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STUDY OF THE EFFECT OF ALCOHOLIC EXTRACT OF *DODONAEA VISCOSA* L. LEAVES ON THE LIFE PERFORMANCE OF THE GREATER WAX WORM *GALLERIA MELLONELLA* L. (LEPIDOPTERA: PYRALIDAE)

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Abstract

The study was conducted to evaluate the effectiveness of the alcoholic extract of leaves in the effect on the percentage of eggs hatching for the major wax worm *Galleria mellonella* (L.) as well as the study of the killer effect of the extract in pupa insects. (2, 5 and 10%) that the percentage of hatching was (20, 13.67 and 12.67%) for the three concentrations, respectively, with the superiority of the concentration 10% compared to the control treatment in which the hatching percentage was 95%. The treatment of pupa showed an effect on the killing rate, with a clear superiority of concentration 10%, a killing rate of 69.26%, and a control treatment of 3.33%. The alcoholic extract of the Dodonaea plant also had an effect on some aspects of the adult female emerging from the treated pupa, as it reduced the age of the male and the average age of the males in the extracted treatments was from 11.33 - 12.67 days, while in the control treatment it was 15.33 days, while the female age rate was 8.67 - 10.67 days and in control 11.33 days in control treatment. The effect of the extract showed significant differences in the average number of placed eggs by females, as the average number of eggs in the three concentrations treatments (2, 5 and 10%) 434, 395 and 210 eggs per female, respectively, compared to the control treatment that recorded 1012 eggs per Female, and there was a slight decrease in the rate of eggs hatching, which ranged from 80-88% in the extracted treatments and for the control treatment 95%. It is concluded from the above that the alcoholic extract of Dodonaea leaves has an effect on the rat of eggs hatching and the number of eggs placed by insect adults and in killing pupa and reducing the age of adults, as well as the ease of extracting it and being safe on the environment and does not cause toxicity to humans.

Key words: Dodonaea, *Galleria mellonella*, wax moth, alcoholic extract.

Introduction

Apis mellifera L. lives in various strains in most parts of the world. Honey bee has received more attention than other insects because of its economic importance and its production of substances with nutritional and therapeutic benefits for some pathological conditions such as honey, wax, royal jelly, propolis and bee poison, and the most important benefits. Agriculturally, it is a major pollinator for plant flowers (Al-Jubouri, 2005). Honey bees *Apis mellifera* (L) infects inside and outside hives with many insect pests, the most important of which is economically the major wax worm *Galleria mellonella* L. that attacks wax frames inside bee hives and in the store where they feed on their contents of pollen, brood, ecdysial skins, and honey and also cause damage Wood for frames and bee hives as a result of excavation for the purpose of exclusion is more harmful to weak bee hives or castes (Warhurst and Goebel, 1995). Katsab (2009) and Farmnote (2007) indicated that the main habitat of the great wax worm is the continent of Asia and is widely spread in tropical and sub-tropical regions and in all regions of the Arab world. And it has great economic damage in many countries. The rate of losses due to it in Iran was estimated at 38% and 4 million dollars in America in 1976 and increased to 8 million dollars in 1984 (Farmnot, 2007: Tsurikov, 2006 a ; Tamigi and Akbarzadeh, 1982). Al-Zubaidi (1999) mentioned that it is one of the most important insects of mites that attack wax combs and spread in all countries of the world except for low temperatures and spread in all regions of Iraq, especially

northern because of the spread of beekeeping there and the use of old local bee hives that are suitable hideouts for laying eggs and the development of larvae causing economic losses Great as a result of the primary methods used in beekeeping For the purpose of wax worm resistance, there are several methods used for bee hives and other stored wax frameworks, including conventional, biophysical and chemical methods such as vaporization using sulfur and formic acids (Calderone, 2000(. Because of the development of resistance of pests against pesticides due to their indiscriminate use, the use of plant-based substances has at present become important in pest control (Gonzalez Gomez *et al.,* 2006).And the use of plant extracts to resist the great wax worm and protect bee hives because they are less polluting to bees and humans, in addition to that plant material is cheap and effective for beekeepers, and it can also be used at the same time to resist other pests, including Varroa and mite (Ayman and Atef, 2007). Redwane and others (2002) mentioned that the use of plant materials as pesticides as a substitute for chemical pesticides reduces their harm to humans and other non-target organisms, as well as the effectiveness of plant pesticides against insect pests and their characteristic of rapid decomposition and does not cause pollution to the ecosystem and low toxicity to the customer and the most important feature of the lack of the characteristic Resistance to it by insects. Kayode (2006) used the hot and cold water extract of the *Gliricidia sepium* plant against the cowpea beetle *C. maculatus* where the hot water extract had the greatest effect against the insect.

