Study of The Effect of Hot Water Extract of Some Plants on The Alfalfa Weevil Hypera Postica (Gyllenhal, 1813) (Coleoptera: Curculionidae)

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Abstract

The research was conducted in the laboratory of insects in the Faculty of Agriculture /the University of Muthanna about studying the effect of Hot water extract of the pomegranate peels, eucalyptus leaves and basil leaves in different stages (egg, larva, pupa and adult) for Alfalfa Weevil Hypera postica. The results showed that the pomegranate peel extract superiority the percentage of mortality in all the stages used in the experiment over the other plant extracts mentioned above, followed by eucalyptus leaf extract and then basil leaf extract. The results showed that the pomegranate peel extract was effective in controlling the larval stage of the insect, especially 1st and 2nd larvae instars in addition to the adult stage, the highest percentage of mortality in the larval stage of the insect at the first larval age was (60.0, 46.6 and 26.7%) for all extracts respectively at 1.5 mg / ml after 5 days of treatment. In the pupae stage the highest percentage of mortality was (16.7, 10.0 and 03.3%) at the concentration of 1.5 mg / ml For all extracts respectively after 7 days of treatment. while the effectiveness was very weak in controlling of the eggs and pupa of insect and all extracts. where the highest percentage of mortality in the eggs was at 1.5 mg / ml. It was (20.0, 10.0 and 03.3%) respectively after 7 days of treatment. While The highest mortality rate in adult was (53.3, 40.0 and 16.7%) At 1.5mg/ml for all extracts respectively compared with control treatment (00.0%) for all the insect stages. The plant extracts used in the experiment at a concentration of 0.5 mg/ml did not show any effect on the rates of insect death after a day of treatment and for all stages that reaching (00.0%).

Keywords: Alfalfa Plant Medicago sativa L., Alfalfa Weevil Hypera postica (Gylle.), Plant Extracts.

1. Introduction

The alfalfa Medicago sativa L is a durable leguminous forage crops grown mainly for animal feeding. The total area planted in the world is estimated at 30 million hectares, mostly in the United States, Argentina [1]. As indicated by the World Food and Agriculture Organization that global production of Alfalfa reached 436 million tons in 2006 [2]. The productivity of the alfalfa in Iraq was estimated at 907 thousand tons, equivalent to 64.5% of the total forage crops as a whole [3].

The alfalfa plant is infected with many agricultural pests that affect its productivity and quality and make it unfit for animal consumption, and among the most important pests that affect the alfalfa crop is the H. postica, which is the most destructive pest of the alfalfa plant in the world, including Iraq, which is one of the insects that feed Voraciously on the alfalfa plant, which is its main host and causes severe damage to it, the damage comes from the larvae and adults of the insect through feeding them on the leaves of the plant leaving only the veins and feeding on the stems of the plant, which leads to a reduction in the yield and quality of the crop [4, 5]. In the case of severe infection, this leads to the death of the plant. Therefore, early intervention is necessary to control this pest.

The control programs of this insect depend on the use of chemical pesticides, in spite of its rapid results, but the use of improperly and high concentrations led to contamination of the environment as well as its negative effects on humans and other organisms and the emergence of insect resistance against them [6]. All these reasons led the specialists in the field of control to pay attention to plant extracts and use them as alternatives to pesticides [7]. Being safe, biodegradable and short-term natural substances, as well as their high efficacy against insects and their low toxicity to humans and beneficial insects [8]. [9] indicated in their study on the effect of extracts of eucalyptus plants Eucalyptus camaldulensis, Oleander nerium and Azadirachta indica on H. postica larvae and adults of the alfalfa Weevil, that the extract of A. indica had a greater effect on larvae and adults compared to the other extracts. [10] was showed in their study on the effect of Lavandula stoechas extract
and *Heracleum persicum* extract on adults of *H. postica*, both of the two extracts had significant toxicity against adult insects. Therefore, it became necessary to maintain biological and food security and protect the environment by limiting the use of chemical compounds, and moving towards the optimal use of organic and selective pesticides and environmentally safe biopesticides [11].

Because eucalyptus plant contains phenols and flavonoids, it is observed that it has the ability to form complexes with calcium and magnesium and thus inhibit growth in insects [12]. The basil plant was found to contain some active compounds such as glycosides, alkaloids, tannins, saponins, flavonoids, phenols and terpenoids [13]. While pomegranate husks contain some active compounds such as Tannic acid and alkaloid substances such as Pelletierine [14]. Due to the types of active compounds possessed by these plants, the research aimed to evaluate the efficiency of the aqueous extracts of these plants in the control of alfalfa weevil in the province of Muthanna.

