



Thyme extracts as a natural and environmentally friendly alternative for the biological control of house fly (*Musca domestica* Lin.) in meat processing facilities

Nebras Mohammed Sahi^{1,*}, Ahmed Habeeb Al-Mamoori¹

¹ University of Babylon. College of science for women. Department of Biology, Iraq.

*Corresponding author: Nebras Mohammed Sahi.

University of Babylon. College of science for women. Department of Biology, Iraq.

E-mail: wsci.nebras.m@uobabylon.edu.iq

DOI: <https://doi.org/10.54448/ijn26304>

Received: 03-14-2026; Revised: 05-23-2026; Accepted: 06-17-2026; Published: 06-18-2026; IJN-id: 26304

Editor: Dr Hind Mamoun Beheiry, MD, MPH.

Abstract

Introduction: Houseflies (*Musca domestica*) are one of the most significant sources of microbial contamination in meat processing facilities, as they act as direct mechanical vectors for numerous pathogens that can adversely affect food safety and human health. Given the risks posed by conventional chemical pesticides, this study highlights the need to seek safe and environmentally friendly alternatives, such as plant extracts (thyme extracts), which can help reduce the prevalence of this insect and mitigate its harmful effects. **Objective:** The study aims to evaluate the biological efficacy of alcoholic and aqueous extracts of thyme in controlling the various larval stages of the housefly, with a focus on comparing their respective effectiveness in inducing mortality rates and their impact on prolonging the larval growth period, as well as to study the effect of these treatments on pupal development and the resulting pupal weights. **Methods:** Alcoholic and aqueous thyme extracts were used at various concentrations (15, 20, 25, and 30 mg/mL), and the three larval stages (first, second, and third) of the housefly were exposed to these extract concentrations under strictly controlled laboratory conditions. Mortality rates were recorded for each stage, larval development duration was monitored, and the survival rate and weights of the pupae were assessed, in comparison with a control group that received no treatment. **Results:** The results showed that the alcoholic extract was more effective than the aqueous extract, with mortality rates of 100%, 90.3%,

and 74.3% in the first, second, and third larval stages, respectively, while the mortality rates for the aqueous extract were 82.2%, 60.2%, and 50% for the same stages, compared to a very low rate of 3% in the control group. An increase in larval growth duration was also observed, particularly in the first instar, which lasted 4-4.5 days compared to 2 days in the control group; in addition, the highest mortality rate (100%) was recorded among pupa that emerged from larvae treated during the first and second instar. The results also showed a significant decrease in pupae weights, with the alcoholic extract recording the lowest weight of 11 mg, while weights ranged from 17–18 mg in the aqueous extract compared to 26 mg in the control group. **Conclusions:** The outcomes of the study indicate that thyme extract, particularly the alcoholic extract, is highly effective as a natural insecticide against houseflies, as it caused increased mortality rates in the larval stages, slowed growth, and negatively affected the development and weight of pupae, making it a promising and environmentally friendly option that can be adopted within integrated pest management programs to limit the spread of this insect.

Keywords: *Thymus vulgaris*. Housefly control. Meat hygiene. Aqueous extract. Alcoholic extract.

Introduction

The house fly (Diptera: Muscidae) is one of the most common and widespread fly species in the world,

abundant in both rural and urban areas with tropical and temperate climates [1,2]. The house fly belongs to the group of flies often called "dirty flies". Other members belong to the families Calliphoridae and Fanniidae [3]. The house fly has been present since the beginning of human life [4] and is well adapted to life in human dwellings [5]. Its presence is linked to the direct mechanical transmission of pathogens to humans and animals, these are bacteria, viruses, fungi, helminths and protozoa, including coliforms and Shigella [6, 7]. Houseflies undergo complete metamorphosis, where their ecology, physiology and behavior are specific to the stage [8,9]. Therefore, the larvae usually prefer to live in moist environments (hydrophilic places) such as corpses, manure or garbage, and may die if they are present in dry environments (hydrophobic). Houses may be prone to dryness and thus will develop a waxy layer, which in turn leads to dry skin [10].

