## Chemical Composition and Fumigant Toxicity of Artemisia absinthium Essential Oil Against Rhyzopertha dominica and Spodoptera littoralis

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## ABSTRACT

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Natural products are excellent alternative to synthetic pesticides due to their reduced harmful impacts on human health and environment. Pesticides based on plant essential oils or their constituents have demonstrated efficacy against a range of pests and pre- and postharvest diseases. In this study, the pesticide potentiality of the essential oils from the absinthe wormwood *Artemisia absinthium* (Asteraceae) was investigated against two insect pests i.e. *Rhyzopertha dominica* and *Spodoptera littoralis*. Essential oil of the aerial parts was obtained by hydrodistillation and was analyzed by GC– MS in order to determine its chemical composition. The major components identified were: camphor (24.81%), camazulene (13.17%), bronylacetate (5.89%), and myrcene (5.83%). The essential oil of *A. absinthium* exhibited strong fumigant toxicity against *R. dominica* adults, a stored product pest, with a LC50 value of 18.23  $\mu$ /l air and LC90 value of 41.74  $\mu$ l/l air. The wormwood essential oil showed high fumigant activity against *S. littoralis*, one of the most dangerous pests of protected crops, with a LC50 value of 10.59  $\mu$ l/l air and a LC90 value of 17.12  $\mu$ l/l air.

Keywords: Artemisia absinthium, chemical composition, essential oil, fumigant toxicity, Rhyzopertha dominica, Spodoptera littoralis

The widespread use of synthetic insecticides has led to severe damage on human health and on environmental equilibrium. There is an urgent need to develop safer, more eco-friendly and efficient alternatives (18). The

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#### **Tunisian Journal of Plant Protection**

valorization of natural resources such as plant extracts and secondary metabolites, particularly those used by the popular tradition and readily available, acquire a great interest due to their biologically active compounds. Among plant secondary metabolites, essential oils extracted from aromatic plants have been widely investigated and were found to have a broad spectrum of activity against insect and mite pests, plant pathogens and nematodes (5). They have considerable potential as crop protectants against pests of stored products and protected crops.

Plant essential oils are produced from different plant organs (leaves, flowers, buds, wood...). The richest plants belong mainly to Apiaceae, Lamiaceae, Lauraceae, and Myrtaceae families. Essential oils are complex mixtures of monoterpenes. sescuiterpenes, and aromatic compounds (4). Due to these compounds, essential oils possess fumigant, antifeedant and repellent properties against various insect species (1).These plant-originated insecticides may have the advantage over synthetic ones terms in of low mammalian toxicity, rapid degradation and local availability (5, 6). The lack of persistence of essential oils under field conditions could provide some measure of temporal selectivity favoring nontarget species, especially natural enemies.

Essential oils have been largely employed for their properties already observed in nature. Thus, it was shown that essential oils might constitute new alternatives to currently used insecticides not only against stored product pests but also against protected crops pests (19, 20) such as aphids, moth or others. Essential oils of cumin, anise, oregano and eucalyptus were effective as fumigants against the cotton aphid (Aphis gossypii) (19). Pavela (14) reported on fumigant toxicity of essential oils from Mentha citrata, Nepeta cataria, Salvia sclarea, Origanum vulgare, Origanum compactum, Melissa officinalis, Thymus mastichina, and Lavandula angustifolia against S. littoralis.

Insecticidal activities of essential oils against stored product pests were cited in several studies. In fact, Yoon *et al.* (21) reported a repellent activity of six essential oils from Carum carvi, Salvia sclarea. Fragaria vesca. Thymus satureoïdes. and Cananga odorata against Sitophilus oryzae. Contact and fumigant activities of essential oils of Pinus sylvestris. Eucalyptus globulus. Coriandrum sativum against the rice weevil S. oryzae and adzuki bean weevil (Callosobruchus chinensis) and the rice moth (Corcvra *cephalonica*) were demonstrated by Pathipati (13). Also, the essential oils of the aerial parts of three Artemisia species (A. absinthium, A. santonicum and A. spicigera) were found to be toxic to adults of Sitophilus granarius (7).

