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Evaluation of the Ovicidal and larvicidal activity of essential oils of three plant species on Fig Moth, *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae)

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Abstract
Most of date palm stores in Iraq are exposing to the infection of fig moth, *Ephestia cautella* (Walker) which caused considerable losses in quality and quantity of these nutritional products. This pest contaminates dates in warehouses, which makes customers to find the infected dates unfit for consumption. Current study covered the efficiency of rosemary, lemongrass and eucalyptus essential oils as ovicidal and larvicidal agents against *E. cautella*. Egg hatching inhibition attained 60.2% as a rate of three concentrations (5, 10,15%) of essential oil of eucalyptus, which statistically exceeded that of rosemary and lemon grass essential oil (33.6, 46.7%) respectively. Effect of three concentrations of tested essential oils on early larval instar of *E. cautella* for 72 hours of exposure revealed that essential oil of lemon grass at concentrations of 10 and 15% realized best results, in which corrected mortality attained 52 and 60% respectively. Regard to mortality of late larval instar of *E. cautella* the rosemary and eucaluptus essential oils achieved best result when had been treated with concentration of 15%.

Keywords: *Ephestia cautella*, Essential oils, larvicidal activity, Ovicidal activity

1. Introduction

Fig moth, *Ephestia cautella* (Walker), has been named with several alternative common names such as a tropical warehouse moth, almond moth, cocoa bean moth etc [1]. Although, fig moth is a cosmopolitan insect pest, it succeeds well in tropical and subtropical regions. As a pest it prefers to infest some types of dried fruits in warehouses, although several articles confirmed that it was found in flour, nuts, beans, wheat, and other grains [2, 3]. In Iraq this pest was first recorded on dates in 1920 [4] and considered one of a serious invader of stored dates. Abdul Hussein [5] described biology and damages of stored dates and fig caused by *E. cautella* furthermore, different measures of its control. Stored product commodities usually are protected from insect pest infestations by many measures, common of which application of insecticides. To yet, the measures to control insect pests in warehouses and stores rely mainly on traditionally insecticides [6] Often, pesticide implications prove adverse effect for killing the beneficial species such as natural enemies, furthermore increasing chances of development of pest resistance to pesticides [7, 8]. Although of its destruction to environment and human health, methyl bromide was most effective compound for confrontation against stored product pests [9]. But the Montreal Protocol have been banned this chemical compound [10] for its environmentally dangerous and ozone layer depletion. The need to develop more environmentally safe control methods, such as using plant essential oils (EOs), had become urgent. The use of EOs, derived from medicinal plants, as low hazard insecticides have increased considerably in last two decades. They have been used effectively to control preharvest and postharvest phytophagous insects [11]. Through a filter paper bioassay Sim *et al* [12] emphasized that 2.4 µg/cm^2 of rosemary EO caused high toxicity to larvae of *Cadra* (*Ephestia*) *cautella*. Prevalent eucalyptus tree species in Iraq are *Eucalyptus camaldulensis* which its leaves possessed as a source of EO in this research. Several researchers revealed that EO of this species contains monoterpenes such as Eucamlol, 1,8-Cineol, Cineol, α pinene and ρ-Cymene [13, 14]. Eucalyptus EO was characterized by insecticidal and repellent activity against specific insect pests of stored products and grains [15]. Furthermore, some researchers confirmed, that lemongrass EO has assigned the most significant insecticidal activity against maize weevil, *Sitophilus zeamais* with a high mortality rate. This EO was found to have high

contact and ingestion toxicity than fumigant / respiratory poison [16]. In the present study, for finding alternative compounds have insecticidal activity useful to control fig moth which infests dried fruits in warehouses, we evaluated the ovicidal and larvicidal properties of eucalyptus, lemongrass and rosemary essential oils against eggs and larvae of *E. cautella*. These secondary metabolites from mentioned plants are easily biodegradable.

