Effectiveness of the alcoholic extracts of leaves and barks of eucalyptus plant (*Eucalyptus camldulenis* L.) against adults of cowpea beetle *Callosobruchus maculatus* (Fab.) (Coleoptera: Bruchidae).

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Abstract

This study aimed to evaluate the effectiveness of three concentrations (1.25, 2.5 and 5%) of the alcoholic extract of leaves and bark of eucalyptus plant (*Eucalyptus camldulenis* L.) against cowpea beetle *Callosobruchus maculatus* (Fab.) for 24 and 48 hours after treatment compared with control treatment. The result showed the effectiveness of leaves and barks extracts on the mortality of insects' adults. The highest mortality reached 60.00 % and 80.00% with the concentration 5% of bark extract after 24 and 48 hours respectively while the lowest mortality was 36.67% and 66.67% after 24 and 48 hours respectively at 1.25 % of leaves extract. The attraction rate of insect was 9.5% for 5% treatment and 3.66% at 1.25% treatment compared to the expulsion rate which was 23.5% at the concentration 5% and 21.83% at 2.5% for leaves and barks respectively.

Keywords: eucalyptus, cowpea beetle, Coleoptera.

فعالية المستخلص الكحولي لأوراق وقلف نبات اليوكالبتوس *Eucalyptus camldulenis* L. في بالغات خنفساء اللوبيا الجنوبية *Callosobruchus maculates* (Fab.) (Coleoptera : Bruchidae).

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المستخلص:-

تناولت الدراسة اختبار تأثير المستخلص الكحولي لأوراق و قلف نبات اليوكالبتوس *Eucalyptus camldulenis* L. باستخدام المذيب العضوي الايثانول وبثلاثة تراكيز (1.25، 2.5 و 5 %). على بالغات

خنفساء اللوبيا الجنوبية . (Fab) *Callosobruchus maculatus* وملاحظة التأثير بعد مرور 24و 48 ساعة من المعاملة ومقارنتها مع السيطرة. وأظهرت النتائج تأثير مستخلص الاوراق والقلف المستخدم في الدراسة على هلاك بالغات الحشرة اذ لوحظ من النتائج ان لمستخلص قلف اليوكالبتوس أعلى نسبة هلاك للحشرة بعد مرور 24 و 48 ساعة بلغ 60.00% و80.00% بالتتابع بتركيز 5% واقل نسبة هلاك كان لمستخلص الأوراق بلغ 36.67% و66.67% بالتتابع بتركيز 1.25%. كانت نسبة جذب 9.5 بتركيز 5 % و 3.66 بتركيز1.25% بالمقارنة مع نسبة طرد التي بلغت 23.33% بتركيز 5% و 21.83% بتركيز 2.5 % للأوراق والقلف بالتتابع.

الكلمات المفتاحية : اليوكالبتس، خنفساء اللوبياء ،غمدية الاجنحة.

Introduction:-

Cowpea beetle *C. maculatus* (Fab.) (Coleoptera: Bruchidae), is one of the serious insect pests infesting seeds of many legume crops including cowpea, chickpeas, lentils, beans and soybeans (Hill, 1990). This insect can cause an estimated loss about 25-30% in the field and 80% in the silo (Hill, 1990).The infestation process begins in the field and then transferred to the storage. The importance of this insect is due to the nutrition and development of its larvae inside the seeds and the consumption of all its contents which lead to increase the percentage of seed damage and reduce their nutritional value and germination rates (Jouki & Khazaei, 2010).