Various plant extracts are known to have insecticidal properties, but the industry has not made much progress in isolating the toxic elements from them or in applying further scientific investigation to increase their biosynthesis level. As a result, their marketability to increase income for the nation and society as a whole is still lacking [11]. So, use the thyme plant (*Thymus vulgaris*) which is a plant belonging to the Lamiceae family and it is an evergreen fragrant shrub that grows in several regions of the world [12]. The thyme plant has been used since ancient times as a flavoring for cheese [13].

In addition, it is considered a herbal medicine that treats alopecia, dental calcifications, skin infections, bronchitis, cough, and digestive system disorders [14]. It has been used as an antiseptic, gas repellent, antimicrobial, and antioxidant [15]. It has also been used as a pesticide that is safe for the environment and humans against various types of insects [16,17]. Therefore, plant extracts are considered safer alternatives to synthetic pesticides in light of the increasing concerns about the effects of these chemicals. on the environment and human health. Their potential to manage a range of insect pests appears promising, given that they are generally considered less hazardous to humans and wildlife than conventional insecticides [18]. Research has shown that the structural diversity of the major components in essential oils may suggest several different mechanisms of action. For example, the major components of thyme and cedar wood oils, respectively, are thymol and nootkatone, which alter GABA receptors in fruit flies in diametrically opposed ways [19,20].

Several chemicals derived from essential oils have

also been proposed to target other neural targets, such as the cholinergic, tyramine, and octopaminergic systems [21,22]. Eradication and biological control measures using chemical or synthetic insecticides are also challenging. Repeated exposure to these pesticides has led to resistance and undesirable negative effects, so this study was developed to find an alternative to chemical insecticides, which are new environmentally friendly pesticides of natural plant origin (*Thymus vulgaris*), against *M. domestica* with four different concentrations of each extract. The results obtained showed good rates of insect mortality when using alcoholic and aqueous extracts through dipping and feeding techniques.

Materials and Methods Insect collecting and raising method

Adult houseflies were collected from various areas of meat-processing facilities in the city of Hilla (AlAkramin, Al-Karama, and Al-Dabat) during July 2024. They were transferred to the insect rearing room at the Advanced Entomology Laboratory of the Department of Biology for rearing and for use in the experiments of this study. The adult flies were placed in parallel rearing cages, the cages measured 40 × 40 × 35 cm, and their bases were made of wood. The four side faces and the upper surface were covered with tulle fabric, and a circular hole with a diameter of 14 cm was made in one of its side sides to allow the hand to enter and deal with the insect. Pieces of the insect were placed in Petri dishes. Cotton, 5 cm thick, was moistened with a solution consisting of 250 mL of distilled water, milk powder, and 80 g of sugar for the purpose of feeding the adults and laying eggs on them. They were raised at a temperature of 30 ± 2 C and a relative humidity of 65 ± 5%, with a 12-hour light period [23]. Collect the eggs using a soft brush and transfer them to plastic containers, each with a capacity of 1000 ml, containing 500 grams of artificial nutrient medium modified according to the method [24], consisting of 60 grams of cow dung, sterilized in an autoclave at a temperature of 121 °C, a pressure of 15 lbs/in², and 10 grams of sugar. Table and 5 gm yeast. The larvae were raised at a temperature of (30 ± 2) °C and relative humidity (65 ± 5)%, while providing conditions of 24 hours of complete darkness by covering the boxes with a black cloth until they reached the pupal stage.

In addition to this medium, the larvae were grown using another medium prepared according to the method of [23], which consisted of strips of paper tissue 5 cm thick, moistened with a solution composed of distilled water, milk powder, and yeast, placed inside 500 ml plastic containers, and after placing the eggs on

the surface of the tissue. It was covered with a layer of dry paper tissue. It was raised at a temperature of (30±2)°C and a relative humidity of (65±5)%, while providing conditions of 24 hours of complete darkness by covering the boxes with cotton.

Preparation of Thyme leaf extract

This research was conducted at the College of Science for Girls (Biology Department) / University of Babylon. Dried leaves of thyme (*Thymus vulgaris*) were collected from Al-Hikma Herb Shop, a shop specializing in medicinal herbs in the city of Hilla. A specialist from the Department of Biology identified the species of thyme.

