57b |
| 16 | 5.33 ± 0.33a | 2.00 ± 1.00a | 2.67 ± 0.88 |
| Control | 0.667 ± 0.33c | 0.00 ± 0.00a | 9.33 ± 0.33 |
| L.S.D. | 1.331** | NS 2.369 | 1.882** |
| P- value | 0.0003 | 0.335 | 0.0003 |

NS (Non-Significant), ** ($P < 0.01$)

The mean bearing different letters within the same column differ significantly among themselves

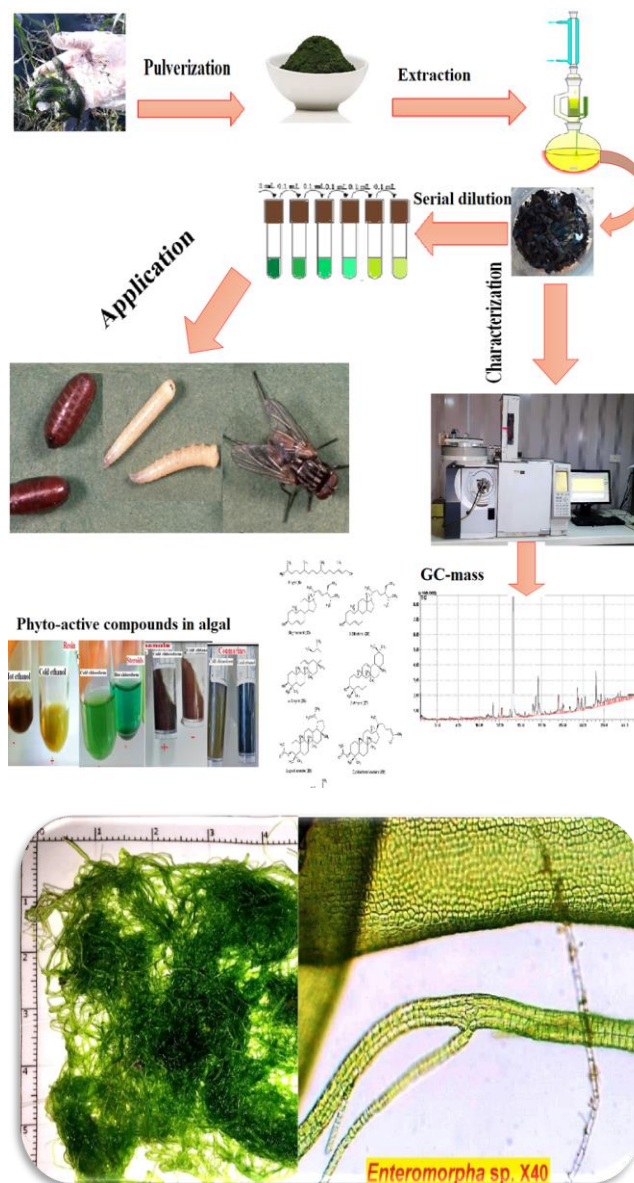


Fig. 1: Phenotypic structure of *E. ralfsii* in nature and under a microscope at 40X magnification

ralfsii, such as alkaloid compounds, which are known to be highly toxic to many insects and that may act as inhibitors of feeding and thus starvation to death of larvae (Zandavar

and Babazad 2023).

The high potency of the green algal extracts could be attributed to the presence of toxic compounds, specifically

Table 5: Effect of different concentrations of hot alcoholic extract of *E. ralfsii* on third instar larvae of house flies (treatment as direct spray)

| Concentration (mg/mL) | Larval mortality (Mean ± SD) | Natural emergence (Mean ± SD) |
|-----------------------|------------------------------|-------------------------------|
| 4 | 1.33 ± 0.88ab | 6.67 ± 0.88ab |
| 8 | 2.33 ± 0.88ab | 6.0 ± 0.88ab |
| 16 | 3.33 ± 0.67a | 6.67 ± 0.67b |
| Control | 0.67 ± 0.33b | 9.33 ± 0.33 |
| LSD | 2.369* | 2.369* |
| <i>P</i> - value | 0.0426 | 0.0426 |