The control of this storage insect pest relies mainly on chemical insecticides belong to chlorinated hydrocarbons and organic phosphorus such as Actellic 50 EC (Ayad & Alyouse, 1994). However, the wide use of these insecticides had led to insecticide resistance in cowpea beetle populations and environmental pollution with negative side effects on human health (Al-Kanani, 2014). Therefore, using the natural alternatives for controlling cowpea beetles, such as vegetable oils, plant extracts and plant powders (Ranjana & Beenam, 1999) can avoid the drawbacks of the chemical insecticides. The effects of serial concentrations of harmala *peganum harmala* L. and fenugreek seeds *Trigonella foenum granecum* oil against the southern cowpea beetle in cowpea seeds (reddish, white, reddish- brown and black) revealed that there was a significant toxic effect of all concentrations on immature stages and adult emergence (Hayder, 2015). The hot water extract of the yellow cassia plant *Cassia sophera* caused a decrease in the first-generation progeny of cowpea beetle eggs *C. maculatus* which were fed on treated cowpea seeds where the number of adults was about 380 compared to 520 adults for control treatment (Kestenholz, Stevenson, & Belmain, 2007) It has been indicated that the alcoholic extracts of Anethum seeds *Aniseum graveolens* and Cumin *Cumin cyminum* with a concentration of 2% led to an expulsion rate of cowpea beetle about 100% and 99.6% respectively when they were added to chickpea seeds followed by garlic extract *Allium sativum* 95.3% and eucalyptus leaves *Eucalyptus spp.* 93.3% (Ibrahim & AL- Naser, 2009) . The results also clarified that some volatile oils had significant effects on insect mortality reached to 100% after 72 hours of treatment with 8 $gm.ml^{-1}$ of (Essa, 2016).

Because of the environmental issues and the toxicity of the chemical compounds, this study was conducted on the southern cowpea beetle to determine the effectiveness of the alcoholic extract of leaves and bark of eucalyptus plant (*Eucalyptus camldulenis* L.) using three concentrations (1.25, 2.5, and 5%) against cowpea beetle *Callosobruchus maculatus* (Fab.). The percentage of the adult mortality, the percentage of attraction, and the percentage of expulsion were measured in this study.

Materials and methods:

Insect collection, identification and rearing

The infected seeds with the cowpea beetle was obtained from the local markets in Kerbala city . The insect was identified in the Museum of Natural History (Bagdad). Cowpea seeds were sterilized using the electric oven at 60 C° for two hours. Ten pairs of identified cowpea beetle (10 males + 10 females) were added to 250g of sterilized and non-infected cowpea seeds placed in 300ml glass jars which were secured with a muslin cloth and rubber bands (Appel, Moar, & Tanley, 1999). Then, the treated seeds were incubated at 28 C° ± 2 C° and relative humidity 60 ± 5% taking into account the constant renovation of the colony (Appel et al., 1999).

Preparation, diagnosis and storage of plant samples:-

The leaves and barks of the eucalyptus were collected from the College of Agriculture at University of Kerbala in 2018. They were washed from dust and dirt and dried in the electric oven at a temperature of 50 C°. Then, the dried leaves and barks grinded by a large electric herb mill Herbal Grinding/FW177 (China) to obtain a very fine powder that placed into paper bags that kept in a cool place until usage.

Preparation of alcoholic extracts:-

Ethyl alcohol was selected as a polar solvent in the extraction method. The method described by (Ladd, Jacobson, & Buriff, 1978) and a little modification was used. A 10 g of powder plant parts was taken and added to 200 ml of alcohol and placed in an electric shaker device for two hours. Then, the sample was dried in an oven at 45-50 C°. The process was repeated several times to obtain sufficient amount of raw material. Then, the samples were kept in the refrigerator at 5 C^{o} until usage. To examine the effectiveness of alcoholic extracts of eucalyptus leaves and barks, a 5 gm of the dry residue of each extract was taken and dissolved with 5 ml of the used solvent (ethyl alcohol). The volume was completed with 90 ml of distilled water to get a 5% as stock solution. Concentrations of 2.5% and 1.25% were prepared from the stock solution while the control treatment was only a mixture of 5 ml solvent (ethyl alcohol) and 95 ml of distilled water.

Laboratory experiments:-

Effect of plant extracts of eucalyptus leaves and barks on adult mortality of Cowpea beetle:-

Ten Adults per replicate were taken from the insect stock culture and replaced in a disposable plastic petri dish. Then, the subsamples were treated directly with the prepared concentrations (0, 1.25, 2.5 and 5%) using a 100 ml sprayer. The suspensions were applied from 25cm height and incubated at temperature 28 ± 2 C°, and 60 ± 5% of relative humidity. Each treatment was replicated three times. The mortality rates were

recorded after 24 and 48 hours after treatments and the results were corrected according to the Abbott's equation (Abbott, 1925).

Attraction and repellent effects of the alcoholic extracts of eucalyptus leaves and barks on adults of cowpea beetle using the chemotropometer.

