Effect of aqueous extract of *Vicia faba* peels on the mortality of immature stages and the inhibition of emergence of *Culex quinquefasciatus* (Diptera: Culicidae) mosquitoes.

Sarah Kadhim Al-Rahimy1,*, Alaa Sajjad AlKhafagi2,*, Balqees Hadi Al-Musawi1,*, Rafid Abbas Al-Essa1,*, and Iqbal Khawwam Khshayyish1

1 College of Science, University of Kerbala, Iraq
2 General Directorate of Education of Holy Kerbala, Iraq
3 College of Education for Pure Science, University of Kerbala, Iraq

Correspondence: sarah.k@uokerbala.edu.iq, Dr.alaa77alaa77@gmail.com, Balqees.hadi@uokerbala.edu.iq, Rafid.abbas@uokerbala.edu.iq

Abstract: The effectiveness of the aqueous extract (cold and boiled) of bean peels was studied, and the active compounds were determined by Fourier Transform Infrared Spectrophotometer (FT-IR), and concentrations (1, 3, 5) mg/ml were used when treating fourth-instar larvae and the pupae of Cx. quinquefasciatus mosquitoes and its effect on the percentage of the mortality of the fourth larval instar, the pupa stage and the inhibition of emergence of adults when treating the fourth larval instar and pupa stage of the insect in Kerbala governorate for March 2022 to control mosquitoes.

Keywords: *Culex quinquefasciatus*, Plant extracts, *Vicia faba*.

Introduction

Insects are widespread organisms in the environment, and the order Diptera is one of the most significant insect orders in the world. Mosquitoes, one of the insects that feed blood by sucking, return to Diptera. Mosquitoes, one of the insects that feed blood by sucking, return to Diptera1, a

It is considered a Cx.quinquefasiatus Mosquito, belonging to the familyCulicidae, is of medical importance for transmitting many diseases to human, including western encephalitis, Rift valley fever, elephantiasis, meningitis and other disease central and southern region. 2 It can be said that all the efforts to comat this didn’t lead to the achievement of all planned goals for several reasons, including the nature of the life of this insect because it is one of the household insects that lives very close to humans, so it require caution when using chemical pesticides to controlling it, 3 as a chemical pesticides are quick way to control them and thus control the pathogens that cause or transmit them, and because of the mortality of non-target organisms and it appearance of resistance to these pesticides 4,5; therefore, has been resorted to the use of safe materials for humans and for non-target organisms; and among these material is the use of plant extracts resulting from plant or their waste, which are natural and inexpensive products it acts as an alternatives to chemical control 6,7; as the peels of the banana plant Musa acuminate had lethal effects on mosquito larvae and pupa 8. *Vicia faba* plant belongs to the Fabaceae family. It possesses many active compounds 9, and due to a lack of studies of the extract of bean peels...
in the control of mosquito larvae, this study included the effect of aqueous extract of bean peels on:

1. Study the effect of bean peels' cold and boiled water extract on the percentage of fourth larva instar mortality and the pupal stage of culex quinquefasciatus mosquito.

2. A study of the cumulative mortality of the immature stages of the insect and the percentage of inhibition of adult emergence.

3. Identification of the active compounds in plant extracts by (FT-IR)

Material and Methods:

Collection and diagnosis of plant samples:
Samples of the Vicia faba plant bean peels were collected from the Kerbala governorate markets in March 2022. It was dried ground and placed in a glass bottle, and the name of the Vegetable part was recorded and kept in the refrigerator until use. Dr. Balqees Hadi Hashem diagnosed the plant at the University of Kerbala -College of Science –Department of Biology.

Preparation of plant extracts:
Adopted method (10) modified from (11) in the preparation of aqueous extract (cold and boiled).

Collecting and diagnosing samples of culex quinquefasciatus mosquitoes and laboratory breeding:
Mosquito egg boats collected from an open pool of water measuring (2*2) m² in one of the orchards in Al-Hur district, Kerbala governorate on 19/2/2022. To diagnose mosquitoes within the study region, Samples were taken from the fourth instar larvae and diagnosed using the taxonomic key (12).