2. Materials and Methods

2.1 Source and Rearing of Insect

Many samples of dried date fruits, with insect infestations, have been collected in July 2016 from local markets in Baghdad, Iraq and from stores belong to Iraqi Dates for Processing & Marketing Company in Al-Shalichiyah, Baghdad in the same period. In the Central Laboratory of Entomology, University of Baghdad, College of Agriculture infested fruits were transferred to the glass jars size of which 618 ml, which maintained at 25 ± 2 ºC, 65±5% RH and light-dark of 10-14 hs until the emergence of moths. Several moths collected, mounted and sent to the Research Center and Natural History Museum belong to the University of Baghdad for detection and classification.

2.2 Collection and identification of plant materials

Eucalypus and lemon grass plants used in current research had been collected at fields belonging to the University of Baghdad in Al-Jaderyah. Rosemary dry leaves brought from local markets. Sample of used plants was sent to the National Herbarium, Department of Seed Testing and Certification for detection and classification.

2.3 Preparing the Essential oils.

In October 2016, dried leaves of tested plants were powdered by electric milling equipment (Pharmaceutical Mill)**.** After milling milled materials of each plant species were distributed in labeled glass jars for each plant species. Milled plant samples were conserved in laboratory conditions for short-duration until extraction date. Distillation of EO achieved by Clevenger Apparatus, where 25-30 grs of milled plant material were put in the flask of the apparatus and added to it 250-300 ml distilled water. The flask was heated to 90-100 ºC for eucalyptus and lemon grass whereas temperature was 70-80 ºC for rosemary. Distillation process had been taken about 3-4 hrs, in which from 30 grams of each mild sample were obtained 0.8, 0.7 and 0.2 ml of EO for eucalyptus, lemon grass and rosemary respectively. Through research duration EOs extraction had been replicated several times as the experiments required. Every time the produced EOs were placed in dark glass vials, which conserved in the refrigerator at 4-6 ºC for future applications.

2.4 Preparation EOs concentrations

For preparation the concentrations of tested EOs acetone 10% was used as solvent. Acquired concentrations obtained using dilution equation.

2.5 Eggs Provision

To prepare a suitable place for adults mating and egg laying, three lantern glasses have been provided. Each glass was put on the underside of a Petri dish's lid provided with a piece of black paper, which preferable for egg laying. Underside this glass was provided with 10 pairs of moth (24-72 h age) which detected as *E. cautella*. The orifice of the lantern glass was closed by a piece of muslin cloth, which had been bound with rubber bands. The lantern glasses kept under controlled conditions of 25 ± 2 ºC, 65±5% RH and light-dark of 10-14 h. After one day collection the ages had been initiated for bioassay.

2.6 Larvae Provision

Each 10 larvae of 1st. generation of classified *E. cautella* were transferred to 618 ml glass jar (4 replicates) contained 50 gr. of artificial food which composed of 81% grind wheat kernels, 1% dry yeast, 6% date syrup (dibs) and 12% glycerin. This artificial medium was recommended by researchers of insect's laboratory, Department of Entomology, Department of Agricultural Research, Incorporated Ministry of Science and Technology. These jars with larvae were maintained at the same mentioned above abiotic ecological factors. Culture rearing continued for many generations for supplying different insect stages.

2.7 Bioassay

2.7.1 Effect of Essential oils on egg Hatchability

A factorial experiment had been carried out according to completely randomized design with three replicates. First experimental factor was three types of EOs (rosemary, lemon grass and eucalyptus), second factor was four concentrations (0, 5, 10, 15%) of each essential oil EO. These factors tested as ovicidal agents against 10 eggs of *E. cautella* for each replicate. Three jars of 204 ml size, were supplied for each concentration. Each of three filter papers was treated with 1 ml of each concentration of EOs, when check treatment was treated with 1 ml of 10% acetone. The treated filter papers exposed to laboratory condition for 20 minutes to get rid of solvent effect. Each jar was supplied with one treated filter paper and 10 eggs of *E. cautella*. All jars were well covered with muslin cloths and transferred to incubator and maintained at mentioned above factors. Exposure time was 24 hrs. After 96 hrs all replicates had been tested for counting the hatched larvae. The percentage of dead embryos in treated eggs were corrected according to Abbott's formula [17].