Alcoholic extract was prepared as follows: Thirty grams of dried plant leaves were used to prepare the ethanolic extract of thyme leaves and placed in one liter of ethanol (90%) absolute alcohol, then the mixture was shaken and incubated for one day (24-48 hours) at room temperature. The mixture was then filtered and centrifuged at 3000 rpm. Ethanol was also evaporated (using a rotary evaporator). Also, the aqueous extract of the plant under study was prepared as follows: This extract was prepared by adding 20 grams of thyme to 300 ml of distilled water in a conical flask and leave on the device at temperature Magnetic stirrer Magnetic stirring the room for 24 hours, then the extract was filtered using leaves Using a filtration device under whatman No 1. The filtrate was then concentrated using vacuum pressure rotary vacuum Rotary evaporator device under vacuum pressure the solid aqueous extract was obtained. Evaporator Store in a sterile, opaque, tightly sealed plastic bottle. Finally, the extracts were kept in the refrigerator until used [25].

Treating the larval stages of the house fly with aqueous and alcoholic extracts of thyme

Using forceps, the first stage larvae - which ranged in length from (1.4 to 3) mm were gathered as soon as the eggs hatched, to conduct this experiment, the food was treated with thyme plant aqueous and alcoholic extract at a rate of three replicates for each of the following concentrations (15- 30) mg/mL. Ten larvae were transferred to Petri dishes containing their previously prepared food medium, which was primarily composed of cow dung, and each of the aforementioned concentrations was treated separately, adding 5 mL of each concentration to every (5) grams of the rearing medium while keeping track of the larval stage, in addition to the control group, which was represented by three Petri dishes containing untreated food that was sprayed with distilled water only and ten larvae were added to each of them. All control and

treatment dishes were transferred to the incubator at a temperature of (2±30) C and relative humidity. 5±65) % [24]. The mortality rates were calculated after 24 hours of treatment, while the remaining larvae were followed for seven days in order to record the duration of larval growth, pupal mortality rates, pupal weight average, and adult emergence rates. In the same way, the second and third larval stages were treated, which were identified by their adult lengths (3-6 and 6-9), respectively.

Statistical analysis

A factorial experiment with a fully randomized design and the global experiments model were used to examine the data. The significance of the findings was assessed using the least significant difference (LSD) test at a probability level of 0.05 using Probit analysis software [26]. The Abbott equation [27] was then used to correct the mortality rates.

$$\text{Percentage of mortality} = \frac{(\text{PMC}\% - \text{PMT}\%)}{(\text{PMC}\%)} * 100 \dots \dots (1)$$

Where:

PMC%= Percentage of mortality in control% PMT%= Percentage of mortality in treatment %

Results

The results of this study indicate that applying different concentrations of the plant's alcoholic and aqueous extract had a negative impact on the average weights and growth periods of the pupae after the larvae were treated with the extracts, as well as the mortality rates of the first, second, and third larval stages of the house fly. Tables (1-7) illustrate this negative effect, a rise in the mortality rates of the larvae, an extension of their growth period, an increase in the mortality rates of the pupae, and a decrease in the average weights of the pupae that emerged from the larvae were among the other negative effects, an inverse relationship was found between the concentrations used and each of the average weights of the pupae with the extract while they are in the first, second, and third larval stages, While the relationship between the concentrations of the extract and the average age of the larvae, their mortality rates, and the mortality of the pupae resulting from treating the larvae was directly proportional.

Rates of mortality of larval stages

The results of Tables (1 and 2) indicated an increase in the mortality rates of the different larval stages treated with increasing concentrations used, where the highest mortality rates were recorded (80.2,60.2 and 50)% in the first, second and third

larval stages treated with a concentration of 30 mg/ml of the aqueous extract, respectively, while the rates reached (100,90.3 and 70.4) in the first, second and third stages treated with the same concentration of the alcoholic extract, respectively, compared to the control, which reached (3 , 3 and 0.0)% for the three stages, respectively. The statistical analysis also showed an increase in the mortality rates of the first stage larvae compared to the second and third stages and at all concentrations used in the two extracts.

Table 1. Effect of interaction of different concentrations of aqueous extract of (*Thymus vulgaris*) on the mortality rate of different larval stages of the house fly *M. domestica*.

Concentration Mg/mL	% Mortality of the first larval stage	% Mortality of the Second larval stage	% Mortality of the Third larval stage
Control	3.0	3.0	3.0
15	30.3	20	9
20	45.3	30	26.7
25	60	45	35
30	80.2	60.2	50

L.S.D value below (0.05) level of interaction effect = 2.6. Source: Own authorship.