The Artemisia genus, small herbs and shrubs, is one of the largest and most distributed widely aromatic plants. Members of this genus are found growing naturally in large areas or can be cultivated, thus, readily available. Among them, A. absinthium, is a yellowflowering perennial plant which has been used as an herbal medicine throughout Europe, the Middle East, North Africa, and Asia (18). This plant is found to be of botanical and pharmaceutical interest (7, 8, 15). Medicinal value of plants is related to their phytochemical components and their secondary metabolites such as essential oils. The aim of this study was to assess the chemical composition and the insecticidal potential of the essential oils isolated from the aerial parts of A. absinthium against a stored product pest (R. dominica) and a greenhouse pest (S. *littoralis*).

### MATERIALS AND METHODS Plant material and essential oil extraction.

This study was performed using the aerial part of the wormwood *A*. *absinthium*. This plant was cultivated in

an organic parcel belonging to the Technical Center of Organic Agriculture of Chott-Mariem (CTAB). The samples were used immediately after their harvest (March, 2013) for the extraction of essential oils.

Essential oils were obtained by hydrodistillation method using a Clevenger-type apparatus. About 200 g of the aerial parts of the plant were mixed in a flask with 400 ml distilled water. The mixture was boiled for 4 h at 350°C. The extract was condensed in cooling vapor to collect the essential oil. The volatile distillate was collected until no oil drop out. The obtained oil was weighed in order to calculate its extraction yield and refrigerated prior to analysis.

## GC-MS analysis of the essential oil.

The essential oils were analyzed by gas chromatography (GC) (model: HP 6890N) coupled with mass spectrometry (MS) (HP model 5975B). They are equipped with a flame ionization detector and capillary column with HP-5MS 5% phenylmethyl siloxane (30 m 0.25 mm  $0.25 \mu m$ ). The GC settings were as follows: the initial oven temperature was held at 50°C for 2 min and ramped at 7°C/min to 250°C for 2 min. The injector temperature was maintained at 240°C. The samples  $(1 \ \mu l)$  were injected neat, with a split ratio of 1: 50. Helium gas (100% pure) was used as carrier gas at a constant flow rate of 1.2 ml/min. Mass transfer line and ion source temperatures were set at 150 and 230°C, respectively. Most constituents were identified by comparison of their retention indices and

their mass spectra, in the stationary phase, with those of the bank Willey data 175.L.

## Insect rearing.

Third instar larvae of *S. littoralis* were obtained from a laboratory colony maintained on artificial diet (150 g chickpea powder, 20 g agar, 750 ml water, 5 g ascorbic acid, 1 g benzoic acid, 1 g nipagin, 30 g yeast). The colony came from laboratory rearing at  $28 \pm 2^{\circ}$ C and  $60 \pm 5\%$  of relative humidity (RH), in the Regional Center of Research on Horticulture and Organic Agriculture (Chott-Mariem, Sousse-Tunisia).

*R. dominica* adults were provided with maize grains as food in a plastic container and were maintained in the dark in growth chamber set at  $28 \pm 2^{\circ}$ C and 50-60% humidity.

All experiments were carried out under the same environmental conditions.

## Fumigant toxicity.

In order to assess the Α. absinthium essential oil fumigant toxicity, different concentrations 25, 50, 100 and 200 µl/l air and 5, 12.5, 25 and 50 µl/l air were tested respectively against the third instar larvae of S. littoralis and newly hatched unsexed adults of R. dominica. Mortalities in both cases were compared untreated control individuals to maintained under the same conditions.

Ten pests were put in a 40 cm<sup>3</sup> container. A Whatman filter paper (3 cm in diameter), impregnated with essential oil, was placed on the underside of the screw cap. Control insects were kept under the same conditions without any essential oil. Each concentration and control was replicated five times. Mortality was assessed after 24 h of exposure to the essential oil.

These bioassays were designed to determine the median effective dose causing 50% of mortality ( $LC_{50}$ ) and the

effective dose causing 90% of mortality  $(LC_{90})$  using Probit analyses.

## Statistical analyses.

Statistical analyses were performed using SPSS program (version 20) (17). Mean's differentiations were carried out using Duncan's multirange test at P < 0.05.

## RESULTS

# Extraction yield and chemical composition of *A. absinthium* essential oil.

The hydrodistillation of *A. absinthium* aerial parts yielded dark blue colored essential oil with a strong odor. In general, essential oils are very complex natural mixtures which can contain about 20-60 components at quite different

concentrations (5). They are characterized by two or three major components at concentrations (20-70%) fairly high compared to the other components present in trace amounts (2). Table 1 lists the composition of wormwood essential oils. According to the GC-MS results, 47 compounds have been identified, in which camphor (24.81%), camazulene (13.17%).bornyl acetate (5.89%).myrcene (5.83%), trimethylnaphthalene (5.09%).1.4-terpeniol (4.97%). camphene (3.74%),  $\gamma$ -terpinene (3.59%)and linalool (3.53%) were major components. Qualitative similarities were found in chemical composition of Tunisian wormwood investigated bv Riahi et al. (15) but there were some notable quantitative differences.