2. Materials and Methods

2.1 Rearing and Diagnosis of Insect

Adults and larvae of alfalfa weevil were collected from a field planted with an insect-infested alfalfa plant at Al-Bandar Agricultural Research Station of Al-Muthanna University. The insect was diagnosed by some of the professors specialized in the field of insects at the Faculty of Agriculture, and by using the insect classification keys, the insect was reared in the laboratory on a food medium consisting of alfalfa or kari plant leaves from its cultivation areas, The food should be changed every 48 hours to provide fresh and soft leaves, For the purpose of obtaining colony, certain numbers of adults were placed in 1.5-liter glass bottles and Her nozzles were covered with boring cloth and tightened with rubber belts and placed in an incubator at a temperature of 2 ± 30 °C and humidity of 75%.

2.2 Collection of Plant Samples

Plant samples were collected as follows: basil leaves and pomegranate peels were collected from the markets after purchasing them. Eucalyptus leaves were collected from the planted areas in Al Muthanna province.

2.3 Preparation of Aquatic Plant Extracts

[15], adapted and modified from [16] in the preparation of aqueous extracts, the plant leaves have been pulverized for plants shown in Table (1) were grinded with an electric mill after drying by placing 100 g of vegetable powder into 500 ml of boiling distilled water in a 1000 ml glass beaker. Then, shake the mixture manually for 15 minutes to obtain a homogeneous solution, leave the mixture for 48 hours and then filtered using two layers of damp cloth and then transfer the filtrate to the centrifuge at a speed of 3000 rpm and for 10 minutes to obtain a clear solution and neglect the precipitate and then dried in 45 °C Electric Oven and Keep in Refrigerator Prepared the base solution at a concentration of 2 mg / ml by dissolving 2 g of dry matter in 100 ml distilled water to become the concentration of 2%, from which the concentrations (0.5, 1 and 1.5) mg / ml were prepared, the control treatment was boiled distilled water only.

| Number | The used part of plant | The scientific name | The family | The English name |
|--------|------------------------|---------------------|------------|-----------------|
| 1      | Crusts                 | *Punica granatum*   | Lythraceae | Pomegranate     |
| 2      | Leaves                 | *Ocimum sp*         | Lamiaceae  | Basil           |
| 3      | Leaves                 | *Eucalyptus sp*     | Myrtaceae  | Eucalyptus      |

2.4 The Effect of Hot Water Extract for Some Plants on Stages of Insect

2.4.1 The Effect of Hot Water Extract for Some Plants on Eggs

The insect eggs were obtained at the age of one day from the colony that was prepared previously to raise the insect, the eggs were collected by a small soft brush and placed inside the petri dishes diameter (9 cm) at the rate of 10 eggs per dish (repeated) at a rate of three replicates per treatment (concentration) 30 eggs for each treatment, and by three treatments in addition to the control treatment (zero concentration), the dishes were treated with the three concentrations (0.5, 1 and 1.5) mg / ml of hot water extract for both pomegranate peels and eucalyptus and basil leaves, while the comparison treatment was spread with water only by a manual sprinkler of 1/2 liter capacity with a distance of 3.5 cm from the plate to be treated, and at 1 ml per repetition then the treated dishes were transferred to the incubator at a temperature of 30 ± 2 °C and 75% RH. And
the percentage of egg mortality was calculated after 7 days of treatment, and the mortality percentage was calculated based on Abbott equation (17).

2.4.2 Effect of Hot Water Extract for Some Plants on Larvae Instars

Took 10 larvae from freshly hatched first age larvae (at the age of one day) for each repeater and were placed inside petri-plastic diameter plates (9 cm) each dish contains soft alfalfa leaves and then the dishes are sprayed with the same concentrations and in the same way mentioned above in the treatment of insect eggs, As for the comparison treatment it was spread with water only. The dishes were placed in the incubator conditions mentioned previously with three replications for each treatment. The percentage of mortality was recorded in the first larval stage after (1, 3, and 5 days) of treatment and the total percentage of death was calculated, and the same process was repeated for the second and fourth larval stages separately and determined immediately after the Moulting and the mortality ratios were calculated in each of them, and the mortality percentage was calculated based on Abbott equation (17).

2.4.3 Effect of Hot Water Extract for Some Plants on The Pupae

I took 10 pupae (at the age of one day) for each repeater and placed them inside a petri-plastic diameter dish (9 cm) Each dish contains insect food from the leaves of the alfalfa and the dishes are sprayed with the same concentrations and in the same way as mentioned above. As for the comparison treatment they were only sprayed with water and the dishes were placed in the incubator conditions mentioned previously with three replications for each treatment. The percentage of mortality in the treated pupae was recorded after 7 days of treatment and the total death rate was calculated.

