Chemical Composition and Bio-Insecticidal Activity of the Dill, *Anethum graveolens*, Essential Oils against the Red Flour Beetle, *Tribolium castaneum*

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ABSTRACT

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Stored products are considered main food source for humans and domestic animals. They were always targeted by insects mainly moths and beetles. The use of natural substances such as essential oils and extracts of aromatic plants constituted an alternative to chemicals. This work aims to highlight the chemical profile of the dill, *Anethum graveolens,* seeds and study their toxicity against red flour beetle, *Tribolium castaneum*. The essential oils were obtained by hydrodistillation and analyzed using the GC/MS technique. A total of 45 compounds are identified in which dillapiole (37.86%), carvone (22.59%), trans-isolimonene (10.01%), dihydrocarvone (6.85%), camphor (5.06%) and α-phellandrene (2.77%) are major compounds. Dill seed essential oils exhibited an insecticidal activity against adults of *T. castaneum* which increased proportionally with the applied dose and the time of exposure. Fumigant bioassays resulted in lethal doses LD_{50} of 232.89 µl/L air and LD_{90} of 328.28µl/L air after 12 h of exposure. These values decreased after 24 h of exposure to 132.57 and 202.01 µl/L air, respectively. The insecticidal activity of these essentials oil can be the result of the existence of dillapiole, carvone, isol-limonene, and other compounds. Due to these promoting results, the essential oils of dill seeds may be used as a natural product to manage this pest in stored products.

Key words: *Anethum graveolens*, dill, essential oils, fumigant toxicity, repulsive activity, stored products, *Tribolium castaneum*

Grain storage has constituted the most important source of staple foods used to feed the world population. It represents a vital sector in the economies of both developed and developing countries. Post-harvest losses are recognized as being one of the critical constraints on food security. This sector was faced with several problems mainly those related to the protection of the stocks against pests and diseases in post-harvest stages. The major threat to stored grains food supplies is still insect pests. Annually and without chemical treatment, damage caused to the world harvests of cereals and legumes

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from their invasion by insect pests during their storage may amount to 20–40% (FAO 2016). The nature of insect damage to stored commodities is very variable. Their direct-feeding activity caused quantitative damages and losses mainly by reducing grain weight and the loosing of their germination power. In addition, indirect damages may appear in post-feeding-activity and reside on fungal contamination of stored grains that diminish the quality and the nutritional value of grains (bad odor, mold, and heat-damage problems) making them unfit for transformation into food and improper for humans and/or animals consumption (Bosly and El-Banna 2015). Between insects that invaded stocks, those belonging to the genus *Tribolium* which comprises 36 described species, ten of them are listed as major pests of stored grain products throughout the world. The two most cosmopolitan major coleopteran species of this genus are *Tribolium castaneum* and *Tribolium confusum* in hot and dry climates of the world. Their widespread was the result of international trade development mainly by the exchange of infested products such as grains, seeds, and flour (Nakakita 1983).

To protect grains, forage crops and other farming commodities and to reduce insect attacks in stored products, diverse methods are used throughout the world. However, the most effective way to secure these products was based on the use of chemicals, either synthetic insecticides, or fumigants such as methyl bromide and phosphine. In numerous storage systems, the use of fumigants is the major economical method for controlling a wide range of pests. The efficiency of fumigation resides in the use of chemical products that enter deeply and cover the maximum if not the entire stored-grain volume.

The excessive and indiscriminate use of synthetic pesticides led to several problems related to environmental pollution, ozone depletion, pest resistance, toxicity to non-target organisms, and high toxicity toward human health (Pavunraj et al. 2016). These problems oriented researchers towards new horizons using natural substances which present a low risk to the environment and human health. For this purpose, the recourse to the use of essential oils to control pests during the last decades seems to be a promoted way, firstly because plants which represent a rich source of bioactive molecules are available, and secondly for their low toxicity and rapid degradation (Amaobenget al. 2019; Ismanand Machial 2006). Additionally, these essential oils act in multiple sites in insects, which highly reduces the appearance risk of resistance (Tangtrakulwanichand Reddy 2014).

Anethum graveolens belonging to the family of Apiaceae, commonly known as dill, is an annual aromatic plant native to Mediterranean and West Asia. This species is a popular aromatic herb and has a long history of cultivation and use as a culinary and medicinal herb. It is widely cultivated in several countries such as India, Pakistan, Afghanistan, Thailand, Africa, China, Egypt, Iran, Turkey, USA, Canada, the Middle East, and other countries (Chahalet al. 2017). In Tunisia, it is called “Shapt” or savage fennel. The major components of *A. graveolens* are flavonoids, phenolic compounds, and essential oils (Stavri and Gibbons 2005; Amin and Sleem 2007). Many studies were interested in the biological activities of its essential oils, like as its antibacterial activity (Delaquis et al. 2002; Jirovetz et *al.* 2003; Rafii and Shahverdi 2007; Ruangamnart et al. 2015; Dimov et al. 2017), antifungal activity (Zeng et al. 2011; Tian et al. 2011) and insecticidal properties (Chaubey 2007; Babri et al.