Compound	Peak N°	TR (min)*	Percentage (%)
Tricyclene	1	6.11	0.12
α-thujene	2	6.21	0.43
α-pinene	3	6.37	3.02
Camphene C10	4	6.9	3.74
Sabinene	5	7.24	0.27
β-pinene	6	7.32	0.19
6-methyl-5-hepten-2-one	7	7.55	0.23
Myrcene C10	8	7.63	5.83
α-phellandrene	9	7.93	0.45
α-trpinene	10	8.2	2.09
Ortho-cymene	11	8.39	0.57
Limonene C10	12	8.48	1.16
1,8-cineole	13	8.55	0.31
γ-Terpinene	14	9.15	3.59
Trans-sabinene hydrate C10	15	9.37	1.52
α-terpinolene	16	9.81	0.67
2-nonanone	17	9.87	0.53

**Table 1.** Volatile constituents identified in the essential oils of Artemisia absinthium aerial parts.

Linalool	18	10.07	3.53
butanoic Acid	19	10.12	0.56
P-menth-2-en-1-ol	20	10.58	0.27
δ-terpinene	21	10.98	0.18
Camphor C10	22	11.13	24.81
Borneol	23	11.55	0.26
1,4-Terpeniol	24	11.78	4.97
Myrcenol	25	11.93	0.53
α-terpineol	26	12.06	0.33
2-methylheptyl acetate	27	12.9	0.8
Perilla aldehyde	28	13.77	0.17
Bornyl-acetate C12	29	13.97	5.89
2-undecanone C11	30	14.06	1.27
Methyl eugenol	31	16.21	0.16
β-caryophyllene	32	16.58	0.71
α-dodecylene	33	16.65	0.2
Germacene	34	17.69	1.53
Trimethyl-dihydronaphtalene	35	18.25	1.03
Trimethyl-dihydronaphtalene	36	18.33	0.10
Caryophyllene oxide	37	19.49	0.33
Geranyl isovalerate	38	19.73	0.23
Diethyl-dimethyl-tricyclo-hexane	39	20.19	0.27
Trimethylnaphtalene	40	20.45	5.09
(2S,5E)-caryophyll-5-en-12-al	41	20.45	0.6
1,2-dimethyl-4-methylene-3- phenyl-cyclopentene	42	20.58	0.25
Trimethyl-dihydrocyclo-	43	20.64	3.12
propainden-6(6a)-one	4.4	21.47	12 51
Camazulene	44	21.47	15./1
(8) paracyciopnane-2.4-diene	45 46	21.18	0.51
2,4-uiiicuiyiuan Bromoacetonitrile	40	25.40	0.22
Total	4/	23.13	0.33 07 34
10141	-	-	97.34

\*RT: retention times

A. absinthium is characterized by a wide range of phytochemical variability (12) associated with different factors (geographical origins of the samples, extraction method used, seasonal fluctuations or plant parts used for extraction).

#### Fumigant toxicity.

Experiments were conducted to assess the fumigant activity of *A*. Tunisian Journal of Plant Protection

*absinthium* essential oil against *R. dominica* adults and *S. littoralis* third instar larvae. In all cases, considerable differences in insect's mortality exposed to essential oil vapor were observed with the different concentrations.

Results of the bioassay (Table 2) were recorded after 24 h of exposure. According to this study, significant differences among all treated concentrations of the essential oils were Vol. 9, No. 1, 2014

61

obtained (P < 0.01). The mortality increased with the increase in the concentrations of the essential oils. In fact, high fumigant toxicity against the third instar larvae of *S. littoralis* was noted with a LC<sub>50</sub> value of 10.59 µl/l air and a LC<sub>90</sub> value of 17.12 µl/l air.

As shown in Table 2, essential oil of *A. absinthium* exhibited fumigant toxicity against *R. dominica* adults with a  $LC_{50}$  value of 18.23 µl/l air and  $LC_{90}$  value of 41.74 µl/l air. The essential oil causes quick knock down followed by mortality.