Mosquito breeding:
The collected egg boats were isolated from the site covered by the study, and each boat was placed in a plastic container (500)ml containing tap water. Pupa was transferred to a breeding cage with metal wire on four sides and tulle on the fifth side. The adult males were fed a sugar solution with a concentration of (10)% and placed in a glass dish, While the females were fed using a pigeon that had it removed in the chest area. It is fixed above the cage by tying its wings and legs so that the females can quickly get the blood meal needed for feeding. The culture was purified for three generations before conducting experiments on it. This breeding method was used to obtain the fourth larval instar and pupae to conduct the experiments mentioned later.

Effect of aqueous extract of bean peels on the biological performance of the insect:
(10) larvae/duplicate was taken from the fourth instar larvae, and (10) pupa/duplicated with a rate of (3) replicates for each concentration. They were transferred to plastic containers with a capacity of 100 ml containing the aforementioned extracts (1, 3and 5)mg/ml for aqueous extracts (cold and boiled) with breadcrumbs to feed the larvae and without breadcrumbs when treating the pupae. As for the control treatment, only distilled water was used. She was transferred to the laboratory, and her life was monitored. The mortality rate was recorded in the fourth larval instar, the pupal stage until they reached adulthood.
The rates representing the percentages of mortality were recorded according to the Abbott formula:

$$\text{Corrected mortality} \% = \frac{\text{mortality in treatment} - \text{mortality in control}}{100 - \text{mortality in control}} \times 100$$

The percentage inhibition of adult emergences was calculated by the percent of inhibition emergence (I.E.):

$$\text{I.E}=100 - (T \times 100/C)$$

T: The percentage of the emergence of the treatment
C: The percentage in control

The relationship between these values and concentrations was represented graphically on logarithmic sheets to draw the toxicity line and calculate the LC$_{50}$ value.

The active compounds were identified from the plant extract by light absorbed by the Fourier Transform Infrared spectrometer. These Samples were diagnosed in one of the laboratories of the College Of Science-Department of the University of Kerbala.

**Statistical Analysis**

The study experiment results were analyzed according to the factorial experiment model, and with a completely randomized design, the mortality rates were adjusted according to equations $^{13,15}$, the corrected values were adjusted into angular values for inclusion in the statistical analysis.

**Results:**

The effect of cold and boiled water extract of bean peels on the percentage of cumulative mortality of immature stages and percentages of inhibition of adult emergence of culex quiquefasciatus mosquitoes:

Table 1 shows the effectiveness of cold and boiled extract of bean peels on the cumulative mortality of immature stages of the insect; the percentages of cumulative mortality of immature stages in the cold extract of bean peels were (60,90,100)% respectively in the concentration (1, 3, 5) mg/ml, and (70,95,95)% respectively in the same concentration when using the boiled extract of the bean peels compared with 0% in the control treatment.

The result of drawing the Toxicity lines of the cold and boiled water extract of the bean peels indicated an effect on inhibiting the emergence of adult Culex quiquefasciatus when treating the fourth instar larvae, as it was observed that there was a direct relationship between the rate of inhibition of emergence IE% and the concentration used. When using cold water extract, the percentage rate of inhibition of emergence reached (83.34,98.45)% when using concentration (1,3)mg/ml, respectively. This percentage increased to 100% at the (5)mg/ml concentration. When calculating the value of half-lethal concentration (LC$_{50}$), it was (0.75,0.8)mg/ml when using cold and boiled aqueous extract of bean peels, respectively.

<table>
<thead>
<tr>
<th>Con. mg/ml</th>
<th>% cumulative mortality of immature stage of cold water extract</th>
<th>% cumulative mortality of immature stage of boiled water extract</th>
<th>% inhibition emergence of the adult stage of cold water extract</th>
<th>% inhibition emergence of the adult stage of boiled water extract</th>
</tr>
</thead>
</table>
From the preceding, the effect of the two extracts was approximately a large percentage to some extent in the percentage of the cumulative mortality of the immature stages. Regarding the percentage of inhibition of the emergence of adults, it was noted that the cold extract was more effective than the boiled extract of bean peels. The results of the statistical analysis indicated that there were significant differences in the results obtained.