2.4.4 The Effect of Hot Water Extract for Some Plants on Adults

I took 10 individuals from the adult of the insect and put it in the dishes of the insect's food container from the leaves of the alfalfa. Then these dishes were treated with the same previously mentioned concentrations of plant extracts to know the extent of its effect on the adults of the treated insect by three replicates per treatment in addition to the comparison treatment that was sprayed with water only. A boring cloth was attached to a rubber band to prevent the adult women from leaving. Then it was transferred to the incubator under the same conditions mentioned previously and the percentage of mortality in the adults of insect were recorded after (1, 3, 5 and 7 days) of treatment and the total death rate was calculated.

3. Statistical Analysis

Follow in the design of experiments complete design randomization (CRD). To compare the results, a test used the least significant difference under the level of significance (0.05), and corrected the percentages of perish according to [17] and then converted the corrected percentages into angle values to be included in the statistical analysis [18].

4. Results and Discussion

4.1 The Effect of Hot Water Extract for Some Plants on Eggs

The results of Table (2) showed a presence of significant differences in the percentages of the mortality of alfalfa weevils eggs at the two concentrations (1 and 1.5) mg / ml in relation to the hot water extract of each plant (pomegranate, eucalyptus and basil) after 7 days of treatment. Where the extract of the pomegranate peels gave the highest percentage of mortality in the eggs of the alfalfa weevil, which reached (13.3 and 20.0%) and for the same concentrations mentioned above respectively, while the lowest mortality percentage of insect eggs when treated with the basil plant extract which reached (90.0 and 03.3%), respectively. As for the extract of the eucalyptus plant it gave a mortality rate which reached (06.7 and 10.0%) to the insect eggs respectively Compared to the control treatment which reached (00.0%) and for all the above plant extracts respectively. While there were no significant differences in the mortality rates of insect eggs at concentration (0.5) mg / ml which reached (00.0%) and for all treatments respectively.

4.2 The Effect of Hot Water Extract for Some Plants on The larval Instar

The results of Table (3) indicated that The pomegranate peel plant extract was superior on the rest of the plant extraxts, as it gave good efficiency in the mortality percentage of larval instar (first, second and fourth) of the insect at concentration (1.5 mg / ml) and after 5 days of treatment. As the mortality rate for the larval ages (first, second and fourth) of the insect with pomegranate peel extract was (60.0, 56.6 and 46.7%) respectively after 5 days of treatment. It is followed by the extract of eucalyptus leaves, which reached at the same concentration and for the same ages mentioned above (46.6, 43.3 and 33.3%)
respectively. While the basil leaf extract did not show good efficiency in the mortality rates of the larval ages mentioned above, which amounted to (26.7, 26.7 and 16.7%) respectively and at the same concentration mentioned above. As for the lowest mortality rates in the larval ages of the insect, it was at a concentration (0.5 mg / ml) for each of the pomegranate plant extract and eucalyptus, which was (16.7, 13.3 and 06.0%) and (13.3, 10.0 and 00.0%) after 5 days of treatment. Whereas, there was no effect of basil leaf extract on the insect’s larval ages that were mentioned above at (5 mg / ml), which were (00.0, 00.0 and 00.0%) respectively, compared with the control treatment, which amounted to (00.0%). The results also indicated that all plant extracts did not show any effect on mortality rates for the larval ages of the insect and for all the concentrations used in the experiment after one day of treatment, which amounted to (00.0%). The results indicated that the mortality rates for the larval ages of the insect and treatment with pomegranate plant extract, eucalyptus and basil at (1 mg / ml) reached (26.7, 26.7 and 20.0%) and (23.3, 20.0 and 16.7%) and (13.3, 10.0 and 03.3%) respectively after 5 days of the treatment compared to the control treatment which amounted to (00.0%). The effectiveness of the hot aqueous extract of pomegranate peel may be due to its possession of a group of important compounds, including tannins and alkaloids, which act as inhibitors to nutrition, as [19] stated that the effect of dietary inhibitors may not be through attracting or expelling the insect, but rather through loss of appetite. Previous studies have shown that the efficacy of eucalyptus is due to the possession of toxic compounds that have a toxic effect on the larvae, whose effects increase over time [20, 21, 22, 23] in their study on the effect of extracts of <i>Eucalyptus camaldulensis, Oleander nerium, and Azadirachta indica</i> on <i>H. postica</i> larvae and adults of the alfalfa Weevil, found that the extract of <i>A. indica</i> gave the highest death rate in the insect larvae of 73.33% within 4 days.