Table 2. Effect of interaction of different concentrations of alcoholic extract of (*Thymus vulgaris*) on the mortality rate of different larval stages of the house fly *M. domestica*.

Concentration Mg/mL	% Mortality of the first larval stage	% Mortality of the Second larval stage	% Mortality of the Third larval stage
Control	3.0	3.0	3.0
15	55	40.3	11.3
20	75	53.3	30.2
25	91.3	74.5	60.2
30	100	90.3	70.4

Note: L.S.D value below (0.05) level of interaction effect = 10.7. Source: Own authorship.

Average larval growth period

The results of Tables (3 and 4) showed the effect of the interaction of different concentrations of aqueous and alcoholic extracts of thyme on the average growth period of the first, second and third larval stages, as an increase in the average growth period of the three larval stages was observed, and there was a direct relationship between the average growth period for each larval stage and the concentration used, as the highest rates were recorded when treated with the alcoholic extract at a concentration of 30 mg/mL (4.5, 5) days in the second and third larval stages, respectively, while the highest average growth period of the larvae was when treated with the aqueous extract of 30 mg/mL (4.5, 3.7 and

3.4) days in the first, second and third larval stages, respectively, compared to the control larvae, which were (2 , 2.3 and 2.7) days for the three stages, respectively. The age rates of the three larval stages treated with the alcoholic and aqueous extracts were significantly higher at this concentration period compared to the ages of the control larvae.

Table 3. Effect of interaction of different concentrations of the alcoholic extract of the plant (*Thymus vulgaris*) on the growth rate of the different larval stages of the house fly *M. domestica*

Concentration Mg/mL	First larval stage growth period	Second larval stage growth period	Third larval stage growth period
Control	2	2.3	2.7
15	3.4	3	3.7
20	4.2	3	4
25	5.2	3.8	4.1
30	5.7	5	4.5

The value of L.S.D below the level of (0.05) for the interference effect = 0.9. Source: Own authorship.

Table 4. Effect of interaction of different concentrations of the aqueous extract of the plant (*Thymus vulgaris*) on the growth rate of the different larval stages of the house fly *M. domestica*

Concentration Mg/mL	First larval stage growth period	Second larval stage growth period	Third larval stage growth period
Control	2	2.3	2.7
15	3.4	3	3.7
20	4.2	3	4
25	5.2	3.8	4.1
30	-----	5	4.5

The value of L.S.D below the level of (0.05) for the interference effect (none significantly). Source: Own authorship.

Percentage of the death of pupa

The results of Tables (5 and 6) indicate a significant increase in the mortality rates of pupae resulting from larvae treated with different concentrations of alcoholic and aqueous extracts of thyme in the first, second and third larval stages compared to no mortality recorded in control pupae, except for the insignificant increase in the mortality rate of pupae resulting from third-stage larvae treated with alcoholic extract at a concentration of 15 mg/ml and control pupae.

The highest mortality rates of pupae (100, 100 and 62.5)% were recorded when treated as first, second and third stage larvae, respectively, at a concentration of 30 mg/ml of alcoholic extract, while the highest mortality rates of pupae (100, 42.8 and

19)% were for first, second and third stage larvae, respectively, at a concentration of 30 mg/mL of aqueous extract. Some pupae showed clear signs of deformity and failed to shed their pupal sheath.

Table 5. The effect of the interaction of different concentrations of alcoholic extract of (*Thymus vulgaris*) plant on the mortality rate of pupae resulting from different larval stages of the house fly *M. domestica*.

Concentration Mg/mL	%Death of pupae resulting from the first larval stage	%Death of pupae resulting from the second larval stage	%Death of pupae resulting from the third larval stage
Control	0	0	0
15	30	18	8
20	70	28.4	14.6
25	100	70	36.2
30	100	100	62.5

L.S.D value below (0.05) level of interaction effect = 11.8.
Source: Own authorship.

Table 6. The effect of the interaction of different concentrations of aqueous extract of (*Thymus vulgaris*) plant on the mortality rate of pupae resulting from different larval stages of the house fly *M. domestica*.