Study 16 confirmed the superiority of aqueous extract of Nerium Olteande larvae in the vital efficiency of the cumulative inhibition of larvae Cx.quiquefasciatus mosquitoes; the 3000pp concentration gave a 100% mortality rate.

A study 17, confirmed that increasing the concentrations of the extract led to an increase in the rates of mortality for immature stages when studying the effect of concentration of cold and boiled water extract of Eucalyptus microthica F. Muell on the percentage of cumulative inhibition of Cx.molestus mosquitoes when using concentrations (1,5,10) mg/ml and their effect on some aspects of mosquitoes life. The effect of boiled water extract of Eucalyptus leaves was more effective than boiled water extract of roots and stems of Eucalyptus and the effect of cold water extracts of Eucalyptus leaves was more effective than cold water extract of roots and leaves of Eucalyptus in the percentage of cumulative mortality, and the percentage of inhibition of adult emergence positive relationship to the concentration used, as for the effect of the fourth instar of larvae and pupae inhibition. We notice that the highest percentages of inhibition happened when using cold and boiled water extract. It was also observed that the leaf extract caused many morphological distortions during the experiments.

The effect of cold and boiled water of bean plant peels on the corrected percentage of fourth instar larvae and the pupa stages of Cx.quiquefasciatus:
Table 2 shows the effect of cold and boiled extract of bean plant peels on the corrected percentage mortality of the fourth larval instar and the pupa stage of the insect. The percentage of the insect mortality was (47, 78, 100)% respectively, in the concentrations (1, 3, 5) mg/ml used when treated in the cold extract, the percentage of the fourth larval stage mortality of the insect was (48.33, 65, 82)% respectively for the same concentration when treated with boiled extract compared with (0)% in the control treatment.

The value of the lethal concentration for half of the individuals (LC50) treated with cold and boiled extract was (1.2, 1.3) mg/ml respectively.

The percentage of mortality of the pupa stage when treated with cold extract was (25, 31, 62)% in the concentrations (1, 3, 5) mg/ml, while in the boiled extract, the percentage of mortality of the pupa stage was (15, 22, 65) respectively in the concentrations (1, 3, 5) mg/ml compared with (0)% in the control treatment.

The value of the lethal concentration for lethal of individual (LC50) treated with cold and boiled extract was (4.5, 4.1) mg/ml respectively.

<table>
<thead>
<tr>
<th>Concentration mg/ml</th>
<th>% mortality fourth instar larvae to insect at treated with the cold extract</th>
<th>% mortality fourth instar larvae to insect at treated with the boiled extract</th>
<th>% mortality pupa stage to treated with the cold extract</th>
<th>% mortality pupa stage to treated with the boiled extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>47</td>
<td>48.33</td>
<td>15</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>78</td>
<td>65</td>
<td>22</td>
<td>31</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>82</td>
<td>65</td>
<td>62</td>
</tr>
<tr>
<td>L.S.D.</td>
<td>1.94</td>
<td>2.86</td>
<td>0.93</td>
<td>0.54</td>
</tr>
<tr>
<td>LC50</td>
<td>0.8</td>
<td>0.2</td>
<td>4.5</td>
<td>4.1</td>
</tr>
</tbody>
</table>

Table 2. The effect of cold water extract of bean peels on the corrected percentage of fourth instar larvae mortality and pupa stage of Culex quinquefaciatus.

From the results presented in Table 2, it was found that the cold extract of bean peels was more effective than the boiled extract, and the effect differed according to the concentration of the extract when treating the fourth larval instar. The effect on the insect pupa was more significant in the boiled water extract than the cold extract of the bean peels, and the statistical analysis results indicated no significant differences in the results obtained.