Al-Jurani and others (1998) studied the effect of various botanical powders in control the major wax insect, including the eucalyptus powder, by evaporating wax combs using a blower, as it gave complete protection to the wax frames from infection for seven months. Sabr *et al.,* (2003) found that the treatment of a 24-hour wax larvae ages with an alcohol extract of eucalyptus plant by 10% caused a significant increase in the duration of the larval phase which reached 32.30 days while it was 27.24 days in the control treatment. Al-Jurani and others (2004) also showed in a study that exposing wax eggs, larvae, and adult greater wax moth, to the smells of eucalyptus leaf oil had a clear effect on them. Where the Arab Organization for Agricultural Development (1988) stated that *Dodonaea viscosa* (L.) is characterized by the presence of citrullus, tannins, saponins and alkaloids in addition to cyclosides as secondary compounds. Dodonaea in its chemical composition contains biochemical compounds including phenols, resins, coumarin, oils, sterols and undiagnosed alcohols (Sachdev and Kulshreshtha, 1986; Van *et al.,* 2000; Abdalmogib *et al.,* 2001.(

Al-Ardi and Taha (2009) conducted a study of the effect of extracting cold, boiled and alcoholic water of Dodonaea leaves on an insect of green peach aphid *Myzus persicae*. Insect decomposition of toxic compounds of cold and boiled water extractors, as well as the toxicity of alkaloid compounds in Dodonaea leaves. And to not use the Dodonaea plant as a pesticide to control the major wax worm and to reduce the use of chemicals in controlling the insect, this research was conducted to study the effectiveness of the alcoholic extract of the Dodonaea leaf in affecting the greater wax moth.

Materials and Methods

Collection and drying of Dodonaea leaves

Modern leaves were collected from Dodonaea trees planted from Baghdad Governorate - Dora from public parks during the flowering period starting from March 2019, after collection the leaves are cleaned from soil and brushed on newspapers at the laboratory temperature with ventilation and continuous stirring to prevent the occurrence of rot until they dry Completed up to ten days. After drying, the dry leaves were ground with a mill (BUHLER SPA MILANO VIA. GOLDONI, 17), at a temperature of 30-40c°. The powder was not divided into nylon bags, each bag contained 300 g, the sample information was recorded and stored in the freezer until extracting.

Preparation of an alcoholic extract for Dodonaea

Take the powder of the previously prepared Dodonaea leaves and put 300 g in a 2 liter glass jar (flask), add ethyl alcohol (ETHANOL, abs. 96% en C_2H_5OH, Belgian) which is a high polarity solvent added so that it covers the powder in the range of 2-3 In which a 1 liter ethyl alcohol / 300 g powder was added, the beaker nozzle was covered with cotton well and left for three days with constant stirring and for certain periods of mixing the powder with alcohol until extracting and melting the powder with alcohol. After three days, the dried leaves of Dodonaea leaves with ethyl alcohol are taken and filtered in Buchner's funnel. To obtain a pure leachate of impurities, it was then filtered in a glass funnel by placing a damp cloth in its mouth. Put a cotton under it. The filtrate was taken after the filter and represented the active substance of the powder with alcohol concentrated or dried to get rid of Solvent (ethyl alcohol) using a rotary evaporator (ROTARY WITH VACCUME EVAPORATOR) with a suction pump (cold air) with cold water to accelerate extraction and increase condensation (Harborne, 1973) To obtain the raw extract,

where the flask of the evaporator containing the leachate is placed in a water bath at a temperature of (40 - 60 c°) after approximately 3 hours we obtain an extract with a viscous leaching, placed in sealed glass cans with the weight of the extract obtained. An average of 40.75 g / 300 g powder as all the plant specimen information was recorded on it and placed in the freezer until concentrations are prepared and used in the experiment treatment.