Concentration mg/mL	%Death of pupae resulting from the first larval stage	%Death of pupae resulting from the second larval stage	%Death of pupae resulting from the third larval stage
Control	0	0	0
15	5	4.1	3.9
20	15.6	6	5
25	45.3	20.4	11
30	100	40.8	19

L.S.D value below (0.05) level of interaction effect = 4.4.
Source: Own authorship.

Average weight of pupae

The results of our current study, as illustrated in Tables (7 and 8), showed a decrease in the weight rates of the pupae treated with different concentrations of the alcoholic and aqueous extracts of the plant under study. The relationship between the weight rates of the pupae and the concentrations used in the experiment showed an inverse relationship. The lowest weight rates (14, 19, 22) mg were recorded in the pupae resulting from the first, second and third larval stages at a concentration of 25 mg/ml of the aqueous extract, while the lowest weight rates (15 and 18) mg were recorded in the pupae resulting from the larvae treated in the second and third stages at a concentration of 25 mg/ml of the alcoholic extract compared to the weight rate of 26 mg in the control pupae. The lowest weight rates were recorded in the pupae resulting from the first stage larvae treated with

different concentrations of the aqueous and alcoholic extracts.

Table 7. Effect of interaction of different concentrations of alcoholic extract of (*Thymus vulgaris*) on the average weights of pupae (mg) resulting from different larval stages of the house fly *M. domestica*.

Concentration mg/mL	Weights of pupae (mg) resulting from the first larval stage	Weights of pupae (mg) resulting from the second larval stage	Weights of pupae (mg) resulting from the third larval stage
Control	26	26	26
15	16	21	24
20	12	19	20
25	-----	15	18
30	-----	-----	11

L.S.D value below (0.05) level of interaction effect = 1.6.
Source: Own authorship.

Table 8. Effect of interaction of different concentrations of aqueous extract of (*Thymus vulgaris*) on the average weights of pupae (mg) resulting from different larval stages of the house fly *M. domestica*.

Concentration Mg/ml	Weights of pupae (mg) resulting from the first larval stage	Weights of pupae (mg) resulting from the second larval stage	Weights of pupae (mg) resulting from the third larval stage
control	26	26	26
15	21	26	29
20	19	25	27
25	14	19	22
30	-----	17	18

L.S.D value below (0.05) level of interaction effect = 2.2.
Source: Own authorship.

Discussion

In this study, the results we have reached can be interpreted as there being a direct relationship between the larval mortality rates and the concentration used, as it was found that the extract at low concentrations prevents feeding, but with increasing the concentration of the extract, the active substances in it have effects similar to the effect of the moulting hormone analogues, which have a fatal physiological effect on the generating cells and their failure to perform their functions, as these analogues work on the processes of moulting and development [28].

Study explained that the cause of death of larvae treated with some plant extracts occurs when toxic substances reach their mid gut, which leads to damage to the epithelial layer lining it, obstructing the secretion of digestive enzymes and thus the death of the larvae

[29], while [30] attributed the cause of death of rice leaf worm larvae *Cnaphalocrocis medinalis* when treated with saffron extracts to the effect of the active substances on the physiology of nutrition, especially on the activity of enzymes present in the digestive system. The reason why mortality rates are higher in the first stage of larvae than in later stages may be because the cuticle layer surrounding the larvae is weaker at the beginning of their development or because newly hatched larvae require a lot of food to grow. This leads to a lot of extract entering the digestive tract with the food, causing the larvae to die early from malnutrition [31]. While [32] offered an explanation for why the first stage is more sensitive than the second and third stages because the first stage lacks this enzyme system and is unable to accomplish this.

The reason for the increased growth period of the larval stages is the inhibition of oxidative phosphorylation of the mitochondria present in the cells of the mid-gastrointestinal tract tissues. This was explained by [33]. The growth period of immature stages of the house fly treated with the ethyl alcohol extract of the *Peganum harmala* plant increased to 8.12 days at a concentration of 5 mg/ml compared to 9 days in the control treatments, this is what was found [34], which is consistent with the results of our current study. An increase in the growth duration of immature stages of the house fly was observed as a result of treatment it with the ethyl alcohol extract of the leaves of the *Clerodendrum Inerme*, which reached 14.8 days at a concentration of 20 mg/mL compared to 9 days in the control treatment [35]. There are other studies that support the results of the current study, which indicated an increase in the average lifespan of larvae as a result of treating them with plant extracts, such as the study [36], which found an increase in the growth period of the larval stages of the watermelon fly *Bactrocera cucurbitae* when treated with a concentration of 125 ppm of the acetone extract of *Acacia auriculiformis*, where ages were recorded at 24.9, 11.0 and 2.27 days for the first, second and third larval stages, respectively, compared to the ages of the control larvae at 25, 20 and 6.58 days.