2012; Oliveira et al. 2013; Rosa et al. 2019; El-Sayed et al. 2020). Other activities are studied for dill essential oils as an antioxidant (Dimov et al. 2017; Ksouri et al. 2020) and also its anti-inflammatory activity as cited by Ksouri et al. (2020).

Due to the widespread of *A. graveolens* as a spontaneous plant in the farming land of the region of Sidi Bouzid in central-western Tunisia and its limited use as a condiment and medicinal plant, it would be very interesting to explore other of its bio-activities. This work aims in part to study the chemical composition of dill seed essential oils by gas chromatography-mass spectrometry (GC/MS) and in another part to evaluate its insecticidal activity by assessing its fumigant and repellent properties against adults of *Tribolium castaneum*.

MATERIALS AND METHODS

Plant material and essential oil extraction.

Dried seeds of the spontaneous plant A. *graveolens* were collected from natural populations growing in the field during June 2020 in the region of Sidi Salem situated 3 km from Sidi Bouzid town in central western Tunisia. Essential oils were extracted from 100 g dried seeds immersed in 600 ml of water (quotient = 1:6, i.e., 100 g of plant material for 600 ml water) and for 90 min by hydrodistillation using a Clevenger apparatus. The extracted essential oils were collected at the end of the process, put in an Eppendorf tube to calculate the yield of extraction, then wrapped in aluminium paper to make darkness conditions, and kept in a refrigerator at 4°C until use in experiments.

Gas Chromatography-Mass Spectrometry analyses.

The chemical analyses of the essential oil composition were performed by GC/MS using gas chromatograph (Agilent 7890 A, Hewlett Packard, CA, USA) coupled with a mass spectrometer detector (Agilent 5972 C model) with electron impact ionization (70 eV). The temperature of column was initially maintained at 50°C for 3 min, then raised gradually to 250°C at a rate of 5°C/min where it was left for 10 min. The temperature of injector was 220°C and of the MS interface was 280°C. Chromatographic separation was performed on the HP-5 MS (30 mm × 0.25 mm, 0.25 µm film thickness) columns. Carrier gas was Helium which was used to inject samples with a rate flow of 1 ml/min. The mass spectrometry conditions were operated as follows: ionization voltage 70 eV scanning for electron ionization mass spectra from 50 to 500 m/z. The injected volume was 1 µl and the total run time of analysis was estimated to 57 min.

Components in the essential oils were identified by comparing the mass spectra of each one with the values stored in the Wiley (7 n) library as well as in the National Institute of Standards and Technology (NIST) mass spectral database (Adams 2001). The percentage composition was calculated based on the separated peak areas using the normalization method.

Insect rearing.

Adults of the red flour beetle, *T. castaneum*, were initially collected from infested wheat to initiate rearing in the laboratory. The breeding led to obtain homogeneous generation for bioassays at any time. Insect rearing was carried out inside plastic containers filled with wheat grains or commercial wheat semolina as substrates and maintained within an incubator at 30±1°C, 65±5% relative humidity, and in total darkness. Selected insects for the bioassays belonging to the same generation (10-15 days age) were chosen randomly from the rearing boxes.

Toxicity measurement of essential oils.

The toxicity of dill essential oil was measured by fumigant bioassays. Ten unsexed adults of red flour beetle (10-15 days old) were placed into a transparent plastic cylindrical vial of 70 ml volume as a fumigant chamber. In the screw cap of each vial was placed a 3.5 cm diameter filter paper (Whatman n°1) which was impregnated with one of the tested essential oil doses using a micropipette. In this study, the fumigant toxicity of *A. graveolens* pure essential oils was experienced at various doses. Used doses were 2, 4, 8, 16 and 32 µl. Then, caps were screwed quickly and tightly on the vials which permitted to generate internal equivalent fumigant concentrations between 28.57 and 457.14 µl/L air. Each assay plus a control group without essential oil concentration was replicated three times. Then, mortality of insects was recorded at different times of exposure during the next 72 h. Insects were considered dead when no antenna or leg movements were observed. The percentage of mortality was calculated according to Abbott's (1925) correction formula as follows:

$$\mathrm{CM} = \frac{\mathrm{Mt} - \mathrm{Mc}}{100 - \mathrm{Mc}} \times 100$$

where CM: Corrected Mortality, Mt: Mortality of treated insects, and, Mc: Mortality of control insects.