**Table 2. Effect of hot water extract for some plants in eggs of the alfalfa weevil insect <i>H. postica</i> after 7 days of treatment.**

| Plant extracts | Concentrations of extracts (mg / ml) | Percentage rate of Mortality after 7 days of treatment | L.S.D (0.05) for interference |
|----------------|--------------------------------------|--------------------------------------------------------|-----------------------------|
|                | 0.5                                   | 1                                                      | 1.5                         |
| Pomegranate    | 00.0                                  | 13.3                                                   | 20.0                        |
| Eucalyptus     | 00.0                                  | 06.7                                                   | 10.0                        |
| Basil          | 00.0                                  | 00.0                                                   | 03.3                        |
| Control        | 00.0                                  | 00.0                                                   | 00.0                        |

**Table 3. The effect of hot water extract of some plants on some larval stages of <i>H. postica</i>.**

| Plant extracts | Concentrations (mg / ml) | After 1 day | After 3 days | After 5 days | After 1 day | After 3 days | After 5 days | After 1 day | After 3 days | After 5 days | Average of total mortality rates for larval ages |
|----------------|--------------------------|-------------|--------------|--------------|-------------|--------------|--------------|-------------|--------------|--------------|-----------------------------------------------|
| Pomegranate    | 0.5                      | 00.0        | 10.0         | 16.7         | 00.0        | 06.7         | 13.3         | 00.0        | 00.0         | 06.7         | 05.9                           |
|                | 1                        | 00.0        | 26.7         | 00.0         | 23.3        | 26.7         | 00.0         | 20.0        | 20.0         | 20.0         | 15.2                           |
|                | 1.5                      | 00.0        | 60.0         | 00.0         | 43.3        | 56.6         | 00.0         | 40.0        | 46.7         | 46.7         | 32.2                           |
| Eucalyptus     | 0.5                      | 00.0        | 33.3         | 13.3         | 00.0        | 03.3         | 10.0         | 00.0        | 00.0         | 00.0         | 03.3                           |
|                | 1                        | 00.0        | 20.0         | 00.0         | 13.3        | 20.0         | 00.0         | 06.7        | 16.7         | 16.7         | 10.4                           |
|                | 1.5                      | 00.0        | 46.6         | 00.0         | 30.0        | 43.3         | 00.0         | 20.0        | 33.3         | 33.3         | 22.6                           |
| Basil          | 0.5                      | 00.0        | 00.0         | 00.0         | 00.0        | 00.0         | 00.0         | 00.0        | 00.0         | 00.0         | 00.0                           |
|                | 1                        | 00.0        | 06.7         | 13.3         | 00.0        | 06.7         | 10.0         | 00.0        | 03.3         | 03.3         | 04.4                           |
|                | 1.5                      | 00.0        | 26.7         | 00.0         | 10.0        | 26.7         | 00.0         | 10.0        | 16.7         | 16.7         | 11.5                           |
| Control        | 00.0                     | 00.0        | 00.0         | 00.0         | 00.0        | 00.0         | 00.0         | 00.0        | 00.0         | 00.0         | 00.0                           |
| L.S.D (0.05)   | for interference          | N.S         | 2.771        | 11.333       | N.S         | 2.667        | 5.228        | N.S         | 3.523        | 3.112        | 3.045                           |

### 4.3 Effect of Hot Water Extract for Some Plants on The Pupae

The results of Table (4) with respect to the effect of plant extracts on the percentage of mortality of insect pupae when treated with it, indicated that there was little effectiveness for the extracts in the mortality ratios after 7 days of treatment, as the results showed the superiority of the pomegranate crust extract in the mortality ratios where the highest mortality was at concentration 1.5% as it reached (16.7%), followed by the extract of Eucalyptus and basil leaves, where the percentages of loss reached (10.0 and 03.3%), respectively, compared with the control treatment, which reached (00.0%). While the results indicated that there was no effect of plant extracts on the percentage of insect adult mortality at concentration (0.5) mg / ml, as the percentage of perishing reached (00.0%), while the percentage of perishing (06.3 and 00.0%) in insect adults at
concentration (1) mg / ml for each of the plant extract (pomegranate, eucalyptus and basil), respectively. The results indicated that there were no significant differences for the extract of basil leaves and for all concentrations in the rate of insect virgins mortality. [23] indicated in a study on the use of some plant extracts to combat H. postica that Eucalyptus leaf extract had an effect on the percentage of killing of second-year larvae at concentration 40% after four days of treatment at 50.0%. The efficacy of the water pomegranate peel extract may be due to its possession of tannin, [24] indicated that the medicinal properties of pomegranate belong to tannin. As the phenolic compounds present in the pomegranate peels work in their ability to precipitate the proteins present in the cell membrane or inside the living cell and then form hydrogen bonds between free and multiple hydroxyl phenolic groups and nitrogenous compounds or proteins, which leads to the inhibition of the action of some necessary enzymes in the organism [25].