## DISCUSSION

The main aim of this research was to investigate *A. absinthium* essential oils composition and insecticide potentialities. Essential oil of the aerial plant parts was obtained by hydrodistillation and analyzed by gas chromatography-mass spectrometry (GC-MS). A total of 47 components of the essential oil were identified. Several studies have been reported on the chemical composition of the essential oils from *A. absinthium* belonging to different regions in the world. Analysis the chemical of composition of Α. absinthium oils extracted from plants grown in USA showed beta-thujone (17.5-42.3%) and cis-sabinyl acetate (15.1-53.4%) as the main components (9). Other investigation from Turkey (7) indicated chamazulene (17.8%), nuciferol butanoate (8.2%), nuciferol propionate (5.1%).and carvophyllene oxide (4.3%) as the main components in A. absinthium oil. The Canadian Α. absinthium oil was characterized by high amounts of myrcene (10.8%), trans-thujone (10.1%)and trans-sabinyl acetate (26.4%) (10). Those from Tajikistan were markedly different from those from European, Middle Eastern, or other Asian locations and likely represent new chemotypes (18). This phytochemical variability in A. absinthium is normally associated with different geographical origins of the samples (12). Jointly or independently, these identified compounds are involved in the bioefficacy of the essential oils used with a range of effects i.e. insecticidal, repellent, antifeeding, and ovicidal activities.

Insect	Concentration (µl/l air)	Mortality (%) after 24 h	LC <sub>50</sub> (µl/l air)	LC <sub>90</sub> (µl/l air)
R. dominica	0	0 a		
	5	$35 \pm 12.91$ b		
	12.5	$45 \pm 12.91$ b	18.23	41.74
	25	$67.5 \pm 17.08 \text{ c}$		
	50	$92 \pm 9.57 \text{ d}$		
S. littoralis	0	$0^{\mathrm{a}}$		
	2.5	$2 \pm 4.47$ a		
	5	$18 \pm 19.24$ a	10.593	17.122
	10	$52 \pm 32.71 \text{ b}$		
	20	$94 \pm 8.94$ c		

**Table 2.**  $LC_{50}$  and  $LC_{90}$  values of Artemisia absinthium essential oil against adults of Rhyzopertha dominicaand larvae of Spodoptera littoralis.

\* For each insect, mortality values followed by the same letter are not significantly different based on Duncan's multirange test at P < 0.05.

The insecticidal propriety of many essential oils are mainly attributed to monoterpenoids (4) which are typically volatile and rather lipophilic compounds that can penetrate into insects rapidly and interfere with their physiological functions (2, 4, 5). Due to their high volatility, they have fumigant and gaseous action which are very important in controlling the stored-product insects.

In vitro experiments undertaken to assess the fumigant activity of *A*. *absinthium* essential oil that they possessed high fumigant toxicity against a stored product insect, as well as a greenhouse pest and its activity varied with insect species and concentrations of the oil used.

The toxic effects of *A. absinthium* could be attributed to its major components. However, the insecticidal effects of the essential oils cannot be explained by the action of their major components only, suggesting that these actions are the result of a synergistic interaction between all components (4).

Previous studies demonstrated that *Artemisia* species essential oils have very important insecticidal activities (7). A recent investigation by Martin *et al.* (11)

highlighted the insecticidal effect of *Artemisia* essential oils against three greenhouse pests, *S. littoralis*, *Myzus persicae* and *Rhopalosiphum padi* via an antifeedant action. Concerning stored products pests, the essential oil of *A. vestita* showed strong fumigant toxicity against *Sitophilus zeamais* adults with a LC50 value of 13.42 mg/l air. Further, it possessed also contact toxicity against this pest with a LD50 value of 50.62 mg/adult (21).

Pavela (14) reported the high fumigant toxicity of Mentha citrata, Nepeta cataria, Salvia sclarea, Origanum compactum, vulgare, 0. *Melilotus* officinalis. Thymus mastichina, and Lavandula angustifolia essential oils against S. littoralis. Moreover. the essential oils of aerial parts of three Artemisia species (A. absinthium, A. santonicum and A. spicigera) were found to be toxic to adults of Sitophilus granarius (7).

The above findings suggest that the essential oil of *A. absinthium* can play an important role in pest control and reduce the need for synthetic insecticides and also the risks associated with their use.

#### RESUME

Dhen N., Majdoub O., Souguir S., Tayeb W., Laarif A. et Chaieb I. 2014. Composition chimique et toxicité par fumigation des huiles essentielles d'*Artemisia absinthium* contre *Rhyzopertha dominica* et *Spodoptera littoralis*. Tunisian Journal of Plant Protection 9: 57-65.

