Efficacy of Certain Selective Plant Essential Oils against *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae)

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Abstract: *Ephestia cautella* (Walker), is one of the serious insect pests to dates in both field and storage houses, which causes considerable losses in quality and quantity of these nutritional products. Control of this pest using fumigants and chemical insecticides are not recommended because of their harmful to the human health and environment.

Therefore, the current study conducted to evaluate the efficiency of four essential oils (Peppermint, Lavender, Star anise and Nutmeg) and their combinations against *Ephestia cautella* under laboratory conditions. Fumigant toxicity results revealed that, Peppermint was the most effective oil (with LC_{50} = 0.18 µl/L and TI= 100%), while Nutmeg was the least toxic oil (with LC_{50} = 1.64 µl/L and TI= 10.98 %). Also application of Lavender in combination with Nutmeg or Peppermint to *Ephestia cautella* moth showed positive effect than when applied all of them separately, whereas antagonistic effect was observed when applied Anise in combination with Nutmeg. The highest repellent effect (86.36%) was recorded with Nutmeg oil, while Anise showed the low repellent effect (7.14%). In contrast peppermint and lavender exhibited attractant activity with percent 104.17 and 16.22 %, respectively against *Ephestia cautella*. In conclusion, our results indicated that, the tested plant essential oils had insecticidal effect, suggesting the possibility of using them as an alternative to conventional insecticides for *Ephestia cautella* control.

Keywords: *Ephestia cautella;* essential oils; repellent activity; fumigant toxicity; Co-toxicity.

1. Introduction

Date fruit (*Phoenix dactylifera* L.) is a native fruit of arid region **(Elkablawy, AR; Sheik *et al*. 2013)** According to the statistics, the global production volume of dates increased from 7.53 million metric tons in 2010 to reach about 8.53 million metric tons in 2018. Egypt, Saudi Arabia, Iran, Algeria and Iraq being the first five main producing countries, producing on an estimated 1.542, 1.224, 1.202 1.058 and 0.618 million tons, respectively **(FAO, 2018)**. Date fruits are severely infested by many insect species belonged to different orders. Lepidoptera is one of the most important orders, which contained many economic date fruits insect pests such as the Cadra (*Ephestia*) spp. (El-Shafei 2018). The Date moth, *Ephestia cautella*, is a serious pest of dates in both field and storage houses. These devastating insect cause losses of weight and downgrading of the commercial value of the fruit **(Dhouibi, 2000)**. the seriousness of date moth on dry dates come from its ability to develop resistance to insecticides **(Al-Taweel, 1990).** Where, several storage systems depend on the use of synthetic insecticides and fumigants like methyl bromide (MeBr) and phosphine (PH3) to control stored-product pests. These synthetic insecticides and fumigants are the most economical and convenient methods for managing these pests **(Mueller, 1990)**. Phosphine and methyl bromide have been used as chemical fumigants to control stored product insect pests because of their broad-spectrum potential. Recently, the use of methyl bromide is being restricted because of its potential to damage the ozone layer. Also, the further use of phosphine may be threatened by the building of insecticides resistance in insect strains with high levels **(Tutuncu, 2019)**. However, their negative side effects such as environmental and human health hazards, effect on non-target organism and the expansion of insect resistance have been documented. The use of plant compounds, such as essential oils and plant extracts, is an increasing interest due to their fewer side effects on non-target organisms and low-risk to environment compared to conventional insecticides. Botanical essential oils show different types of bioactivities such as contact and fumigant toxicity, antifeedant activity and repellency **(Yeom, Kang *et al*. 2012)**. Plant extracts have been widely used for pest control. Moreover, they were developed to get new generation pesticides with more safely applications **(Cantrell, F.E. Dayan *et al*.; 2012, Gerwick, 2014)**. Although there are a great varity in plant species, only near to 10% have content the essential oils **(Kalemba, 2003)**. Essential oils could produce from all plants parts; stems, leaves, flowers, fruits and seeds.

So this study aims to develop eco-friendly botanical insecticides from four plant species (Peppermint; Lavender; Star anise and Nutmeg) against *Ephestia cautella* moths, by evaluated the fumigant toxicity and repellent activity of theseessential oils under laboratory conditions.