Preparation of plant extract concentrations

Concentrations 2, 5 and 10% were chosen to prepare them from the alcoholic extract. Each weight of the extract was dissolved for the three weights in ethyl alcohol, and complete the volume to 100 ml of alcohol to obtain the above concentration

Insect breeding

To provide the phases of the insect on a continuous basis, a wooden breeding box was made with dimensions (30 x 30 x 30 cm), the base of which is wood, the three sides of the metal wire, and the facade of the millimeter cloth, in which wax frames were severely infected with the major wax worm brought from different apiaries in Baghdad and placed in the laboratory For adults and other phases (Al-Jurani, 1991)

Study the effect of ethyl alcohol extract for Dodonaea leaves on eggs percentage hatching for greater wax worm

Female and male puberty were isolated no later than 24 hours from the insect farm prepared at the beginning of the work by 3 males and 3 females in plastic boxes 15 cm high and 9 cm in diameter sterilized with alcohol at a concentration of 99%, provided cans with cotton saturated with a sugar solution with a concentration of 10% in a glass tube placed In the box and replace every 2-3 days when they dry and prevent contamination with fungi, with a 5 x 5 cm black paper folded in the form of folds and a dull cloth at the base to put the female on it eggs so it is easy to collect and count and see the eggs easily, The cans were sealed with a boring cloth with a rubber band and put the cans in an incubator type (Nahita-639/150) at a temperature of 30 ± 1 m and a relative humidity of 70 ± 5% and completely dark. After mating and laying eggs, they are collected at the age of one day by cutting the paper or the fabric of the millet placed on the eggs and distributed on the repeaters for each treatment in appropriate numbers to calculate the rate of hatching, where the eggs were treated with the three concentrations of the alcoholic extract (2, 5 and 10%) with the control treatment using ethyl alcohol only, so it is 4 treatments and each treatment has 3 replicates. Putting eggs in Petri dishes (1.5X9 cm) with filter papers (Whatmon No. 1 The eggs were treated directly using (Micro applicator), ¼ ml for each of the three concentrations and the alcohol was left to dry and then the treated eggs were placed in sterile plastic boxes 6 cm high and 14 cm in diameter and equipped with pieces of sterilized wax by cooling, put the cans in the incubator on the same previous conditions where Monitor them within 7-10 days to calculate the percentage of hatching eggs per repetitive equals (number of eggs hatched / total number of eggs X 100) and then hatch rate is extracted for each treatment.

Study of the effect of ethyl alcohol extract of Dodonaea leaves on the death of pupa of the greater wax worm and the life performance of adults produced from pupa treated with the extract

Larvae were isolated in the last phase before pupation and placed in sterile boxes 6 cm in high and 14 cm in diameter to obtain pupa at the age of one day, 10 pupa were taken for each repeater (4 females and 6 males). The pupa were placed in a 1.5X 9 cm Petri dish at their base (Whatmon No. 1) filter paper. The pupa were treated with alcohol extract concentrations (2, 5, 10%) and with three replicates per concentration (treatment). The control treatment was sprayed with ethyl alcohol only, the treatments were sprayed. With a hand sprinkler of 10 ml, 15 cm away vertically, at a solution of 3 ml of each concentration, the treatments were allowed to dry and evaporate the alcohol, and then placed in sterile plastic boxes 9 cm in diameter and 16 cm in height, covered with mull cloth and attached with a rubber band, and placed in the incubator under the same conditions until The emergence of adults and the calculation of dead pupa and their impact on adults,

Statistical design and analysis

The experiments were designed according to the design of complete randomized design (CRD). The results were statistically analyzed according to the method of ANOVA Analysis Of Variance. The mean differences between the averages were compared with the Least Significant Difference_ LSD at 0.05 (Sahuki and Wahib, 1990),

Results and Discussion

Effect of ethyl alcohol extract of Dodonaea leaves on egg hatching percentage of the greater wax worm

It is clear from table 1 that the concentrations of alcoholic extracts of Dodonaea leaves have an effective effect in inhibiting egg hatching ratios, as the effect of the extract clearly on egg hatching ratios which were 20, 13.67 and 12.67% for concentrations of 2, 5 and 10%,

respectively. There were no significant differences between the concentrations while there was a clear significant difference between the concentrations treatments of the extract with the control treatment and with a ratios of hatching of 95%, and this was confirmed by the statistical analysis. Sabr *et al*., (2004) confirmed that the alcoholic extract of Eucalyptus leaves and fruits in concentrations 1, 5 and 10% reduced the percentage of hatching in eggs of worm insects treated superficially with the concentrations of the extract at one day of age as it reached 8.7% either in control treatment was 91.66%.

