Chemical composition and repellent activity of lavender and Nutmeg essential oils against *Ephestia cautella* walker

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Abstract: The aims of this study were to identify the chemical composition and repellent activity of lavender (*Lavandula angustifolia*) and Nutmeg (*Myristica fragrans*) essential oils. The extract was obtained using heat distillation of tested plant extracts. The essential oils were prepared and evaluated for repellent activity against *Ephestia cautella* walker. The chemical composition of *lavender and nutmeg* was analyzed by gas chromatography/mass spectroscopy (GC/MS). Results showed that lavender and nutmeg essential oils were a rich source of many effective compounds. Repellency was -16.22 % and 86.36 % for lavender and nutmeg essential oils, respectively. The results revealed that the nutmeg essential oils can be used in biological pest control as alternative to be synthetic chemicals. Results indicates that nutmeg essential oil can be introduced as a repellant against date pest E. *cautella* walker, so these results sign the interesting assurance of designing a potentially active repellent agent from nutmeg essential oils.

Keywords: Chemical composition; repellent activity; essential oils; lavender; Nutmeg; Ephestia cautella.

1. Introduction

Essential oils (EOs) are volatile constituents obtained from aromatic plant material including leaves, rhizomes, flowers, roots, bark, seeds, peel, fruits, wood and whole plants **(Hyldgaard, Mygind *et al*., 2012)**. Essential oils have been traditionally employed in traditional medicine since ancient times also in the manufacture of foodstuffs, cosmetics, herbicides insecticides (Mafra-Neto 2015). More-over, in the last decades, scientific studies have related many biological properties (antioxidant, anti-inflammatory, antiviral, antibacterial, stimulators of central nervous system, etc.) of several plants and herbs to some of the compounds present in the essential oil of the vegetal cells **(Sarac and Ugur 2008, Mechergui, Coelho *et al*., 2010)**. Some species from plant families have been identified as promising sources of compounds with pesticide properties.

Lavender oil obtained from *Lavandula angustifolia*, has a broad spectrum of biological activity **(Mechergui, Coelho *et al.*, 2010)**, antimicrobial and antioxidant properties, So it is used in the food, pharmaceutical, and cosmetic industries to replace or reduce the amount of preservatives **(Bassole and Juliani; 2012, Turek; 2013)**.

Nutmeg (*Myristica fragrans*) is a well-known spice from the nutmeg tree. Nutmeg is widely used as spices and in alternative medicine as it has been reported to have aphrodisiac **(George, Kulkarni *et al.* 2015, George, Alonso-Gutierrez *et al*., 2015)**, memory enhancer, antidiarrheal property **(Bassole and Juliani, 2012)**, anti-inflammatory and anti-cancer property **(Helmy, Morsy *et al.*, 2017).**

The Date moth; *Ephestia cautella* walker., is a serious pest of dates in both field and storage houses **(Fadil, 2015)**. These devastating insect cause losses of weight and downgrading of the commercial value of the fruit **(Dhouibi, 2000)**. Two important factors that contribute to its seriousness on dry dates including its ability to develop resistance to insecticides **(Al-Taweel, 1990)**.

The main objectives of present study were to study the chemical composition of lavender and nutmeg essential oils and identified their constituents by gas chromatography-mass spectrometry (GC–MS), Also investigated the repelling activity of these essential oils against one of the main date pests; E. *cautella*

2. Materials and methods

2.1. Collection of plant material

Two medicinal plants; Nutmeg *Myristica fragnas* (seed), and Lavender *Lavandula angustifolia* (flower) were collected from local markets in Unaizah city, Saudi Arabia. The main reason to choose these essential oils for this study is that, in spite of traditional use in numerous medical aspects. Biological properties of many plants are still unknown; these could probably have potential to use as an alternative to synthetic insecticides for agricultural and public health pest control. Collected plant material (Table1) were washed by distilled water and dried under shade at room temperature (25±5C°). All samples were milled (particle size about 2 mm) and stored at -18˚C prior to analyses.

Table (1): Plants essential oils and used parts.

No.	Plant name	Scientific name	Family	used parts
1	Lavender	*Lavandula angustifolia*	Lamiaceae	Flower
2	Nutmeg	*Myristica fragnas*	Myristicaceae	Seeds

2.2. Extraction 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, and then dried with anhydrous sodium sulfate (Na_2So_4) to remove all water. 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 a deep freezer at -20 °C.