2. Materials and methods

2.1. Rearing of Insects

The colony of *Ephestia cautella* was obtained from laboratory of food and agricultural science at King Saud University. The culture was reared by using a method which described by **Aldawood, Rasool *et al*. (2013),** with minor modification, larvae of *E. cautella* were reared on sterilized artificial diets contained from barley, layer diet, broiler diet, glycerin. Larvae kept in one-liter plastic container. The adult collected when it was emergence and kept in plastic jar. The screw cap was covered with double mineral mesh (120mm×2mm) for ventilation and collection eggs. An impregnated cotton with 10% sugar solution was introduced to the adult for feeding. Eggs were transferred to new tube with media. All the colony kept on 27±2C°, 65±5% relative humidity and darkness.

2.2.Collection of plant material

Leaves of Peppemint, *Mentha pipertia* ;seed of Star anise, *Illicium verum* ; Nutmeg, *Myristica fragnas* and flower of Lavender, *Lavandula angustifolia* were collected from local supermarket in Unaizah city (Table 1).

Table (1): Classification of tested plants and their parts used:

No.	Plant name	scientific name	Family	used parts
1	**Lavender**	*Lavandula angustifolia*	Lamiaceae	Flower
2	**Nutmeg**	*Myristica fragnas*	Myristicaceae	Seeds
3	**Pepper mint**	*Mentha pipertia*	Lamiaceae	Leaves
4	**Star anise**	*Illicium verum*	Illiciaceae	Seeds

2.3. Preparation of essential oils

A total of 200 gm of coarse dried powder from each plant was mixed with 700 ml of distilled water in a round bottom flask standing on a heating mantle and connected to the Clevenger-type apparatus. The volatile oil distillates were collected for 3 hours, then dried with anhydrous sodium sulfate (Na_2So_4) to remove all water **(Elyemni; Louaste *et al*., 2019)**. The processes were repeated many times for each plant to collect at least 10 ml of the volatile oils. The oil samples were stored away from the light in -20 °C freezer.

2.4. Identification of essential oils component by mass spectrometry

The identification of the essential oils chemical composition was performed based on the comparison of retention rates experimentally obtained and found in the literature, as well as the comparison of the compounds mass spectra with those from the NIST library. The retention index was obtained by applying an oil sample with a C11-C24 linear hydrocarbons mixture **(Gusmão; de Oliveira *et al*., 2013)**. The main active component which identified in tested essential oil were presented in (Figure 1-2).

2.5. Bioassay

2.5.1.Fumigant toxicity on *Ephestia cautella*

Fumigant toxicity of the essential oils was tested by using 10 adults of *Ephestia cautella*. filter paper 2.5cm (Whatman no.1) was impregnated with doses of tested oils to release fumigant concentration. Each filter paper was attached under surface of screw caps of 500 ml plastic bottle. All treatments as well as control were replicated five times. Mortality was recorded after 24 hours of treatments for 7 days. The entire bottle kept on 27±2C°, 65±5% relative humidity and darkness. Mortality percentage was calculated using Abbott's correction formula **(Abbott, 1925)**. The LC_{25}, LC_{50}, LC_{90} and slope values for each oil were calculated according to Finney 1971 **(Finney, 1971)**, using SAS software. To evaluate the toxicity index (TI) of the tested oils, the following equation **(Sun, 1950)** was applied:

$$\text{Toxicity index} = \frac{LC50\ of\ the\ most\ effective\ compound}{LC50\ of\ the\ used\ compound} \times 100$$

2.5.2. Impact of joint toxicity of various oils mixtures to *Ephestia cautella* moth

The investigation of combined effect of different essential oils, LC_{25} from each oil which determined previously mixed doses of tested then applied on filter paper (2.5cm) to release fumigant concentration. Each filter paper was attached under surface of screw caps of 500ml plastic bottle then the adult was left to exposure one day to mixed with the essential oils (Lavender, Mint, Anise, and Nutmeg oils) that previously recorded by then all the moth transferred to non-treatment bottles. percentage of moth mortality was calculated after 7 days. The same LC_{25} of the moth on each oil was calculated separately, to determine the effect of mixing oils on LC_{25}, whether to increase or decrease this ratio by used 40 moths in every test. Co-toxicity factor (CTF) was calculated using the equation recorded by **Mansour, Eldeferawi *et al*. (1966)** as follows:

$$Cotoxicity\ factor = \left(\frac{Observed\ \%mortality - Expected\%\ mortality}{Expected\ \%\ mortality}\right) \times 100$$

If the value of Co-toxicity factors (CTF) ≥ +20 will indicate potentiation effect; and CTF < -20 meant antagonism; and those between -20 and +20 meant additive effect.