Repellent activity evaluation.

The repellent activity of *A. graveolens* essential oils on *T. castaneum* adults was evaluated using the preferential zone method on filter paper described by McDonald et al. (1970). Assays were carried out inside Petri dishes of 9 cm diameter and a height of 0.5 cm.

Thus, 9 cm diameter filter paper discs are cut into two equal parts; one-half of the paper is treated with essential oils diluted in acetone, and the other half is treated only with absolute acetone. Five test solutions were prepared by dissolving 2, 4, and 8 µl of the essential oils in 2 ml of acetone. Volumes were converted to give equivalent concentrations of, respectively, 0.063, 0.125 and 0.25 µl/cm^2. Then, 0.5 ml of each prepared solution was spread evenly over one half of the filter paper disc using a micropipette while the other half was only soaked with 0.5 ml of acetone. Both treated and untreated halves of filter papers were dried to ambient air for 5 min and then were reattached with adhesive tape to reconstitute the filter paper disc which was placed in the Petri dish. Twenty adult beetles of both sexes were released inside the Petri dish, at the middle of each filter paper disc.

Each treatment was replicated three times. The number of insects presents on control (Nc) and treated halves (Nt) were recorded after 15, 30, 60, 90, and 120 min of exposure for each tested concentration. The Percent Repellency (PR) values were computed using the following formula:

$$\mathrm{PR}\ (\%) = [(\mathrm{Nc}-\mathrm{Nt})/(\mathrm{Nc}+\mathrm{Nt})] \times 100.$$

To categorize the repellent activity of essential oil, five classes were distinguished based on the mean of percent repellency (PR) as follows: Class 0: PR= [0-0.1]%, Class I: [0.1-20]%, Class II: [20.1-40]%, Class III: [40.1-60]%, Class IV: [40.1-60]%, Class V: [60.1-100]% (McDonald et al. 1970).

Data analyses.

Statistical analyses were performed using the software IBM SPSS (version 20). Data related to fumigant toxicity are subject to statistical analyses using the one-way analysis of variance (ANOVA). This type of analysis is used to test the effect of the dose and the exposure duration of the essential oils on the adult mortality. A Tukey test was applied to the concentration means, after fixing the exposure time, to detect significant differences at a 5% level.

The efficiency of the essential oils was determined based on the Probit analysis, described by Finney (1971) to determine in part the lethal concentrations causing 50% and 90% mortality of the exposed insects (LC_{50} and LC_{90}) and in other parts the lethal times (LT_{50} and LT_{90}) values with their 95% confidence limits. The Probit analysis was also applied to the repellent test to calculate the repellent concentration causing 50% and 90% repellency (RC_{50} and RC_{90}).

RESULTS

Chemical composition analyses.

The yield of essential oils extracted by hydrodistillation from dried seeds of *A. graveolens* was 2.5% ± 0.23%. The essential oil composition of dill seeds, as obtained by GC/MS analysis, as illustrated in Table 1. It allowed the identification of 45 compounds representing 100% of the essential oils. The major three constituents in this essential oils (up to 10%) were dillapiole (37.86%), followed by carvone (22.59%), and (+)-trans-limonene (10.014%). A second group of constituents, with concentrations comprised between 1% and 10%, included dihydrocarvone (6.846%), camphor (5.055%), dillether (4.939%), and α-phellandrene (2.774%), whereas most compounds of the essential oils, of a number of 25, with concentrations ranging between 0.1% and 1%, represented 9.35%. They were considered as minor compounds such as α-pinene, β-pinene, sabinene, β-myrcene, gamma-terpinene, and more other compounds illustrated in Table 1. The remaining 10 compounds of the essential oils were present in trace amounts (less than 0.1%), and all of them summed together represented only 0.617% of the total composition.

These compounds belong to six chemical classes (Table 1). Dill seed essential oils were particularly rich in phenylpropanoids (38.93%) and ketones (34.9%). Monoterpenes represent 23.59% partitioned between monoterpenes hydrocarbons (16.41%) and oxygenated monoterpenes (7.18%).

Fumigant toxicity measurement.

Fumigant bioassays of *A. graveolens* seed essential oils showed marked and variable toxicity against adults of *T. castaneum* at several concentrations and exposure times. The evolution of the mortality percentage for different applied concentrations for different times of exposure is illustrated in Fig. 1.

Monitoring of bioassays has been performed continuously since the beginning of the experiment to show the onset time of mortalities for different concentrations, i.e. the biological effectiveness of the tested oils on the red flour beetle. Results revealed the very rapid response of adults to essential oils after 2 h of application for the highest concentration (457.14 µl/L air). However, this action was registered after 6 and 12 h, respectively, for the concentrations 228.57 and 114.28 µl/L air, respectively. When the essential oils were applied at the lowest concentrations of 57.14 and 28.57 µl/L air, first mortalities were observed after 24 and 36 h, respectively.