2.3.Yield of Essential Oils

The yields of essential oil of lavender and nutmeg were expressed in g relative to 100 g of dry vegetable matter; it was calculated according to Equation (1):

$$\text{Yield (\%)} = \frac{Amount\ of\ extracted\ oil\ (g)}{Amount\ of\ dray\ plant\ mass\ (g)} * 100$$

2.4.Chromatographic analysis and mass spectrometry

The chromatographic analysis of essential oils was performed using an Agilent 7890A gas chromatograph equipped with MSD-5975C detector, coupled directly to an Agilent 7890A gas chromatograph split–split less injector, Agilent model 7693 autosampler and analytical column DB-Wax capillary column (polyethylene glycol, 30 m × 0.25 mm i.d, film thickness 0.25 μm, Agilent Technologies, USA). The column's temperature was programmed at 40 °C for 4 min, then ramped to 220 °C, at rate of 4 °C/min, finally, ramped to 280 °C, at rate of 4 °C/min 20 °C/min, respectively. Approximately 0.2 µl of each sample diluted in diethyl ether (5% solution in diethyl ether) was injected using the split injection mode; the split flow ratio was 10:1. Helium was used as the carrier gas, with a flow rate of 1.0 mL min^{-1}. The detector temperature was maintained at 300 °C. The mass spectrum was obtained for each compound at 70 eV, and the reading speed was 0.5 scan s^{-1} m/z 40-650. The temperatures of the electronic-impact ion source and the MS quadrupole were 230 °C and 150 °C, respectively. The MSD transfer line was maintained at 280°C for the analysis. 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 2013).

2.5. Repellency effect of essential oils

In the repellency test, the Sidney et al. 2006, (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% R.H.. This test was applied to 24-hour moths that were inserted from main arm of the olfactometer. The end of other two tubes were connected through a sealed lid 500 ml cylinder plastic bottles. For each essential oil, one dose (0.25 microliter / 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.

2.6. Statistical analysis

From the obtained data repellency was calculated using the following formula (Huang, Qian et al. 2018):

% of repellency = [(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 LDP line software to calculate Probit analysis according to Finney (Finney 1971).

3. Results and discussion

3.1. Identified compounds from extract of essential oils

With the increase in the incidence of resistance to pesticides, alternative natural products of plants could be of interest. Some plant extracts and phytochemicals are identified as promising sources of compounds with pesticide properties, which could be have a great importance role in integrated pest management programs. In the last years, various studies have been conducted in different countries, demonstrating the efficacy of this type of treatment (Huang, Qian et al. 2018). The chemical composition of tested essential oils is given in Tables 2-3. These *tested essential oils* are a rich source of many effective compounds. Table 2 illustrates the identified compounds of essential oil extracted from lavender. These results showed that 31 compounds were detected in lavender essential oil, which eleven of them had the highest abundant and represents 92.4 % while other 20 compounds only represented 7.6 % of total amount of identified compounds. Lavender essential oil is a rich source of many effective compounds as monoterpenes

Table (2): The identified compounds from extract of lavender essential oil.

Peak	R.T. (min)	Chemical name	Formula	% of total	Kovat Index
1	8.062	Alpha-Pinene	$C_{10}H_{16}$	2.109	1018.376
2	9.435			0.16	1060.923
3	12.932	Beta-Pinene	$C_{10}H_{16}$	1.581	1163.795
4	14.095			0.865	1196.976
5	14.412			0.289	1206.074
6	14.517	Eucalyptol	$C_{10}H_{18}O$	2.866	1209.096
7	15.443			1.933	1235.751
8	15.78			0.148	1245.452
9	16.02	1,3,6-Octatriene, 3,7-dimethyl-Z)	$C_{10}H_{16}$	1.866	1252.36
10	16.143			0.268	1255.901
11	16.801			0.129	1274.842
12	17.08			0.289	1282.873
13	20.117			0.174	1373.532
14	20.377			0.183	1381.361
15	21.592			0.27	1418.933
16	23.964			0.217	1494.282
17	24.751	Camphor	$C_{10}H_{16}O$	2.815	1520.315
18	25.775	Carene	$C_{10}H_{16}$	34.687	1554.585
19	25.992	Carene	$C_{10}H_{16}$	30.225	1561.847
20	26.417			0.184	1576.071
21	27.174	Cayophyllene	$C_{15}H_{24}$	8.355	1601.478
22	27.404	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyle)	$C_{10}H_{16}$	3.832	1609.574
23	29.068			0.791	1668.145
24	29.228			0.647	1673.777
25	29.512			0.245	1683.773
26	30.099			0.812	1704.651
27	30.25	Borneol	$C_{10}H_{18}O$	2.099	1710.225
28	30.328			0.3	1713.104
29	30.795			0.506	1730.343
30	31.632			0.897	1761.24
31	34.118			0.256	1855.637