* (*P* < 0.01)

The means bearing different letters within the same column differ significantly among themselves

Table 6: Effect of different concentrations of the hot alcoholic extract of *E. ralfsii* on the pupae (24 h old) of houseflies

| Concentration mg/mL | Pupa (24 h old) | | |
|---------------------|-----------------------------|-------------------------------|-------------------------------|
| | Pupal mortality (Mean ± SD) | Partial emergence (Mean ± SD) | Natural emergence (Mean ± SD) |
| 4 | 2.67 ± 0.33b | 2.00 ± 0.57a | 5.33 ± 0.88b |
| 8 | 4.33 ± 0.67a | 2.00 ± 1.52a | 3.67 ± 1.85b |
| 16 | 5.00 ± 0.57a | 2.00 ± 0.57a | 3.00 ± 0.57b |
| Control | 0.67 ± 0.33c | 0.00 ± 0.00a | 9.33 ± 0.33a |
| LSD | 1.630** | NS 2.824 | 3.522** |
| <i>P</i> - value | 0.0012 | 0.330 | 0.010 |

NS (Non-Significant), ** (*P* < 0.01)

The mean bearing different letters within the same column differ significantly among themselves

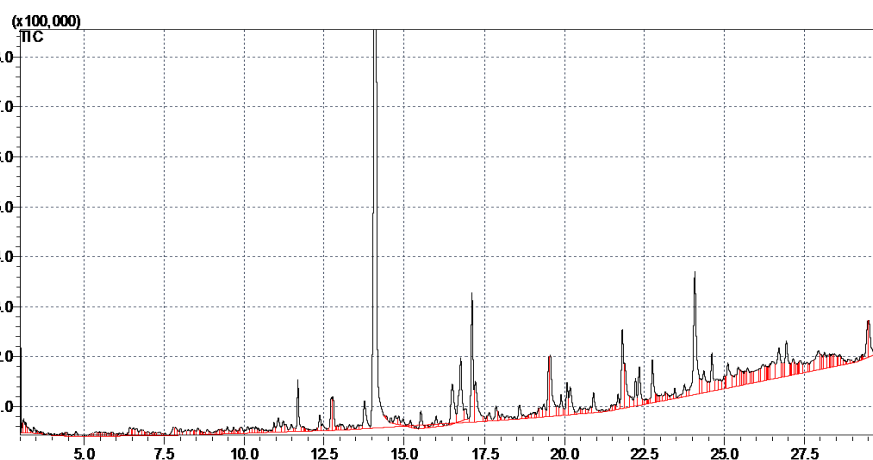


Fig. 2: The main Compounds detected by the gas chromatography (GC-Mass) technique of a hot alcoholic extract of *E. ralfsii* algae

Nonadecane and Tetradecane, which inhibit the AChE enzyme. Furthermore, green algae are rich in saponin and flavonoid compounds (Adesina and Rajashekar 2018). These flavonoids inhibit the protein responsible for cholesterol transportation during larval development resulting in larval mortality. In addition to the presence of Salicylic acid that prevents the natural emergence of insects, it is an exfoliating material that causes changes to the pupal shell through surface scratches that reduce larval performance by preventing the insect from exiting the pupal (Lortzing *et al.* 2019).

Nadeem *et al.* (2022) mentioned that larvae die of starvation was due to the presence of terpenoid compounds, including saponins, which are considered feeding inhibitors (Mazid *et al.* 2011; Kachhwaha 2017). It was also found that these compounds affect the gastrointestinal tract, especially the epithelial cells, and the occurrence of a state of poisoning

and death after a period of feeding and the death of larvae may be due to the presence of flavonoids because of their ability to bind with digestive enzymes in the insect's body and thus lead to reduced metabolism (Palaniyappan *et al.* 2023), or these compounds may interfere with the work of the endocrine system, which leads to a defect in the process of growth and increases in the destruction of the insect (Kreem and Annon 2018). Through the union of these compounds with the fatty substances present in the gastrointestinal tract, these fatty substances are expelled without the benefit (Shaker 2019; Gao *et al.* 2022). The effect of compounds including Phenolic, including Flavonoids, increases the rate of larval mortality, which may be due to a decrease in the rate of food digestion, which leads to the killing of larvae due to lack of nutrition (Kazim 2013; Salman and Ahmed 2017; Nadeem *et al.* 2022).