According to Fig. 1, the total mortality (100%) during all the assays was registered only for the highest concentration after 12 h of exposure compared to 93.33% registered for the concentration 228.57 µl/L air after 24 h and 70% for 114.28 µl/L air after 48 h of exposure. However, fumigation using the two lowest doses, i.e. 28.57 and 57.14 µl/L air, can be considered as not effective against tested adults even after 72 h of exposure time and resulted in 6.67% mortality. Therefore, it is clear from the observation of Fig. 1 results, that the efficiency of the essential oils toward red flour beetle increases quickly and proportionally with the increasing applied concentration and exposure time.

Table 1. Chemical composition of *Anethum graveolens* seed essential oils

N	TR (min)	Compound	Concentration
1	12.265	α-pinene	0.59%
2	12.831	Camphene	0.083%
3	13.643	Sabinene	0.531%
4	13.831	β-Pinene	0.386%
5	14.117	β-Myrcene	0.851%
6	14.466	Isoterpinolene	0.064%
7	14.614	α- terpinene	0.587%
8	14.797	α- phellandrene	**2.774%**
9	15.512	O-cymene	0.382%
10	15.780	(+)-Trans-Isolimonene	**10.014%**
11	16.746	γ- Terpinene	0.152%
12	17.106	Trans-sabinene hydrate	0.168%
13	17.889	Terpinolene	0.276%
14	18.821	1,3-Pentadiene, 2,4-dimethyl- (CAS)	0.093%
15	18.924	Heptylideneacetone	0.499%
16	18.924	1-terpineol	0.150%
17	19.593	Cis-Limoneneoxide	0.042%
18	19.753	Trans-Limoneneoxide	0.06%
19	19.913	2-Norpinanol, 3,6,6-trimethyl- (CAS)	0.364%
20	20.038	Artemisiaketone	0.264%
21	20.244	Camphor	**5.055%**
22	20.736	Cyclopropane, trimethylmethylene-(CAS)	0.153%
23	20.804	2-Methylene Vinyl Cyclopentane	0.137%
24	21.039	Trans-3(10)-Caren-2-ol	0.702%

25	21.296	4-Terpineol	0.325%
26	21.650	Dillether	**4.939%**
27	22.370	Dihydrocarvone	**6,846**
28	22.988	Iso-Dihydrocarveol	0.129%
29	23.182	Trans-Carveol	0.104%
30	23.571	Dihydro-carveol	0.382%
31	23.851	Carvone	**22,59%**
32	24.897	Isophorone	0.104%
33	25.171	Thymol	0.126%
34	25.525	Carvacrol	0.056%
35	27.143	Piperitenone	0.046%
36	32.778	Myristicine	0.379%
37	33.613	Elemicine	0.605%
38	36.276	Dillapiole	**37.861%**
39	37.465	Apiole	0.083%
40	39.917	Cinnamic alcool	0.114%
41	44.621	Dibutylphtalate	0.048%
42	52.565	Vinhaticoicacid	0.141%
43	52.851	Xanthine, 8-[2-cyclohexenyl]-1,3-dipropyl- Spongia-13(16),14-dien-19-oic acid	0.579%
44	55.600	3-Methylheneicosane	0.042%
45	56.703	Bis(2-ethylhexyl) phthalate	0.131%

Chemical classes

Monoterpenes hydrocarbons	16.41
Oxygenated monoterpenes	7.18
Oxygenated sesquiterpenes	0.10
Ketones	34.9
Phenylpropanoids	38.93
Phenol	0.18
Others	2.30

RT: Retention Time.