2.5.3. Repellency effect of essential oils

In the repellency test, the **Sidney, Gries *et al*. (2006),** method was applied with a few minor modifications. A vertical Y-shaped pyrex glass with three arms olfactometer were used. The length of each arms was 10 cm and 1 cm diameter at 27±2C°, 65±5% relative humidity. This test was applied on 24-hour moths that is inserted from main arm of the olfactometer. The end of other two tubes were connected through a sealedlid 500 ml cylinder plastic bottles. For each essential oil, one doses of 0.25 microliter in 100 micro liters acetone was tested separately. In one arm, Whatman N°1 filter paper (2.5cm) were impregnated with doses of tested oils in the other arms was control added filter paper with only acetone. Filter papers have been exposed to open-air until the acetone evaporates for a minute before being used. The experiment was repeated 8 times, with 10 adults for each time. The insects are counted in the treated and the control bottles. To determine the percentage of oil repellency, the fallowing formula was used **(Huang; Qian *et al*., 2018)**:

$$\% \, of \; Repelncy = \frac{T - C}{T} * 100$$

Where:
T = Number of insects in the treated arm
C = Number of insects in the control arm.
Chi-square comparison between the number of insects attracted to the oil treatment and control were performed separately with SAS software (SAS Institute 1997).

3. Results and Discussion

3.1. Fumigant toxicity on Ephestia cautella

Data in Table (2) represented LC_{25}, LC_{50}, LC_{90}, TI and slope values of the tested plant essential oils (EOs) on *Ephestia cautella* moth after 7 days of exposure. Data revealed that, the fumigant toxicity of the tested (EOs) varied according to the values of median lethal concentration (LC_{50}) and toxicity index (TI). Amongst all the tested (EOs), Peppermint was the most effective oil as showed the lowest value was $LC_{50} =$ 0.18 µl/L and highest TI value (100%), followed by Lavender (LC_{50} = 0.39 µl/L and TI= 46.15%), while Star anise (LC_{50} = 0.71 µl/L and TI= 25.35%) and Nutmeg (LC_{50} = 1.64 µl/L, and TI= 10.98 %) showed the least effective, respectively to *Ephestia cautella* moth.

Results demonstrated that, the tested essential oils have fumigant toxicity against *E. cautella* moth, and indicated that Peppermint and Lavender oils were more effective to *E. cautella* moth compared to Star anise and Nutmeg oils after 7 day of exposure. This result could be attributed to its chemical composition. Similar findings were obtained by **Karaborklü, A. Ayvaz *et al*. (2011), Tandorost (2012), Mehany (2014), El-Khyat (2017)** who reported that, There was a possitive correlation between the period of exposure to different plant oils and mortality percentage, as the toxicity increased with increasing period of exposure and these results assured by many researchers, **(Tunc; 2000, Karaborklü, A. Ayvaz *et al*.; 2011, Khajehali, Van Nieuwenhuyse *et al*.; 2011**) they revealed that, mostly monoterpenes were very effective on insects due to their active volatiles.

Several studies have been reported that the plant essential oils have a fumigant toxicity activity against stored products pests **(Suthisut, Fields *et al*., 2011; Mishra, Tripathi *et al*., 2012).** In accordance with our study Rajendran **(Rajendran, 2008)** evaluated that the fumigant toxicity activities for some plant essential oils, where many of them showed positive results against stored-product pests. The essential oils may be disrupted the normal respiratory activity of the insect and this may lead to asphyxiation and death of the insect **(Akinneye 2016).** Also **Akinneye, Akinyemi *et al*. (2019)** suggested that ethanolic extract of essential oil of *Eugenia aromatica* was effective for the control *of E. cautella* on Cocoa beans since they completely inhibited development of the storage pest from eggs to adult stage at all concentration used. **As well as El-Khyat (2017)** revealed that, Bitter orange oil was much more toxic as fumigant than Sweet marjoram and German chamomile against *Ephestia cautella* adults. Previous studies revealed that the plant origin, species of insect and exposure duration time can affect the insecticidal activity of essential oils **(Regnault-Roger, Hamraoui *et al*., 1993; Regnault-Roger, Vincent *et al*., 2012).** The tracheal respiration could block due to presence of essential oils with strong odor and leading to insect death **(Pugazhvendan, Ross *et al*., 2012)**. Insect exposure to essential oils could be lead to their suffocation due to the interfering in normal respiration **(Schoonhoven, 1978).** The mode of action essential oils, affects the opening of the trachea through insects breath which usually leads to its blocking that might have been lead to suffocation **(Ileke, 2012).** Also essential oils are presumed to interfere with basic metabolic, biochemical, physiological and behavioral functions of insects **(Mann, 2012)**. As well as essential oils block the spiracles, resulting in blockage of respiratory siphons (asphyxiation) and death **(Rotimi, 2011).**