Les produits naturels constituent une excellente alternative aux pesticides de synthèse à cause de leurs impacts réduits sur la santé humaine et l'environnement. Les pesticides à base d'huiles essentielles ont montré une efficacité contre une large gamme d'insectes et de maladies de pré- et de post-récolte. Dans ce travail, la potentialité insecticide de l'huile essentielle de l'armoise absinthe *Artemisia absinthium* a été investiguée contre deux insectes ravageurs à savoir *Rhyzopertha dominica* et *Spodoptera littoralis*. L'huile essentielle des parties aériennes a été extraite par hydrodistillation et analysée par GC-MS pour déterminer sa composition chimique. Les constituants majoritaires identifiés sont le camphre (24,81%), le camazulène (13,17%), le bornylacétate (5,89%), le myrcène (5,83%) et le trimèthylnaphtalène (5,09%). L'huile essentielle d'*A. absinthium* a montré une forte toxicité par fumigation contre les

adultes de *R. dominica*, un insecte des denrées stockées, avec des concentrations létales  $CL_{50}$  de 18,23  $\mu$ l/l d'air et  $CL_{90}$  de 41,74  $\mu$ l/l d'air. Cette huile a aussi montré une forte activité fumigène contre *S. littoralis*, un des insectes les plus dangereux des cultures protégées, avec des concentrations létales  $CL_{50}$  de 10,593  $\mu$ l/l d'air et  $CL_{90}$  de 17,122  $\mu$ l/l d'air.

Mots clés: Activité fumigène, Artemisia absinthium, composition chimique, huile essentielle, Rhyzopertha dominica, Spodoptera littoralis

## ملخص

## دهان، نجلاء وأنس مجدوب وصلاح الدين سوقير ووفاء التايب وأسماء العريف وإقبال الشايب، 2014. تركيبة الزيوت الأساسية للأفسنتين أو شجرة مريم (Artemisia absinthium) وفاعليتها السمية بالتبخير ضد Rhyzopertha dominica و dominica Ittoralis 9: 57-65.

تمثل المواد الطبيعية أفضل بديل للمبيدات التركيبية وذلك لتأثيرها المنخفض على صحة الإنسان و المحيط. بينت المبيدات المستخلصة من الزيوت الأساسية نجاعتها ضد مجموعة كبيرة من الحشرات الضارة والأمراض قبل و بعد الجني. في هذا البحث، تمت دراسة قدرة الزيوت الأساسية لنبتة الأفسنتين على إبادة آفتين حشريتين وهما Rhyzopertha dominica و Spodoptera littoralis لقد وقع استخراج الزيوت الأساسية من الأجزاء الهوائية للنبتة باعتماد التقطير المائي ووقع تحليلها بواسطة تقنية GC-MS لقد وقع استخراج الزيوت الأساسية من الأجزاء الهوائية للنبتة باعتماد من مادة الكافور S,89%) bornylacetate (13,17%) و 13,17%) و 5,89%) و 5,89% من مادة الكافور خاليها بواسطة تقنية GC-MS تصد تشخيص تركيبتها الكيميائية. تبين أن هذه الزيوت تتكون أساسا من مادة الكافور (5,89%) camphre (8,90%) و 13,17%) و 5,89% (3,9%) و A. absinthium (5,89%) و 5,09%) trimethylnaphtalene (5,83%) مورد سمية هامة عن طريق التبخير ضد الأطوار البالغة من حشرة 6,09%. أظهرت الزيوت الأساسية المواد الغذائية المخزنة، حيث بلغت الجرعات القاتلة 18.23 18.23 مكال و 19.24 مكال. كما أظهرت هذه الزيوت كذلك سمية على معلم قاتلة الجرعات القاتلة 18.23 مكال و 19.24 مكال. كما أظهرت هذه الزيوت كذلك سمية على معرفر و من أخطر الأفات على الزراعات المحمية، حيث بلغت الجرعات القاتلة 10.593 مكال و 10.593 مكال و 10.593 مكال. كما أظهرت و الزيوت كذلك سمية على مكل و 17.22 12.00 مكال.

كلمات مفتاحية: أفسنتين/شجرة مريم، تركيبة كيميائية، زيت أساسي، فاعلية سمية بالتبخير، Rhyzoptrera Spodoptera littoralis dominica

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64

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