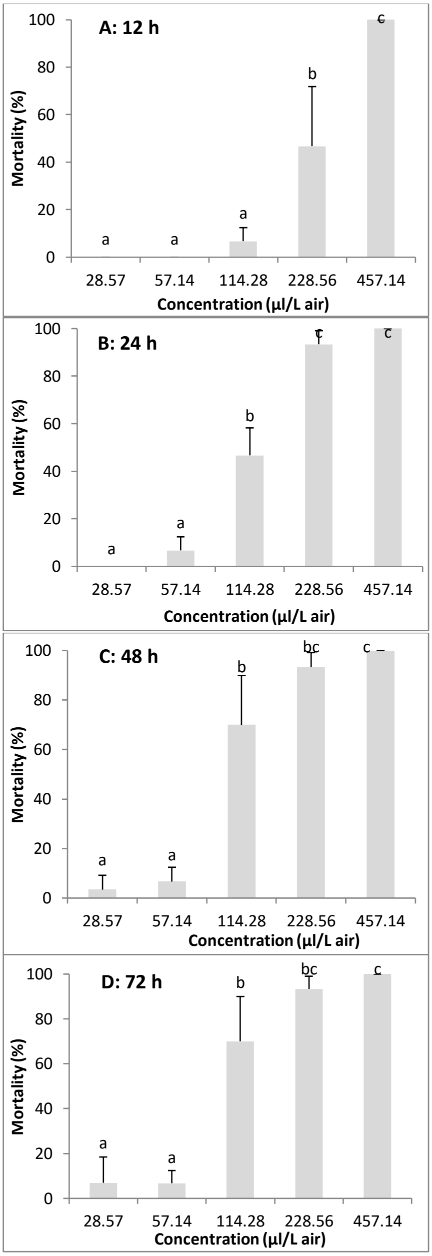

Fig. 1. Percentage mortality ± SE of *Tribolium castaneum* adults exposed to different concentrations of dill seed essential oils at different times in fumigant toxicity bioassays. Different letters above the concentrations indicate significant differences between the concentrations (P = 0.05).

ANOVA analysis revealed a significant difference (df = 4, F = 42.3, sig. = 0 < 0.05) between the responses to these doses after 12 h of adult exposure to the studied essential oil (Fig. 1.A). The use to the Tukey test distinguished three sub-homogeneous groups (2, 4, and 8 µl), (16 µl), and (32 µl). Results of ANOVA after 24 h of exposure to dill essential oils (Figure 1.B) also indicated a significant difference in its toxicity (df = 4, F = 164.33, sig.= 0 < 0.05) and the existence of three distinguishable sub-homogeneous groups (28.57 and 57.14µl/L air), (114.28µl/L air) and (228.56 and 457.14µl/L air). The same results were observed after 48 (Fig. 1.C) and 72 h (Fig. 1.D) of exposure to these essential oils, where significant differences were registered with three sub-homogeneous groups for each time.

Based on the Probit analysis method, the LC_{50} and LC_{90} of dill seed essential oils on the adult of *T. castaneum*,12 and 24 h after applying concentrations, were estimated to be 232.89, 132.57 and 324.28, 202.01 µl/L air, respectively.

The time required to induce 50% mortality (LT_{50}) in adults of red flour beetle at the highest dose of essential oils was 6.7 h (between 6.14 and 7.37 h), whereas the time required to induce 90% mortality (LT_{90}) was 9.33 h (between 8.46 and 10.67 h). For the other doses, lethal times results are illustrated in Table 2.

Table 2. Calculation of LT_{50} and LT_{90} for different concentration

Volume	LT_{50} (h)	Fiducial interval at 95%		LT_{90} (h)	Fiducial interval at 95%		χ2
		Lower	Upper		Lower	Upper	
8 µl	41.96	37.55	47.26	70.16	62.71	80.53	48.49
16 µl	22.7	20.04	25.72	39.99	35.84	45.52	1073.09
32 µl	6.7	6.14	7.37	9.33	8.46	10.67	1.88

LT_{50}: Lethal Time for 50% mortality; LT_{90}: Lethal Time for 90% mortality.

Repellent activity evaluation.

Dill seed essential oils exhibited significant repellent activity against red flour beetle even at low concentrations in all applied doses after 1, 2, and 4 h of exposure. In fact, after 1 h of treatment, the lowest applied concentration (0.063 µl/cm^2) resulted in 66.67% repellency, which increased to 73.33% after 2 h, and then decreased to 60% after 4 h of exposure. This fluctuation in percentage can be attributed to the free mobility of insects in the Petri dishes. This repellent activity becomes more apparent with the increase in essential oil concentration. Indeed, the repellent percentage recorded for the two concentrations of 0.125 and 0.25 µl/cm^2 was higher than 90% at all experimental times (1, 2, and 4 h of exposure). Therefore, according to the classification by McDonald et al., (1970), the lowest concentration was attributed to classes III and IV and the two other concentrations in class V for all experimental times. These results indicated that dill seed essential oils had a strong repellent effect against *T. castaneum*.

After 1 h of treatment, the calculated median repellent concentration RC_{50} was 0.01 µl/cm^2 with the RC_{90} was0.038 µl/cm^2. By increasing the exposure time, these values changed to 0.009 and 0.022 µl/cm^2 after 4 h of exposure. Generally, the repellent concentration values were inversely proportional to exposure time, meaning the repellent concentration decreased as the exposure time to essential oils increased.