3.2.Impact of joint toxicity of various tested essential oils mixtures to *Ephestia cautella* moths

Potentiation, antagonistic and additive interaction effects were observed when applied the LC_{25} value for each one of various tested essential oils in mixtures form with other to *Ephestia cautella* moths as shown in Table (3)

Table (2): Fumigant toxicity of four essential plant oils against *Ephestia cautella*

No	Plant essential oils	LC_{25} values in µl/L (CL)	LC_{50} values in µl/L (CL)	LC_{90} values in µl/L (CL)	Toxicity index	*Slope* ± SE
1	**Peppermint**	0.10 (0.07- 0.12)	0.18 (0.15-0.22)	0.56 (0.42 – 0.93)	100	2.55 ± 0.36
2	**Lavender**	0.12 (0.04-0.21)	0.39 (0.23 - 0.58)	3.92 (2.20 - 12.42)	46.15	1.29 ± 0.25
3	**Star anise**	0.10 (0.01 - 0.22)	0.71 (0.35 - 1.51)	30.48 (7.71- 1431.88)	25.35	0.78 ± 0.20
4	**Nutmeg**	0.7012 (0.09 - 1.21)	1.64 (0.72- 2.24)	8.30 (4.97 - 57.64)	10.98	1.82 ± 0.57

*CL: Confidence limit. SE: Standard error.
*Toxicity index compared with Peppermint at LC_{50} value.

Results indicated that, Lavender exhibited the highest potentiation effect when applied in combination with Nutmeg to *Ephestia cautella* moth, where Co-Toxicity Factor (CTF) value was 88.89%, whereas the potentiation effect was reduced when Lavender applied in combination with Peppermint to reach (CTF) value to 33.33%. On the other hand, Anise gave remarkable antagonistic effect (CTF= -73.33%) when applied in combination with Nutmeg to *Ephestia cautella* moth, while additive effect was observed when applied Anise in combination with Peppermint and Lavender to *Ephestia cautella* moth with CTFs values 16.67 and -6.25 % , respectively. Also additive effect was noticed when *Ephestia cautella* moth exposure to the mixture of Nutmeg and Peppermint with CTF value -11.76%.

The obtained results revealed that, application of Lavender in combination with Nutmeg or Peppermint to *Ephestia cautella* moth showed positive effect than when applied all of them separately (Table 3) by increasing the adult mortality thereby causing potentiation effect and caused additive effect with Anise. Whereas, antagonistic effect was observed when applied Anise in combination with Nutmeg.

Similar finding was reported by **Jayakumar, Arivoli *et al*. (2017)** who found that some essential oils have repellent activity against rice weevil. Also they observed that use essential oils mixtures could enhance the synergistic effect and reduce the insect resistance. They observed, essential oils mixtures could be useful in the protection of stored products and increasing their activity. The current results revealed that, combinations of tested plant oils were useful to enhancement the toxicity of the tested essential oils against *Ephestia cautella* except the mixture of Anise and Nutmeg caused antagonistic effect. In addition, to prevent building the resistant strains, the importance of mixed formulations containing the insecticides and the non-toxic chemicals was well emphasized.

Table (3): Impact of joint toxicity of tested oils mixtures to *Ephestia cautella* moths

Plant essential oils	Expected mortality	Observed mortality	Co-toxicity factor	Type of interaction
Anise	-	27.5	-	-
Lavender	-	12.5	-	-
Nutmeg	-	10	-	-
Peppermint	-	32.5	-	-
Anise + Lavender	40	37.5	-6.25	AD
Anise + Nutmeg	37.5	10	-73.33	AN
Anise + Peppermint	60	70	16.67	AD
Lavender + Nutmeg	22.5	42.5	88.89	P
Lavender + Peppermint	45	60	33.33	P
Nutmeg + Peppermint	42.5	37.5	-11.76	AD
Control	0	0	0	-

***P:** (Potentiation)
***AN:** (Antagonism)
***AD:** (Additive)

3.3.Repellency effect of tested essential oils on *Ephestia cautella*

The repellent effects of the four tested oils against *Ephestia cautella* are shown in Table (4). The result indicated variation among the plant oils tested. Nutmeg oil recorded high repellent activity against *Ephestia cautella* with percent 86.36%, while Anise showed the low repellency with percent 7.14%. In contrast

Lavender and Peppermint exhibited attractant activity with percent 16.22 and 104.17%, respectively. The highly repellency effect that recorded with Nutmeg oil (86.36%) may be due to the chemical components of this oil.