Al-Hamdani (2016) confirmed in a study conducted to evaluate the effect of different concentrations of alcoholic extract (3, 5, 7%) for the *Adhatoda vasica* Nees plant to control the major wax moth insect, *Galleria mellonella*, in laboratory conditions that showed that the extract had an effect in inhibiting the hatching of the insect's eggs, where the average Inhibition rate (60.256, 79.059, 93.331%) for concentrations at respectively and 5.55 in the control treatment.Al-Omairi (2013) confirmed that the use of volatile oil for caraway plant *Carum carvi* in concentrations (1, 1.5 and 2%) had a significant impact on the hatching percentage of the wax worm eggs, where the hatching rate (83, 17.3 and 13.7%) for the above concentrations, respectively, while The percentage of hatching in the control treatment was 99.7%. Between Subashini *et al.*, (2004), the hexane extract of *D. viscosa* reduced fertility and hatch ability in *H. armigera* eggs, caused an increase in the mortality rates of the larvae, and reduced pupal period in the insect. The study agrees with the results of (Al-Qazzaz, 2010) that the ethyl alcohol extract of the leaves of the *Dodonae viscosia* (L.) in controlling the growth and survival of the cowpea beetle *C. maculatus* (Fab.), and indicated that the percentage of hatching due to the insulating layer formed by the extracts on the outer shell of the eggs and prevented the exchange of air between The fetus and the outer circumference. Malaruannan *et al.*, (2004) studied extracts of several plants, including *Dodonaea angustifolia*, against *Helicoverpa armigera* (Hubner). They found that the chloroflum extract of Dodonaea caused no eggs hatching at the age of 24 hours, and hexane and acetone extracts caused the no eggs hatching to age 72 hours.

Effect of ethyl alcohol extract of Dodonaea leaves on the death of pupa of the major wax worm and the life performance of adults resulting from viruses treated with the extract

(Table 2) shows that the alcoholic extract of Dodonaea leaves has a clear effect on the death events of the pupal when treated with concentrations (2, 5 and 10%), and the killing ratios are 37.78, 65.56, and 69.26%, respectively. As for the killing rate in the control treatment, it was 3.33%. With the occurrence of abnormalities in the emerging adults from the treatment of the two concentrations 5 and 10% and the incomplete growth of the adult within the treated pupa, a small size was observed in some of the emerging males of the pupal, and showed statistical analysis, the two concentrations were given more efficient in killing rates with a high significant difference with a control treatment. There were also slight differences that were not significant in the age of the pupal between the treatments, which amounted to 11.1 - 12.83 days, with a significant difference with the control treatment, which reached 9.83 days. It had an effect on reducing the age of the males and reached (12.67, 11.33, 12.33 days) for the three concentrations, respectively and 15.33 in the control treatment. As for females, their average age was 8.67-10.67 days for treatment concentrations and 11.33 in the control treatment. There were no significant differences between the treatments concentrations and with the control treatment. The results showed a clear decrease in the number of eggs laid by females compared to the control treatment, where the number of eggs laid 434,, 395,210 eggs / female for the three concentrations, respectively and 1012 eggs / female in the control treatment with a clear significant difference between the extracts treatment and the control treatment.There were no significant differences in the egg incubation period, which ranged between 6-7 days. As for the eggs hatching rates, there was little effect in reducing the percentage of hatching, which amounted to 80.67 -88.67 for alcoholic concentrations and 95% in the control treatment. Al-Hamdani (2016) confirmed that the alcoholic extract of *Adhatoda vasica* Nees plant led to killing the pupa of the Great Wax Worm in all concentrations. With the occurrence of clear deformations in pupa in high concentrations (5 and 7%), there was a decrease in the emergence ratios of the pupa (53.33, 30, 33.33%) of the above concentrations, respectively. Abbas (2016) reported that the concentrations (1, 3 and 5%) of the alcohol extract of the leaves of ace and eucalyptus affected the age of the male cowpea beetle Fab. They have a clear effect in all concentrations

Table 1: Effectiveness of alcoholic extract of Dodonaea leaves in influencing the eggs percentage hatching of the greater wax worm.