(Alpha-Pinene and Beta-Pinene) which, have a wide range of pharmacological activities, including, antibiotic resistance modulation, antimicrobial, antimalarial and antioxidant **(Salehi; Upadhyay *et al.*, 2019)**. Eucalyptol is a terpenoid oxide isolated from Eucalyptus species, is a promising compound for treating such conditions as it has been shown to have anti-inflammatory and antioxidant effects **(Seol and Kim, 2016)** the cyclic monoterpene (Camphor) and bicyclic monoterpene (Cayophyllene) which, has anti-inflammatory and analgesic properties and is used for its aromatic properties, as an insect repellant **(Hu; Jia *et al*., 2019, Liu; Guo *et al*., 2019)**. The compounds which identified in nutmeg essential oil were presented in Table 3, the obtained results showed the presence of 21 compounds, 12 out of 21 were high abundant with total 91.85% of total detected amount, while other 9 compounds only represented 8.14 % of total amount of identified compounds. Nutmeg shows repellent effect which is due to the presence of many effective compound in the essential oil which contains terpenes (α-pinene, p- cymene, sabinene, camphene, myrcene and γ-terpinene) terpene derivatives (terpinol, and linalool) and phenylpropanes

(**Rejitha T P 2014; Huang, Qian *et al*., 2018**). **Naeem *et al*. (2016)**, was mentioned that the Nutmeg contains fats (30-40%) and essential oils (10%). Essential oil is generally characterized by GC-MS analysis

(Al-Maskri; Hanif *et al*., 2011, Namra Naeem 2016)

Table (3): The identified compounds from extract of nutmeg essential oil.

Peak	R.T. (min)	Chemical name	Formula	% of total	Kovat Index
1	8.055	Alpha-Pinene	$C_{10}H_{16}$	21.325	1018.159
2	8.222	Alpha-phellandrene	$C_{10}H_{16}$	2.099	1023.334
3	9.419			0.319	1060.428
4	10.833	Beta-Pinene	$C_{10}H_{16}$	13.434	1103.909
5	11.343	Beta-phellandrene	$C_{10}H_{16}$	18.385	1118.459
6	12.305			1.032	1145.906
7	12.904	Beta-Myrcene	$C_{10}H_{16}$	2.589	1162.996
8	13.401	-4-Carene		3.934	1177.175
9	14.078	D-Limonene		4.215	1196.491
10	14.394	Beta-phellandrene		2.66	1205.556
11	15.765	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)		5.617	1245.02
12	16.607			1.136	1269.257
13	17.063			1.879	1282.383
14	23.244			0.269	1471.41
15	23.948			0.287	1493.774
16	27.38	Alpha-Pinene		5.611	1608.729
17	30.087			0.768	1704.208
18	34.73	1,3-Benzodioxole, 5-(1-Propenyl)		2.143	1879.349
19	38.17			0.311	2017.74
20	43.926	1,3-Benzodioxole, 4-methoxy-6-(2-Propenyl)		11.987	2250.871

3.2. Repellency effect of tested essential oils on E. *cautella* moth

In the present study, repellant property of two plant essential oils have been assayed against E. *cautella*. Insects were exposed to the tested essential oils for one day and the behavior was observed, the longest reported time exposure period, (24 hour). The repellent effects of the essential oils against E. *cautella* are shown in Table (4). Repellency was determined by comparing number of insects in the treated chamber with the untreated chamber (control). The results indicate variation among the two tested essential oils extracts against the selected insect pest E. *cautella*. Nutmeg oil recorded the high repellent activity against E. *cautella*. The repellency percent was -16.22 % and 86.36 % for lavender and nutmeg essential oils, respectively. In general, lavender essential oil showed attractant activity at tested concentration during 24h time period, while the trend had changed with nutmeg essential oil at the same concentration and duration period. The reason for this kind of both repellent and attractant at two different concentrations is unknown. The high repellency effect that recorded with Nutmeg oil may be due to the active components of this oil.