Other study by **Jayakumar, Arivoli *et al*. (2017)** was tested the repellent activity and fumigant toxicity of ten plant oils at levels of 10-50µL/L against the rice weevil. Results showed that six of the tested essential oils wintergreen, lemon, geranium, citronella, camphor and lavender have varying repellency activity against rice weevil, whereas three plant essential oils rosemary, aniseed and vetiver exhibited attractant activity **(El-Khyat, 2017)** recorded high repellence activity of essential oils (German chamomile, Sweet marjoram and Bitter orange) against *E. cautella*. Also several studies recorded that the essential oils have repellent activity against stored product pests **(Regnault-Roger, 1997, Cosimi; Rossi *et al*., 2009, Nerio; Olivero-Verbel *et al*., 2009)**. In this respect repellency increases the potential value of materials in protecting grains from the attack by stored product pests **(Bekele AJ, 1997)**. EPI value gives an idea about the substance repellency or attractancy activity **(Paulraj, 2002)**. The repellent effect of volatile essential oils their local availability making it an attractive candidate in management of stored product pests **(Oh; Park *et al*., 2006, Kumar; Mishra *et al*., 2012).**

Table (4): Values of repellency effect of four tested essential oils on *Ephestia cautella moths*

Plant essential oils	T	C	ST	% of repellency
Star anise	3.5	3.25	3.25	7.14
Lavender	4.625	5.375	0	-16.22
Nutmeg	5.5	0.75	3.75	86.36
Peppermint	3	6.125	1.56	-104.17

***T** (Number of insects in the treated arm)
***C** (Number of insects in the control arm)

Conclusion

Results in the present study indicated that, the tested essential oils have fumigant toxicity against *Ephestia cautella* moth, and Peppermint and Lavender oils showed high efficacy that may be have properties which cause adult mortality compared to Star anise and Nutmeg oils. The positive effect of oil combinations was observed when applied the four tested essential oils mixtures except the mixture of Anise and Nutmeg caused antagonistic effect. Also, there was variation among the tested essential oils in the repellency percent, where Nutmeg oil recorded high repellent effect against *Ephestia cautella*, while Lavender and Peppermint exhibited attractant effect. So our results suggested that, plant oils may be a useful tool of integrated management of *Ephestia cautella* moth. Moreover, more studies are in demand to use plant essential oils as one of the components in IPM programs

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كفاءة بعض الزيوت النباتية الطيارة ضد حشرة التمر المخزون

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الملخص العربي

حشرة التمر المخزون (*Ephestia cautella*) واحدة من أهم الآفات التي تصيب التمر في الحقل أو المخزن وتسبب خسائر عالية في كمية وجودة التمر. كما انه لايوصي بإستخدام المبيدات والمدخنات الكيميئية في مكافحة هذه الآفة لما لها من تأثير ضار علي صحة الانسان وكذلك علي البيئة. تقوم هذه الدراسة علي تقييم فعالية اربع انواع من الزيوت الطيارة المستخلصة من نباتات النعناع، اللافندر، الينسون وجوزة الطيب علي طور الفراشة تحت ظروف المعمل. أظهرت نتائج السمية ان الزيت الطيار المستخلص من النعناع سجل أعلي درجة سمية، حيث بلغ (التركيز القاتل لـ ٥٠ % من الحشرات المعاملة ٠,١٨ ميكروليتر / لتر ودليل سمية ١٠٠٪) في حين كان زيت جوزة الطيب هو الأقل سمية (التركيز القاتل لـ ٥٠ % من الحشرات المعاملة ١,٦٤ ميكروليتر / لتر ودليل سمية ١٠,٩٨٪). كما أظهر الزيت الطيار المستخلص من نبات جوزة الطيب أعلي نسبة طرد للحشرة حيث سجل نسبة ٨٦,٣٦٪ بينما كانت النسبة في حالة الينسون ٧,١٤ في حين كان التأثير عكسي (جاذب) حيث كانت النسبة ١٠٤,٢٢ و ١٦,٢٢ في حالة كل من النعناع واللافندر، علي الترتيب. عند خلط زيت اللافندر مع زيت جوزة الطيب والنعناع أعطي المخلوط سمية عالية علي الفراشات (تأثير تنشيطي) عن الزيوت المفردة في حين كان التأثير تثبيطي عند خلط زيت الينسون مع زيت جوزة الطيب علي كفاءة الزيوت ضد الحشرة. نستخلص من هذه النتائج انه يمكن التوصية باستخدام الزيوت الطيارة كبدائل للمبيدات في مكافحة هذه الآفة.