Study 16 explained the effect of the aqueous extract of the leaves Datura innoxia, E. microtheca, the Cx. quinquefasciatus, The corrected mortality percentages for fourth instar larvae were (0.20, 71 and 29.82)% using concentrations (1000, 5000 and 7000) ppm for Eucalyptus and Datura, and the corrected mortality percentage for fourth instar larvae was (1.42, 27.14 and 37.14)% for the aforementioned concentrations. The Eucalyptus extract was superior in bio-efficiency on the Datura plant. The effect of the aqueous extract of Eucalyptus leaves was superior to the aqueous extract of Datura in the bio-efficiency of the cumulative effect of Cx. quinquefasciatus larvae; this superiority began with the decrease of the Eucalyptus plant with high concentrations and the superiority of the Datura plant by its effect on larvae in
high concentrations, as the mortality rate reached 63.57% at a concentration of 7000ppm for Eucalyptus plant, while for Datura plant 81.42%

Study a,b showed the effect of concentration of cold and boiled water extracts of Mentha spicata L. when using concentrations (1,5,10) mg/ml when treating fourth instar larvae of Cx. molestus their effect on some aspects of the life mosquitoes and the result of the study confirmed the effect of cold and boiled water extract on leaves, stems and roots of the mint plant, and it was noted the appearance of morphological deformations of the stages treated with aqueous extracts.

Detection of effective chemical compounds in aqueous extracts (Cold and boiled) of the peels Vicia faba plant by using the FT-IR technique:

The technique of infrared spectroscopy FT-IR showed several absorbances in the form of bundles and their peaks, and the effective chemical compounds in the bean peels in Table (3), which showed the most critical effective compounds in the cold aqueous extract which showed the absorption band at a frequency of 3365.90cm⁻¹ due to the stretching of the Hydroxyl group (O.H.). The absorption band at frequency 2931.90 is due to stretching Aliphatic (C-H) bonds, the absorption band at frequency 1643.41 is due to stretching the Carbonyl group (C=O), and the absorption band at frequency 1615.64 is due to stretching the (C=C) bond, and the absorption band at frequency 1527.67 returns to the flexion bond (N-H), the absorption band at frequency 1410.01 cm -1 is due to the stretching of the (C-N) bond, The absorption band at frequency 1242.20 is due to the stretching of the (C-O) bond, and the absorption band at frequency 1057.03 is due to the structural stretch of the single (C-C) bonds.

As for the boiled aqueous extract of bean peels, it was observed there was an absorption band at frequency 3365.90cm⁻¹ due to stretching of the Hydroxyl group (O.H.) and an absorption band at frequency 2928.04 due to stretching of Aliphatic (C-H) bonds. The absorption band frequency 1647.26 is due to the stretching of the Carbonyl group (C=O), and the absorption band at frequency 1616.40 is due to the stretching of the band (C=C). The absorption band at frequency 1518.03 is due to the flexion band (N-H), and the absorption band at frequency 1410.01cm⁻¹ returns flexion aliphatic (C-N) bonds. An absorption band at frequency 1230.63 is due to the stretching of the (C-O) bond, and an absorption band at frequency 1043.52 is due to the structural stretch of single (C-C) bonds.

<table>
<thead>
<tr>
<th>Type of plant extract</th>
<th>band frequency</th>
<th>effective group type</th>
</tr>
</thead>
<tbody>
<tr>
<td>cold extract</td>
<td>3365.90</td>
<td>Stretching Hydroxyl group (OH)</td>
</tr>
<tr>
<td></td>
<td>2931.90</td>
<td>Stretch (C-H) Aliphatic bonds</td>
</tr>
<tr>
<td></td>
<td>1643.41</td>
<td>Stretching Carbonyl group (C=O)</td>
</tr>
<tr>
<td></td>
<td>1615.64</td>
<td>Stretch bonds (C=C)</td>
</tr>
<tr>
<td></td>
<td>1527.67</td>
<td>flexion bonds (N-H)</td>
</tr>
<tr>
<td></td>
<td>1410.01</td>
<td>Stretch bond (C-N)</td>
</tr>
<tr>
<td></td>
<td>1242.20</td>
<td>Stretch bond (C-O)</td>
</tr>
<tr>
<td></td>
<td>1057.08</td>
<td>Structural stretching of single (C-C) bonds</td>
</tr>
<tr>
<td></td>
<td>3365.90</td>
<td>Stretching Hydroxyl group (OH)</td>
</tr>
<tr>
<td></td>
<td>2928.04</td>
<td>Stretch (C-H) Aliphatic bonds</td>
</tr>
<tr>
<td>Boiled extract</td>
<td>1647.26</td>
<td>Stretch Carbonyl group (C=O)</td>
</tr>
<tr>
<td></td>
<td>1616.40</td>
<td>Stretch band (C=C)</td>
</tr>
<tr>
<td></td>
<td>1518.03</td>
<td>flexion bonds (N-H)</td>
</tr>
<tr>
<td></td>
<td>1410.01</td>
<td>flexion bonds Aliphatic (C-N)</td>
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<tr>
<td></td>
<td>1230.63</td>
<td>Stretch band (C-O)</td>
</tr>
<tr>
<td></td>
<td>1043.52</td>
<td>Structural stretching of single bonds (C-C)</td>
</tr>
</tbody>
</table>