Other studies have been showed same trend of results especially with low concentration, the presence of an olfactory stimulus might be the reason of insect behaviour. Some essential oils showed attracting activity of insects when used at low concentration while have been showed moderate or high repellent activity with increasing the concentration (Rejitha T P 2014). In the same line Sabbour and Abd El-Aziz 2018 (Sabbour and Shadia 2018) recorded high repellency effect (71% and 54%) of Rosemary and Eucalyptus, respectively against E. *cautella*. As well as

Table (4): Repellency effect of essential oils

Plant essential oils	T	C	ST	% of repellency
Lavender	4.625	5.375	0	-16.22
Nutmeg	5.5	0.75	3.75	86.36

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

(Jayakumar, Arivoli *et al.*, 2017), tested the repellent activity of ten plant oils against the adults insects of *Sitophilus oryzae* under the laboratory conditions. The obtained results showed varying degree of repellency activity between the ten tested *plant essential oils,* six essential oils Wintergreen, Lemon, Geranium, Citronella, Camphor and Lavender have been showed repellent activity while the other three essential oils Rosemary, Aniseed and Vetiver exhibited attractant activity. El-Khyat et al. (2017) recorded high repellence activity of essential oils (German chamomile, Sweet marjoram and Bitter orange) against E. *cautella*. Also repellent activity of essential oils have been recorded against stored product pests by Regnault-Roger (Regnault-Roger 1997), Cosimi et al. (Cosimi, Rossi et al. 2009) and Nerio et al (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 1997). Maximum repellency of plant essential oils could have expressed as Excess Proportion Index (EPI), which reflect the effect of these essential oils against the exposed insects. The repellent effect of essential oils and their local availability could making them a good candidate in management control of stored-grain insect (Mishra, Tripathi et al. 2012).

Conclusion

The major thrust of this work is to study the chemical composition of lavender and nutmeg essential oils and evaluate their repellent effects against one of the major pest which attack stored date, which considered as a native fruit of arid region using low concentration 0.25 % with relatively long duration exposure period 24h. Among the two tested essential oils high repellency was seen in the case of nutmeg, while opposite trend was seen in case of lavender. In this context the relevance of the study becomes that the essential oils can be alternative to use the other harmful pesticides or chemicals. Nutmeg essential oils can be considered as good alternative to be synthetic and biological method of insect control. Results indicated that nutmeg essential oil can be introduced as repellants against date pest E. *cautella* walker, Study results reflected that the plant essential oils can use as an eco-friendly approach in pest control.

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التركيب الكيميائي والنشاط الطارد لزيوت اللافندر وجوزة الطيب ضد حشرة التمر المخزون

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الملخص العربي:

تهدف هذه الدراسة إلي تعيين التركيب الكيميائي للزيت الطيار المستخلص من نباتات اللافندر وجوزة الطيب، وكذلك إلي دراسة التأثير الطارد لهذه الزيوت ضد حشرة التمر المخزون (*Ephestia cautella*). تم إستخلاص الزيوت الطيارة باستخدام طريقة التقطير من المستخلص النباتي لكل من اللافندر وجوزة الطيب ومن ثم تم تقييم تأثيرها الطارد علي الحشرة محل الدراسة. كذلك تم تعريف التركيب الكيميائي وتحديد المركبات الفعالة في هذه الزيوت باستخدام التحليل الكروماتوجرافي الغازي – مطياف الكتلة. وقد أشارت النتائج إلي ان زيت اللافندر يحتوي علي العديد من المركبات الكيميائية الفعالة إلا انه لم يظهر تأثير طارد للحشرة في حين أظهر زيت الطيار المستخلص من جوزة الطيب فعالية كبيرة في طرد الحشرة مما يجعله بديلا جيدا للمركبات المخلقة كيميائيا في المكافحة الحيوية لحشرة (*Ephestia cautella*).