2.7.2 Effect of essential oils on early and late larvae mortality

The same experiment and design which mentioned above carried out on early larval instar of *E. cautella*. Three jars of 618 ml size, were supplied for each concentration. Each jar was supplied with 20 gr of artificial food and 10 larvae of *E. cautella*. All jars were well covered with muslin cloths and transferred to an incubator and maintained at mentioned above factors. Exposure time was 24 hrs. Another experiment was carried out with the same details except the exposure was extended to 72 hrs to early instar and 5 days for late instar. Percent control mortality was corrected according to Abbott's formula (Abbott, 1925).

2.8 Statistical Analysis

The data of laboratorial experiments regarding to estimating mortality of eggs and larvae of testing insect pest were corrected using Abbott's formula [17]. Resulted values of percentage mortality wer submitted for analysis of variance (two-way ANOVA) using GenStat soft ware. Significance of differences of means tested using the LSD test.

3. Results and Discussion

3.1 Effect of Essential oils on egg hatchability of *E. cautella*

Table-1 showed the effect of concentrations (5, 10, 15%) of EOs on hatching inhibition rates of fig moth's eggs. Results revealed that the mean of concentrations of EO of Eucalyptus was statistically exceeded the other EOs, in which the rate of egg hatching inhibition attained 60.2%. The difference in EO concentrations caused a disparity in the percentages of egg hatching inhibition. This case is described as positive relationship in which hatching inhibition increased with increasing the concentration of the EOs. The concentration of 15% of eucalyptus essential oil showed 89% egg hatching inhibition compared with the two other concentrations. The Researchers have been evaluated the efficacy of EOs with different concentrations as ovicidal agents, where at 1mg/cm^2 of (−)-α-pinene, 2-β-pinene, and γ-terpinene *(Eucalyptus globulus* leaf oil-derived monoterpenoids) exhibited moderate ovicidal activity against eggs of *Pediculus humanus capitis* [18]. Warikoo *et al* [19] observed that the using rosemary essential oil with 10% concentration caused reduction of egg hatching percentage of mosquito, *Aedes aegypti*, to 28%, whereas Siriporn and Mayura [20] mentioned existence of adverse relationship between concentration of EOs, extracted from seven plant species (including rosemary and lemongrass) and percentage of egg hatching of three species of mosquito. Therefore we discussed the tested concentrations of EOs of the plant species that created reduction of egg hatching of fig moth.

Table 1: Effect of the different concentrations of EOs on egg mortality of *E. cautella*

Essential Oils	Corrected Mortality of Eggs of *E. cautella* at concentration of			Mean of concentrations
	5%	10%	15%	
Rosemary	7.1	40.3	53.5	33.6
Lemon grass	22.4	57.1	60.7	46.7
Eucalyptus	23.4	67.8	89.3	60.2
Mean	17.6	55.1	67.8	46.83

LSD ($P \leq 0.05$): 1. for EO types: 9.98; for EO concentrations: 9.98; for EO types x concentrations 17.28

3.2 Effect of Essential oils on larval stage of *E. cautella*

Data of table 2 revealed the absence of significant differences among tested EOs at concentration of 5%. Whereas the data showed a high rate of corrected mortality of *E. cautella* larvae was 40% caused by EO of lemongrass that exceeded the EOs of eucalyptus which attained 17.3%, while no significant difference with EO of rosemary (29.33%). As illustrated from the mentioned table, the high corrected mortality (60%) of early larvae of *E. cautella* was recorded in lemongrass treatment at 10% concentration, that significantly exceeded both of EOs of rosemary (40%) and eucalyptus (16%). As in 15% concentration, high corrected mortality of the larvae of *E. cautella* was 52% achieved by EO of lemongrass which was not significantly different from EO of rosemary (36%), whereas was significantly different from EO of eucalyptus (28%). The statistical analysis of interaction between EOs and concentrations showed superiority percentages of 60% and 52% of EO lemongrass at concentrations of 10 and 15% respectively compared with all EOs at 5% concentration. Other researchers observed that the rosemary oil at 2.4 mg/cm^2 caused 100% mortality after one day of treatment when they followed the contact toxicity bioassay for *E. cautella* larvae, have been [21].