DISCUSSION

Our results are in good agreement with El-Sayed et al. (2020) who noted dillapiole (44.01%) as the major compound of Egyptian dill seed essential oils; added to that a slight similarity in the rest of the composition containing Dl-limonene (19.47%), cis-dihydrochalcone (7.27), myristicin (3.54%) and phellandrene (3.19%). Generally, previous studies of this species mentioned carvone, trans-dihydrocarvone, limonene, apiole constituents as the key compounds. Babri et al. (2012) analyzed the Pakistani dill seed essential oils using GC/MS and reported R-(-)-carvone (38.89%), apiole (30.81%), limonene (15.93% and trans-dihydrocarvone (10.99%) as the major compounds. Meanwhile, Khani and Bassavand (2013) cited carvone (30.2%), trans-dihydrocarvone (11.7%), dillapiole (11.5%) and limonene (10.2%) as major compounds of Iranian dill seed essential oils. Our results are different from Jayakumar et al. (2020) who found a total of 13 compounds in Indian essential oils of dill seeds whose dominant ones are carvone (30.19%), limonene (16.25%), D-carvone (7.56%), D-limonene (6.68%) and others. However, Said Al-Ahl et al. (2015) in the same country (Iran) reported carvone (62.48%), dillapiole (19.51%), and limonene (14.61%) as the major components of dill seed essential oils. The chromatographic analysis of *A. graveolens* from Brazil mentioned by Vieira et al. (2019) permitted to identify 5 principal compounds: carvone (34.89%), apiol (22.02%), D-limonene (18.92%), dihydrocarvone (16.91%) and trans-dihydrocarvone (7.26%). Contrary to Singh et al. (2005) who signaled carvone (55.2%), limonene (16.6%), and dill apiole (14.4%) as the main components with the presence of camphor (11.44%).

Another study conducted by Sharapov et al. (2013) on Tajikistani dill essential oils extracted from aerial parts of the plant using GC/MS, obtained almost a similar composition with different concentrations compared to our finding. They cited carvone as a major constituent with 51.7%, trans-dihydro-carvone (14.7%), dill ether (13.2%), alpha-phellandrene (8.1%) and limonene (6.9%). Other compounds exist in a trace like α-pinene (0.1%), myrcene (0.1%), camphor (0.3%) and dillapiole (0.4%). The differences in chemical composition between the same cultivars are a result of several exogenous and endogenous factors such as geographic location, climate, soil structure, plant growth stage, harvest time, plant drying method, method of extraction of the essential oils which affect the quality and the quantity of compounds (Zandi-Sohaniand Ramezani 2016). So, differences observed in chemical composition between dill seed essential oils can be due to the differences in such factors. Other research made in Thailand indicated that D-carvone (32.94%), carvone (20.73%), dillapiole (19.64%), and limone (18.08%) were found as chief compounds in *A. graveolens* seeds (Peerakam et al. 2014).

Previous studies demonstrated monoterpenoids as the principal insecticidal constituents of many plant extracts and essential oils. Chahal et al. (2017) who made a comparison between the chemical compositions of dill seed essential oils from different countries in different continents reported that carvone and limonene represents the major compounds, whereas they cited also dillapiole, trans-dihydro-carvone and α-phellandrene in appreciable amounts. In these essential oils, other compounds like α-thujene, α-pinene, sabinene, myrcene, p-cymene, γ-terpinene, dill ether, iso-dihydrocarveol, trans-carveol and anethole were also present in trace. Constituents of this chemical family are volatile and lipophilic. These properties offer them a rapid action by easy penetration via airways into insects and interfere with their physiological function causing them damage and their mortality.

Few studies in the literature reported the fumigant toxicity of *A. graveolens* essential oils extracted from its different parts (Kaur et al. 2020). Scientific papers reported the toxicity of dill seed essential oils by continuous exposure bioassays and fumigant toxicity bioassays against stored insects as *Callosobruchus maculatus*, *T. castaneum*, *T. confusum*, *Oryzaephilus surinamensis*, *Rhyzopertha dominica*, *Sitophilus oryzae*, *Ephestia kuehniella* (Ebadollahi et al. 2012; Tripathi et al. 2003; El-Sayed et al. 2020; Khani and Basavand 2013; Jayakumar et al. 2021; Kaur et al. 2020; Najafzedah et al. 2019, Rosa et al. 2019; Su 1985) and other insects like *Periplanata americana*, *Musca domestica* (Kaur et al. 2020).