Table 7: Effect of different concentrations of the hot alcoholic extract of *E. ralfsii* on the pupae (72 h old) of houseflies

| Concentration mg/mL | Pupal mortality (Mean ± SD) | Natural emergence (Mean ± SD) |
|---------------------|-----------------------------|-------------------------------|
| 4 | 1.00 ± 0.57c | 9.00 ± 0.57a |
| 8 | 4.33 ± 0.33b | 5.67 ± 0.33b |
| 16 | 6.67 ± 0.33a | 3.33 ± 0.33c |
| Control | 0.00 ± 0.00c | 10.00 ± 0.00a |
| LSD | 1.215* | 1.215* |
| P- value | 0.0001 | 0.0001 |

*($P < 0.01$)

The means bearing different letters within the same column differ significantly among themselves

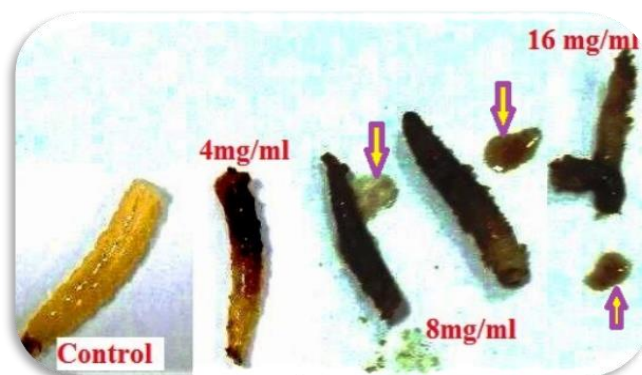


Fig. 3: Housefly larvae after treatment with different concentrations of hot alcoholic extract of *E. ralfsii*. The blackening of larva and exit of digestive juices at high concentrations is clearly visible

The reason for the mortality rates recorded for the pupae treated with alcoholic extract at the ages of 24 and 72 h may be attributed due to the effective properties of terpenes compounds including saponins. The mortality rates of pupae treated with the tested concentrations may be due to the interaction that occurs between the chemical compounds and the alienation hormone in the pupal body (Wink 1988).

Rodrigue *et al.* (2023) showed that the toxic compounds present in the extract such as saponins, and tannins, may affect the cells that are in a state of continuous division, especially in the early stages of the puparium, to complete the growth of the organs and prepare for the exit of the adults, which leads to the death of the pupae while they are inside the puparium. Hayat *et al.* (2010) mentioned the presence of salicylic acid, which is one of the types of phenols that have a physiological effect through changing osmosis and could cause changes in the pupal shells, which could not be opened.

Conclusion

It was concluded that the hot alcoholic extract of *E. ralfsii* is biologically effective in controlling *Musca domestica* L. The hot alcoholic extract contains numerous active compounds and toxic substances such as alkaloids, tannins, flavonoids, and saponins, which interfere with the physiological processes and increase the mortality of larvae and pupae of houseflies. Therefore, it is necessary to think about

increasing the production of algae for its potential use in the control of houseflies at the time of their peak spread and reproduction. Ultimately, the availability of *E. ralfsii* in large quantities is one of the effective sources to eliminate the housefly by spraying it alcoholic extract on the waste, piles of garbage, animal manure waste, and housefly breeding sites.

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Author Contributions

All authors wrote reviews, edited, and contributed equally to the work. All authors have read reviewed and agree to publish the version.

Conflicts of Interest

Authors are responsible for the correctness of the statements provided in the manuscript. The authors declare that they have no competing interests. We hereby confirm that all the Figures and Tables in the manuscript are us.

Data Availability

All data and materials are available if requested.

Ethics Approval

This is an observational study. Mustansiriyah University Research Ethics Committee has confirmed that no ethical approval is required. The study was conducted on the natural occurrence of algae extracts and usage of house flies that are available in the environment and ethical approval is not demanded.

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