Table 3. Type of influential groups and their frequency in cold and boiled water extract of Vicia faba bean peels using FT-IR technique.

IR technology was used to diagnose the active organic groups through the sites and their peaks of bundles from the extracts plants of Lawsonia inermis, Adhatod fascia and Nicotiana tobacco and the study of the biological activity on the life of some economic and medical insects such as the house fly Musca domestica, Cx. quinequefasciatus and Triplioium castoreum. 19

The FT-IR technique was also used to diagnose the influential groups by the type and frequency of the beam in Eucalyptus microtheca extract, as several bundles appeared in the cold. It boiled aqueous extract and chloroform extract and the effect of these extracts on the percentage of mortality of the fourth larval instar of Culex molestus insect and caused the appearance of morphological abnormalities in the treated larvae, pupae and adult that resulted in them. 18, b

Discussion

The high percentage of larval and pupae mortality of Cx. quinequefasiatus mosquitoes may be to this because the bean peels contain many effective compounds that act as feeding deterrents, such as phenolic compounds, tannins and phenols, or it may be due to the effect of these substances on the digestive and nervous system of the insect through the extracts coming into contact with the cuticle the insect or their entry through the respiratory openings of the insect larvae and pupae 16, 20. Furthermore, this indicates the accumulation of substance effectiveness of the plant under study, which led to the appearance of poisoning cases of the immature stages of the insect. 21 Explained the effect of the boiling water causes inhibition of enzymes that degrade the active compound Esterase, phenolase and hydrolase, which were not affected by these enzymes by cold extract, or it may be a result of feeding the larvae on toxic compounds found in the plant extract that affect the target tissues and cause tissue poisoning larvae 22, 23.

The cause of the mortality of fourth-instar larvae may be due to their sensitivity to the presence of toxic substances in the plant. This substance may have an accumulative effect on the larvae, Which leads to a lack of food conversion efficiency and thus the insect's mortality, as a result of its impact on the rate of digestion and the movement of the digestive tract and increased absorption that leads to reduced growth 24.

Alternatively, the cause of mortality of the four larval instar is due to the effect of a group of microsomaloxidae enzymes present in the epithelial cells of the alimentary canal, which works to remove the toxicity of the natural compounds present in the plant extract, poisoning the insect. Then its mortality is 25, or it may be the reason. The aqueous extract of the plant under study contains compounds that prevent chitin formation in the immature insect's stages. Thus, the molted larval stage is unable to build the new cuticle, which leads to the mortality of the insect 26, or it may be the reason for the inhibition of growth and development of the new cuticle and preventing its hardening as a result of
inhibiting the activity of the enzyme Tyrosinase found in mosquito larvae, as it is directly responsible for hardening the cuticle.

Conclusion
The results of the study showed that the effect of the two extracts was approximately a large percentage to a certain extent in the percentage of cumulative mortality of immature stages; as for the inhibition of the emergence of adults, the cold extract was more effective than the boiled extract of bean peels, and it was found that the cold extract of bean peels was more effective from the boiled extract. The effect differed according to the different concentrations of the extract when treating the fourth larval instar, and the effect on the pupae treated with the boiled extract was more significant compared to the cold extract of the bean peels.

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lyptus microtheca F. Muell extracts on morphological


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