Table 4. Effect of hot water extract for some plants in pupae alfalfa weevil H. postica after 7 days of treatment.

| Plant extracts | Percentage rate of Mortality after 7 days of treatment |
|----------------|------------------------------------------------------|
|                | Concentrations of extracts (mg / ml)                  |
|                | 0.5  | 1 | 1.5 |
| Pomegranate    | 00.0 | 06.0 | 16.7 |
| Eucalyptus     | 00.0 | 03.0 | 10.0 |
| Basil          | 00.0 | 00.0 | 03.3 |
| Control        | 00.0 | 00.0 | 00.0 |
| L.S.D (0.05) for interference | N.S | 1.966 | 2.336 |

4.4 The Effect of Hot Water Extract for Some Plants on Adults

The results of Table (5) showed that there were significant differences between plant extracts in the percentage of adult insect mortality, as well as the presence of significant differences between the overlapping concentrations used for the plant extracts (1.5 Mg / ml) as it reached (50.0 and 53.3%) after (5 and 7 days) of treatment respectively. Followed by eucalyptus leaf extract, where the mortality rates were (36.7 and 40.0%) for the same concentration and the same periods mentioned above respectively. Whereas basil leaf extract showed a weak effect on adult mortality rates which reached (16.7 and 20.0 mg / ml) for the same concentration and the above durations respectively, compared to the control treatment, which amounted to (00.0%). The results also indicated that there was no effect of plant extracts on the percentage of adult insect mortality at concentration (0.5) mg / ml, where the mortality rates were (00.0%) for all periods, while the percentage of mortality was (23.3 and 26.7%) in adults of the insect at concentration (1 mg / ml) of pomegranate peel extract after (5 and 7 days) of treatment, respectively. The mortality rate was (16.7 and 20.0%) in adults of the insect at concentration (1 mg / ml) of eucalyptus leaf extract after (5 and 7 days) of treatment, respectively. Whereas, the mortality rate was (10.0 and 16.7%) in adults at concentration (1 mg / ml) of basil leaf extract after (5 and 7 days) of treatment respectively. The results did not show any effect for the three concentrations (1, 0.5 and 1.5 mg / ml) and for all plant extracts used in the experiment on mortality rates of adult insects after one day of treatment which reached (00.0%). The results showed significant differences in the average percentage of total adult insect mortality for each concentration of plant extracts used for all periods, as the pomegranate peel extract outperformed the rest of the extracts at the two concentrations (1 and 1.5 mg / ml), which amounted to (14.9 and 31.7%), respectively. It was followed by the extract of eucalyptus leaves and basil in terms of mortality rates, which were (10.9 and 23.4%) and (05.8 and 10.1%) respectively. Between [23] when using some plant extracts to control the H. postica Alfalfa weevil, the eucalyptus leaf extract had an effect on the adult mortality percentage at a concentration of 40% after six days of treatment, reaching 23.3%.

Table 5. The effect of overlapping concentrations of hot water extract of some plants in adults of H. postica.

| Plant extracts | Concentrations (mg l / ml ) | The percentage rate of Mortality after |
|----------------|----------------------------|-------------------------------------|
|                | 1 day | 3 days | 5 days | 7 days | Total of mortality rate |
| Pomegranate    | 0.5   | 00.0   | 00.0   | 00.0   | 00.0 |
|                | 1     | 00.0   | 13.3   | 23.3   | 26.7   | 14.9 |
|                | 1.5   | 00.0   | 23.3   | 50.0   | 53.3   | 31.7 |
|                | 0.5   | 00.0   | 00.0   | 00.0   | 00.0   | 00.0 |
| Eucalyptus     | 1     | 00.0   | 06.7   | 16.7   | 20.0   | 10.9 |
|                | 1.5   | 00.0   | 16.7   | 36.7   | 40.0   | 23.4 |
|                | 0.5   | 00.0   | 00.0   | 00.0   | 00.0   | 00.0 |
| Basil          | 1     | 00.0   | 03.3   | 10.0   | 16.7   | 05.8 |
|                | 1.5   | 00.0   | 06.7   | 16.7   | 20.0   | 10.1 |
| Control        | 00.0  | 00.0   | 00.0   | 00.0   | 00.0   | 00.0 |
| L.S.D (0.05) for interference | N.S | 2.221 | 6.667 | 7.323 | 4.221 |
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