A chemotropometer, which was manufactured in a workshop related to the Faculty of Science / University of Babylon, was used in this study. It was a wooden box about 48 cm in length and 20 cm in height with a moving cover. This box has two facing openings with a glass tube (100 cm length and 3 cm diameter) passing through them. The tube is divided into centimeters and has a hole in the middle to insert the insects as well as the ends of it are closed by pieces of cotton. Cotton in the right side of the tube was treated with the concentrations of alcoholic extract of eucalyptus leaves and barks (1.25, 2.5, and 5%) while the cotton in the left side was treated with control treatment (95% distilled water and 5% alcohol). Ten adult of insects were placed in the middle of the tube and the number of attracted and repelled insects was calculated 15 minutes after insect releasing.

The experiment was conducted under laboratory conditions. The tube was cleaned between the treatments. Each treatment was replicated three times. The results were then calculated according to the following equations (Al-mousawi, Husain, Ameen, & Fthalla, 2012).

$$\text{Percentage of attraction} = \frac{\text{Insect number directed towards the tested material at 25 cm away from the center}}{\text{Total insect number}} \times 100$$

$$\text{Percentage of repellency} = \frac{\text{Insect number directed inverse the tested material at 25 cm away from the center}}{\text{Total insect number}} \times 100$$

Balance rate = Attractive percentage - Repellent percentage.

Statistical analysis

The results were analyzed using the Completed Randomized Design (CRD). The least significant difference (L.S.D) was used at the probability level (0.05) to test the differences between treatments (Al-Rawi & Khalafullah, 2000). The mortality rates were corrected according to Abbott Formula (Abbott, 1925). The percentages of mortality corrected are calculated according to the following equation:

$$\%\text{Correction of Mortality} = \frac{\%\text{ mortality in the treatment - \% to mortality in the control treatment}}{100 - \%\text{ mortality in the control treatment}} \times 100$$

Corrected mortality was transferred using angle value transformation. Statistical analysis was carried out using (Delwiche and Slaughter, 2013).

Results and Discussion:

The results of table (1) showed that there was no significant effect on the type of plant extract of leaves and barks. There was a significant effect of the used concentrations (5, 2.5, 1.25 and 0.0%) and their interaction in the mortality percentage of adult

cowpea beetle insects *C. maculatus* after 24 hours. The results of the leaf extract rate showed the highest percentage of adult mortality about 33.33%, and the lowest rate of adults mortality was in the bark extract reached 30.00%. The results showed a significant effect of the concentrations of the extracts, compared to the control treatment, with the highest effective mortality at the concentration of 5% of the barks extract reached 60.00%. The lowest effective of mortality of the extract of the leaf was in the concentration 0.0% (control treatment) which amounted to 0.00%. The interactions between the type of extracts and the used concentrations showed a significant effect on the mortality rate of the cowpea beetle whereas the concentration of 5% for the extract of the bark was the highest loss rate of 60.00% and the lowest effective was in bark extract in concentration 0.0% (control treatment) which reached 0.00%.

The study confirmed by (Mahmood, Mzahem, & Hasson, 2016), which found that the higher concentration of leaves extract led to greater mortality.

Many studies have shown that eucalyptus contains several active compounds such as phenolics, tannins, flavonoids, saponins, clicosides glycosids, resins and comarins which might cause this high mortality (Akbar, AL-Mansour, & Nadhum, 2011)

The ethanolic extract of Eucalypt leaves prolonged the period of egg development which increased to 7.33 days by using 10000 ppm while the egg hatching percentage was decreased to 71 % at the same dose of extract (Imeiri & Ali, 2012).

Plant extracts may contain compounds that have diffusion and permeability within the living tissue compared to pesticides (Daoud, Al – Dwlaimy, & Abdal – Jabbar, 2010).

Plant extracts may affect through contact with the body surface so that the chemical compounds penetrate the insect through the flexible areas causing paralysis and then death (Al–Mallah, Mustafa, & Qasseer, 2007). The plant powders of *E. camaldulensis* leaves showed high inhibitory activity against the cowpea beetle and this effect could be due to the fact that the eucalyptus plant leaves contains a stable and volatile oils which can be the killer compounds by causing the trauma of the insect which paralyzes the movement and then death (Al-Jassany, 2007).

Table 1: Effect of different concentrations of the alcoholic extracts of eucalyptus leaves and barks *E. camldulenis* L. in the mortality percentage of cowpea beetle *C. maculatus* after 24 hours.