Treatments	Alcoholic extracts concentrations%				
	Control	2	5	10	LSD 0.05
Egg percentage Hatching %	95	20	13.67	12.67	8.66 **

Repeat Concentrations of alcoholic% LSD 0.05%.

Table 2: Effectiveness of the alcoholic extract of Dodonaea leaves in affecting the wax maiden pupa and some life phenomena of the resulting generation of pupas.

Life phenomena	Alcoholic extracts concentrations				
	Control	2	5	10	LSD 0.05
Killing rate in the pupa phase	3.33	37.78	65.56	69.26	14.99**
Average age of the pupa phase	9.83	11.10	12.83	11.80	2.087*
Male adult / day lifetime rate	15.33	12.67	11.33	12.33	N.S(5.099)
Female adult lifetime / day	11.33	10.67	8.67	8.67	N.S(3.438)
Average number of eggs / female	1012	434	395	210	386.80**
Average egg incubation time / day	6.00	7.00	7.00	7.00	N.S(1.331)
Eggs Hatching rate%	95.00	88.67	88.33	80.67	8.93*

treatments on reducing the age of the treated male, As for its effect on the ratios ages of the Southern Cowpea Beetle *C. maculatus* (Fab.) Treatment superficially. The results showed that the alcoholic extract of leaves of Ace and Eucalyptus had reduced the age of the female treated in all concentrations. The reason for the decrease in the adult average ages may be due to the alcohol extract of the leaves of Eucalyptus and leaves containing compounds and toxic substances that have a deadly effect of the insect through contact and that these materials have the ability to penetrate into the insect's body through the elastic areas or from the spiracles or through the insect's cuticle. Which affected the nervous system and led to paralysis and death of the insect. Al-Omairi (2013) confirmed that the pilot oil of rosemary plant *Rosmarinus officinalis* was highly effective in preventing female greater wax moth, from laying eggs at a concentration of 2% at a 100% expulsion rate when treating wax with a concentration above, while the concentration (1 and 1.5%) was the number of eggs placed 16.66 and 11.33 eggs / females, respectively, and the number of eggs was zero for the same concentrations of volatile oils for caraway *Carum carvi* and cumin *Cuminum cyminum* plants.This is due to the effect of oils on the insect reproductive system directly, while the incubation period for eggs in the treatment of caraway plant reached (9.33, 9.67, 10 days) for treatments concentrations, respectively and 7 days for the control treatment. Subashini *et al.*, (2004) explain that the hexane extract of *D. viscosa* reduced fertility and hatchability in the eggs of *H. armigera* and reduced pupality in the insect. Al-Ardi and Taha (2009) found that the extract of raw alkaline compounds for Dodonaea leaves in concentrations (0, 2.5, 5, 10, 20 mg / ml) had an effect on increasing the period of growth of the immature stages of the nymph stages and reducing female productivity, where the growth periods were (5.6, 6.2, 7, 0 and 0 days) and productivity (65, 25, 17.3, 0 and 0 nymphs / females) for the same concentrations, respectively. Al-Qazzaz (2010) found that the rate of eggs excreted from adults of the cowpea beetle *C. maculatus* (Fab.), that treated with Dodonaea leaf extract at a concentration of 15% and for ages 24 and 96 hours decreased for both ages respectively and the lowest rate for adult emergence. Abdel Karim (2012) explained that the direct treatment of adults with 24-hour-old miniature pills with phenolic extract of *Eucalyptus camaldulensis* Dehnh. With a concentration of 7% led to reducing the number of eggs laid to 25.8 eggs while 319 eggs in the control treatment, as well as the reason of the terpenes extract at the same concentration decreased In the percentage of eggs hatch.

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