The reason for the effect of the alcoholic extract of the plant under study on the death and deformity of the pupae is due to one of the compounds contained in the extract, including (carvacrol and thymol), which may have caused disturbances in the division of the cells that make up the different parts of the body or a malfunction in the work of the hormone responsible for the emergence of adults from the pupal stage, as shown in figure (1), which was explained by [37], and the explanation of [30] about the reason for the death

of virgins treated with neem extract is due to the presence of the compounds azadirachtin and salinin, which belong to the group of limonoids, which are attributed to the lethal effect on insects, through studying the efficiency of Neem extracts against rice leaf worm *C. medinalis*.



Figure 1. Malformations in the Pupa stage. Source: Own authorship.

Stankovic et.al [38] explained the reason for the appearance of deformities in pupae and the adult insect, especially when the exposure period to the extract increases and at high concentrations, it is likely that there are insect growth regulators in the plant, as it was found that these compounds have an effect in controlling the successive formation processes through their effect on the nervous secretion system of the insect sensitive to these compounds, and thus will lead to inhibiting the growth of insect stages, as well as the effect of insect growth regulators present in the plant in inhibiting the effectiveness of the hormone that stimulates the release of the moulting hormone called (PTTH) Prothoracicotropic Hormone, which is found in the cardiac body Corpora Cardiaca, and this in turn leads to a decrease or severe slowdown in the secretion of the moulting hormone necessary for the moulting process to occur.

The results of the current study also agreed with [39] found regarding high mortality rates of housefly pupae reaching 100% when using ethyl alcohol extract of the plant *Melia azedarach* at a concentration of 40 micrograms, in addition to the occurrence of morphological deformities in the pupae. He attributed the reason for this to the effect of the active compounds in the moulting hormone through the inability of the pupae to develop into the adult stage. The morphological deformities were represented by the appearance of complete individuals with short wings,

some with deformed or irregular wings, or the pupae die and do not emerge as adults.

Alkan et.al. [40] explained the reason for the decrease in the weights of the pupae treated with the extracts, which are larvae, as a result of the presence of stomach toxins that obstruct the normal movement of the intestines and thus affect the course of digestion and absorption in the intestines, which negatively affects the weights of the pupae resulting from the treatment [34] also mentioned that the weights of the pupae of the house fly treated in the larval stage with ethyl alcohol extract *Peganum harmala* decreased to 0.06 g at a concentration of 5 mg/mL compared to 0.1 g in the control treatments [35] mentioned that the weights of the pupae of the house fly resulting from treating them as larvae with ethyl alcohol extract *Clerodendron inerme* at a concentration of 20 mg/mL decreased to 0.05 g compared to 0.13 g in the control treatment. These results are consistent with the results of our current study.

Conclusion

The study demonstrated, thyme extracts, whether alcoholic or aqueous, have a detrimental effect on every stage of the insect larval life cycle. Additionally, the plant's alcoholic extract was shown to be efficient against housefly larvae. As a result, thyme extracts can be regarded as an environmentally safe and efficient natural insecticide that attacks insect larvae without harming other plants or organisms.

CRedit

Author contributions: Conceptualization, methodology, investigation, data collection, laboratory work, formal analysis, data interpretation, writing—original draft: All authors; Supervision: Nebras Mohammed Sahi.

Acknowledgment

We would like to thank the department head and his distinguished staff for working in the department's laboratories.

Ethical Approval

Not applicable.

Informed Consent

Not applicable.

Funding

No outside funding was obtained for this study.

Data Sharing Statement

On-demand access to the data is possible.

Conflict of Interest

The authors declare no competing interests.

Similarity Check

It was applied by Ithenticate®.

Application of Artificial Intelligence (AI)

Not applicable.

Peer Review Process

It was performed.

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