Concentrations / Extracts	0%	1.25%	2. 5%	5%	Concentrations rate
Leaves extract	0.00	36.67	46.67	50.00	33.33
Barks extract	0.00	40.00	20.00	60.00	30.00
Concentrations rate	0.00	38.33	33.33	55.00	
L.S.D	To extracts	To Concentrations	To interactions		
	12.492	17.666	24.983		
Significant level	To extracts	To Concentrations	To interactions		
	Not s.	0.0001	0.0001		

Table (2) shows a significant effect of the type of extract, the used concentrations (5, 2.5, 1.25 and 0.0%), and their interaction in the percentage of cowpea beetle adults' mortality *C. maculatus* after 48 hours. The results of the leaf extract rate

showed the highest percentage of adult mortality about 54.1% and the lowest percentage of adults in the treatment of the extract of the bark was 37.5%. The results also showed that there was a significant effect of the concentrations of the extract compared to the control treatment. The highest percentage of insect mortality was at 5% of the barks extract amounted of 80.00% while the lowest rate of mortality in the adult's insect at 0.0% (control treatment) of the leaf extract amounted to 0.00%. The interactions between the type of extract for leaves and the barks with the used concentrations showed a significant effect on the mortality rate of the southern cowpea beetle. The concentration of 5% for the extract of the bark was the highest loss rate about 80.00% and the lowest percentage of the barks extract was in the 0.0% (control treatment) which reached 0.00%. Eucalyptus plant is a source of many secondary metabolic compounds that show an effect on the vital activity of different insect pests (Kestenholz et al., 2007).

It has been reported that the increase in death rate after 48 hours of treatment by the effect of plant extracts than it was after 24 hours of treatment (Kestenholz et al., 2007). The death rate ranged from 93.33 to 100% in the concentration of 6000 ppm full of the plant extracts used in the study, due to the exposure duration to the active compounds that caused the increase in the killing rate (EL-Nahal, Schomidt , &Risha 1989).

Table 2: Effect of different concentrations of the alcoholic extracts of eucalyptus leaves and barks *E. camldulenis* L. in the mortality percentage of cowpea beetle *C. maculatus* after 48 hours.

Concentrations / Extracts	0%	1.25%	2. 5%	5%	Concentrations rate
Leaves extract	0.00	66.67	76.67	73.33	54.1
Barks extract	0.00	43.33	26.67	80.00	37.5
Concentrations rate	0.00	55.00	51.66	76.66	
L.S.D	To extracts	To Concentrations	To interactions		
	14.886	21.051	29.771		
Significant level	To extracts	To Concentrations	To interactions		
	0.03	0.0001	0.04		

Table (3) shows that the extracts gave a higher expulsion force than the attraction force of the insect adults as well as the contrast in attraction force between the concentrations. The highest concentration of leaf extract at 5% was about 9.5% while the balance was -13.83. The lowest attraction force was 2.83% for barks extract at the concentration of 2.5% and the balance reached -19.00. The highest expulsion force of leaves extraction at the concentration of 5% was 23.33% and the balance was -13.83 while the lowest expulsion force of barks extract was at 5% about 16.5% with the balance was -12.84. These results were consistent with the findings of (Al-Jassany, 2007).

The study by Shaaban & Al-Mallah, 1993 determined the expulsion and attraction effect of some volatile oils extracted from the leaves of eucalyptus on the adults of cowpea beetle by using the chemotropometer device, showed that the attraction rate was 17% and 7% and the expulsion rate was 47% and 73% with balance about +23

and -66 for the myrtle and the eucalyptus respectively. It has been reported that the extracts of alkaloids in the succulent wood of cypress and eucalyptus trees had a greater effect in the expulsion of woodworm from the phenols, turbines extract, oils and the watery part of the succulent and intrinsic wood (Mustafa, 2011).

Table 3: The percentage of attraction and expulsion of the leaves and barks extract of eucalyptus onto the adults of cowpea beetle by using the chemotropometer.

Extracts	Concentration%	Attract rate	Expulsion rate	Ambulance
Leaves extract	5	9.5	23.33	-13.83
	2.5	7.66	22.83	-15.16
	1.25	5.66	17.33	-11.66
Braks extracts	5	3.66	16.5	-12.84
	2.5	2.83	21.83	-19.00
	1.25	3.66	17.83	-14.16

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