Table 2: Effect of the different concentrations of essential oils on mortality percentage of early larval instars of *E. cautella* for 24 h. of exposure

Essential Oils	Corrected Mortality of young larvae of *E. cautella* at concentration of			Mean of concentrations
	5%	10%	15%	
Rosemary	12.0	40.0	36.0	29.33
Lemon grass	8.0	60.0	52.0	40.0
Eucalyptus	8.0	16.0	28.0	17.33
Mean	9.33	38.67	38.67	28.89

LSD ($P \leq 0.05$): 1. for EO types: 17.98; for EO concentrations: 17.98; for EO types x concentrations 31.14

Obviously, no significant differences was appeared among the EO types at concentration of 5% which was applied to kill the young larvae of *E. cautella* (Tab.3). The lowest correct mortality (8%) was observed in the treatment of eucalyptus EO and the highest 20% was found in rosemary EO treatment. When the concentration was increased to 10% treatments of the rosemary and lemongrass EOs (36 and 52% respectively) exceeded the eucalyptus EO treatment (24%). At the same time, data of the Tab. 3 revealed the superiority of corrected percentages of the larval mortality by EOs of rosemary and lemongrass which attained 52 and 60% respectively at concentration of 15% on eucalyptus EO treatment (28%). With regard to the interaction between EOs and their concentrations, statistical analysis proved that the corrected mortality percentages of 52% and 60% at a concentration of 15% for EOs of rosemary and lemongrass treatments surpassed on all mortality percentages of all EOs at the concentration of 5%, also on the mortality percentage of 24% of eucalyptus EO at 10% concentration. Our results matched with Baskaran *et al* [22] with regard to superiority of rosemary and lemongrass EOs on eucalyptus EO, where no mortality occured to the 4th. larval instar of *Spodoptera littura* happened when treated with concentrations of 125, 250 and 500 ppm of *Eucalyptus globules* EO. Whereas mortality percentages reached 20 and 46.66% at the treatments of rosemary EO at concentrations of 500 and 1000 ppm respectively. Also for lemongrass EO, the mortality percentages attained 20 and 40% for each concentration respectively. With regard to lemongrass EO, our results agreed with the results of Kabera *et al* [16] who confirmed that EO of lemongrass have high insecticidal activity against maize weevil *Sitophilus ziamais*.

Table 3: Effect of the different concentrations of essential oils on mortality percentage of early larval instar of *E. cautella* for 72 hrs of exposure.

Essential Oils	Corrected Mortality of young larvae of *E. cautella* at concentration of			Mean of Essential Oils at 3 concentrations
	5%	10%	15%	
Rosemary	20.0	36.0	52.0	36.00
Lemon grass	16.0	52.0	60.0	46.66
Eucalyptus	8.0	24.0	28.0	20.00
Mean	14.67	45.33	38.67	32.89

LSD ($P \leq 0.05$): 1. for EO types: 17.98; for EO concentrations: 17.98; for EO types x concentrations 31.14