Chaubey (2007) conducted a study on the toxicity of *A. graveolens* oil against larvae and adults of *T. castaneum*. He discovered that to eliminate 50% of the studied population, an LC_{50} of 14.78 µl was required for larvae, compared to 16.66 µl for adults. However, results obtained by El-Sayed et al. (2020) from Egypt, focusing on the fumigant toxicity of dill seed essential oils on adults of *T. castaneum* and *Oryzaephilus surinamensis*, yielded relatively higher values compared to ours. The registered LD_{50} and LD_{90} values were 76.12, 150.8, and 80.6, 150.63 µl/L air, respectively, indicating that the Egyptian essential oils were more toxic and efficient than ours. Ebadollahi et al. (2012) testing the toxicity of dill seed essential oils on *C. maculatus* indicated an LC_{50} value of 25.48 µl/L air against an LC_{50} value of 0.54 µl/L air by Khani and Basavand (2013). The volatile toxicity of dill seed essential oils were also found against *T. confusum* with LC_{50} = 143.8 µl/L (Khani and Basavand 2013). Results of LC_{50} values revealed almost the same level of susceptibility of *T. castaneum* and *T. confusum* to the tested plant. Also, Babri et al. (2012) reported the dill seed essential oils have fumigant toxicity against *T. castaneum* by inducing a mortality that ranged between 58.3 and 100% in 24 hours of exposure.

In their studies, Majdoub et al. (2022) and Sriti Eljazi et al. (2017) investigated the insecticidal properties of essential oils derived from two Apiaceae. Majdoub team focused on *Daucus setifolius* essential oil, evaluating its effectiveness against two pests, *T. confusum* and *Sitophilus zeamais*. Their research unveiled significant repellent activity, with repellency rates of 72.5% and 75% against *T. confusum* and *S. zeamais*, respectively, following a 120-minute exposure period. However, despite its repellent properties, the essential oils exhibited only moderate toxicity towards adult specimens of both species, resulting in mortality rates of 62.5% for *T. confusum* and 52.5% for *S. zeamais*. In contrast, Sriti Eljazi et al. (2017) explored the efficacy of coriander fruit essential oils against *T. castaneum*, *S. oryzae*, and *Lasioderma serricorne*. They found that exposure to coriander essential oils at an air dose of 625 µl/L for 24 h led to mortality rates of 66.67%, 70%, and 100%, respectively, for the mentioned pests. Additionally, Lopez et al. (2008) reported similar results, demonstrating the effectiveness of coriander essential oils against *S. oryzae*, *R. dominica*, and *C. pusillus*, with mortality rates averaging approximately

56%, 90%, and 100%, respectively, after 24 h of exposure.

The adulticidal activity of essential oils recorded in this study may be attributed to its major compounds dillapiole, carvone, trans-isolimonene, and others. The insecticidal activity of dillapiole was confirmed by Bernard et al. (1995) against second instar larvae of *Aedes atropalpus* and later by Almeida et al. (2009) who recorded 96% mortality with the essential oils of *Piper aduncum* containing 86.9% of dillapiole tested against *Anopheles marajoara* and *A. aegypti* and 100% of mortality using a purified essential oils containing between 95 and 98.9% of dillapiole for the two same species.

Fumigant toxicity experiments conducted by Tripathi et al. (2003) using l-carvone, d-carvone, and dihydrocarvone toward *R. dominica*, *S. oryzae*, and *T. castaneum* adults and *T. castaneum* larvae showed high efficiency of these 3 compounds with high toxicity attributed to l-carvone. So, l-carvone, d-carvone, and dihydrocarvone are considered as insecticidal compounds.

In conclusion, *T. castaneum* is considered among the most destructive insect pests of stored products. The management of this pest was based on the use of synthetic pesticides that have numerous undesirable effects. During the last decades, research focused on finding other alternative pest control practices and using compounds with low risk to control such pests. Researchers were oriented to plants for their richness in natural substances and compounds that might help in finding new molecules. Essential oils represent one of these alternatives known for their rapidity of action that might reduce damage to stored products in a short time. Several specific factors associated with essential oils as the high volatility of these substances permitting the easy diffusion between grains in storage, their pleasant smell, and their non-toxicity could authorize their use as bio-insecticides in certain closed places like storages and greenhouses. In the present study, the possibility of controlling the red flour beetle using dill seed essential oils was investigated and approved. This effectiveness or potential activity can be attributed to its rich chemical composition with 45 compounds. Consequently, Tunisian dill seed essential oils could be valued as a natural biological compound to manage *T. castaneum*. For further studies, it will be important to explore the bioactivity of these essential oils against other storage pests that affect stored products.

RESUME

Soltani R., Ktari R., Barhoumi L. et Chouaibi M.H. 2024. Composition chimique et activité bio-insecticide des huiles essentielles de l'aneth, *Anethum graveolens*, contre le tribolium rouge de la farine, *Tribolium castaneum*. Tunisian Journal of Plant Protection 19 (1): 27-42.