Statistically, the results of larvicidal activity of tested EOs manifested significant superiority of the rosemary and eucalyptus EOs (mean of corrected mortality of 39.77 and 32.20% respectively) on lemongrass, corrected mortality of which counted 21.67% (Table 4). Outcomes of this experiment exhibited minimal corrected mortality of the late instar larvae which happened by tested EOs at concentration of 5%. But the corrected mortality considerably appeared effective with concentration of 10%, of both rosemary and lemongrass EOs with 27.5% corrected mortality of late larval instar of *E. cautella*. Where EO of eucalyptus caused significant depressed corrected mortality attaining 10.3%. Regarding the concentration 15% of all EOs increased in their influence on *E. cautella* larvae specially with the rosemary and eucalyptus EOs, of which the corrected mortality reached 89.6% and 86.2% respectively, accompanied with statistical considerable surpass on the corrected mortality of lemongrass EO which accounted 37.9%. The statistical interaction between types of EO and the concentrations, the data exhibited surpass of rosemary and eucalyptus EOs at the concentration of 15% on all the EOs larvicidal activities at both concentrations of 5 and 10%. This study agreed with Abd-El-Salam [23] who confirmed that mortality percentage of *Callosobruchus maculatus* and *Sitophilus oryzae* increased with increasing concentration of different oils and exposure period. In the same context, Prates et. al [24] revealed that LC_{50} and LC_{99} of cineole against lesser grain borer, *Rhyzopertha dominica* were 0.7 and 2.4 mg/gr. respectively

Table 4: Effect of the different concentrations of essential oils on mortality percentage of late larval instars of *E. cautella* for 24 h. of exposure.

Essential Oils	Corrected Mortality of late larvae of *E. cautella* at concentration of			Mean of Essential Oils at 3 concentrations
	5%	10%	15%	
Rosemary	2.3	27.5	89.6	39.77
Lemongrass	0.0	27.5	37.9	21.67
Eucalyptus	0.0	10.3	86.2	32.20
Mean	0.77	21.77	71.13	31.21

LSD ($P \leq 0.05$): 1. for EO types: 9.55; for EO concentrations: 9.55; for EO types x concentrations 16.54

The results of larvicidal efficiency of tested EOs showed statistically considerable superiority of the rosemary and eucalyptus EOs (mean of corrected mortality of 52.67 and 42.10% respectively) on lemongrass, corrected mortality of which counted 29.80% (Table 5). Results of this experiment showed surpass corrected mortality (27.3%) of late instar larvae happened by EO of rosemary at concentration of 5% on both lemongrass and eucalyptus EOs which counted 13.7 and 9.3% respectively. At concentration of 10%, corrected mortality of rosemary EO which reached 37.7%, was significantly exceeded the mortality of eucalyptus only which counted 24.0%. The concentration of 15% of all the EOs increased in their influence on *E. cautella* larvae especially with rosemary and eucalyptus EOs (corrected mortality of both reached 93.0%) which accompanied with statistically considerable surpass on the corrected mortality of the lemongrass EO (44.7%). The bioactive constituents of EOs had major role as insecticidal agents [25]. The required concentration which realizes the effective mortality depends on several factors, such as temperature, larval instar, capability of EO to penetrate the larval cuticle, as well as the mode of action [26]. Jayakumar *et al* [27] emphasized on other factors affected the repellency and toxicity of EOs, that were the concentration and exposure time. In this regard, our research included these two factors as well as the plant's type EOs to test their toxicity against larval stage of *E. cautella*.

Table 5: Effect of the different concentrations of essential oils on mortality percentage of late larval instars of *E. cautella* for 5 days of exposure.

Essential Oils	Corrected Mortality of late larvae of *E. cautella* at concentration of			Mean of concentrations
	5%	10%	15%	
Rosemary	27.3	37.7	93.0	52.67
Lemon grass	13.7	31.0	44.7	29.80
Eucalyptus	9.3	24.0	93.0	42.10
Mean	16.77	30.90	76.90	41.52

LSD ($P \leq 0.05$): 1. for EO types: 11.06; for EO concentrations: 11.06; for EO types x concentrations 19.16

4. Conclusions

Results of the current study conclude that, the tested essential oils were effective at concentration of 15% against egg and larval instars of *E. cautella*, that these controls agents might be useful for managing fig moth in warehouses.

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