Les produits alimentaires stockés sont considérés comme la principale source de nourriture pour l'homme et les animaux domestiques. Ils ont toujours été ciblés par les insectes, principalement les lépidoptères et les coléoptères. L'utilisation de substances naturelles comme les huiles essentielles et les extraits de plantes aromatiques constituent une nouvelle alternative aux produits chimiques. Le but de ce travail est de mettre en évidence le profil chimique des huiles essentielles des grains de l'aneth, *Anethum graveolens*, et d'étudier sa toxicité contre le tribolium rouge de la farine, *Tribolium castaneum*. Les huiles essentielles ont été obtenues par hydrodistillation et analysées par la technique GC/MS. Au total, 45 composés sont identifiés parmi lesquels l'apiole d'aneth (37,86 %), la carvone (22,59 %), le trans-isolimonène (10,01

%), la dihydrocarvone (6,85 %), le camphre (5,06 %) et l'α-phellandrène (2,77 %) représentent des composés majeurs. Les huiles essentielles de grains d'aneth ont montré une activité insecticide contre les adultes de *T. castaneum* qui augmente proportionnellement avec la dose appliquée et la durée d'exposition. Les essais biologiques par fumigation ont révélé des concentrations mortelles DL_{50} de 232,89 µl/L d'air et DL_{90} de 328,28 µl/L d'air après 12 h d'exposition. Ces valeurs ont diminué respectivement à 132,57 et 202,01 µl/L d'air après 24 h d'exposition. L'activité insecticide de ces huiles essentielles peut être le résultat de l'existence d'apiole d'aneth, de carvone, d'isol-limonène et d'autres composés. En raison de ces résultats promettants, les huiles essentielles de grains d'aneth peuvent être utilisées comme produit naturel pour lutter contre ce ravageur dans les silos des produits stockés.

Mots clés: Activité répulsive, *Anethum graveolens*, huiles essentielles, produits stockés, toxicité par fumigation, *Tribolium castaneum*

ملخص

سلطاني، رسمي وريم كتاري ولسعد برهومي ومحمد الهاشمي شعيبي. 2024. التركيبة الكيميائية ونشاط البيو-مبيد حشري للزيوت الأساسية المستخلص من نبت ة الشبت، *Anethum gravolens*، ضد خنفساء الدقيق الحمراء، *Tribolium castaneum*. Tunisian Journal of Plant Protection 19 (1): 27-42.

تعتبر المنتجات المخزنة المصدر الرئيسي لغذاء الإنسان والحيوانات الأليفة. ويقع استهدافها دائمًا من قبل الحشرات وخاصة منها العثة والخنافس. ويشكل استخدام المواد الطبيعية كالزيوت الأساسية ومستخلصات النباتات العطرية بديلاً جديداً للمواد الكيميائية. الهدف من هذا العمل هو تسليط الضوء على التركيبة الكيميائية للزيوت الأساسية لبذور الشبت، *Anethum graveolens*، ودراسة سمّيتها ضد خنفساء الدقيق الحمراء، *Tribolium castaneum*. تمّ استخراج الزيوت الأساسية عن طريق عملية التقطير المائي وتحليله باستخدام تقنية GC/MS. وقد تمّ تحديد إجمالي 45 مركبًا، منها ابيول الشبت (37.86%)، والكارفون (22.59%)، والترانسآيزوليمونين (10.01%)، والديهيدروكارفون (6.85%)، والكافور (5.06%)، والألفا فيلاندرين (2.77%) كمركبات رئيسية. أظهرت الزيوت الأساسية لبذور الشبت نشاطًا كمبيد حشري ضد البالغين من خنفساء الدقيق الحمراء والذي زاد بشكل متناسب مع الجرعة المطبقة ومدة التعرض. أدّت الاختبارات الحيوية للتبخير إلى تحديد الجرعات المميتة LD_{50} بـ 232.89 ميكرولتر/لتر هواء و LD_{90} بـ 328.28 ميكرولتر/لتر هواء، بعد 12 ساعة من التعرض. انخفضت هذه القيمة إلى 132.57 و202.01 ميكرولتر/لتر هواء، على التوالي، بعد 24 ساعة من التعرض. يمكن أن يكون نشاط المبيد الحشري لهذه الزيوت الأساسية نتيجة لوجود أبيول الشبت والكارفون والإيسولليمونين ومركبات أخرى. وبسبب هذه النتائج المشجعة، يمكن استخدام الزيوت الأساسية لبذور الشبت كمنتَج طبيعي لإدارة هذه الآفة في مخازن المنتَجات المخزنة.

الكلمات المفتاحية: سمية التبخير، زيوت أساسية، نشاط طارد، منتجات مخزنة، *Anethum graveolens*، *Tribolium castaneum*

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