South Western Journal of Horticulture, Biology and Environment
P-ISSN: 2067- 9874, E-ISSN: 2068-7958
Vol.15, No.2, 2024
pp. 113-122
Art.no. e24107

CHEMICAL COMPOSITION AND INSECTICIDAL TOXICITY OF *Artemisia arborescens* and *Rosmarinus tournefortii* ESSENTIAL OILS AGAINST TWO MAJOR COLEOPTERAN PESTS

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ABSTRACT. *Essential oils extracted from plants are of growing interest as natural alternatives for the control of cereal pests. In this study, the essential oils of Artemisia arborescens and Rosmarinus tournefotii were characterised and their insecticidal activity was assessed against adults of Tribolium castaneum and Rhyzopertha dominica. The chemical composition of the essential oils was analysed by gas chromatography coupled with mass spectrometry, and the insecticide tests were carried out by contact. The results revealed the presence of several active compounds in the essential oils, which demonstrated potentially effective insecticidal activity, with mortality rates of 100.0% in T. castaneum and R. dominica using the essential oil of A. arborescens, and 93.33±11.54 % and 100% using the essential oil of R. tournefotii after 72 hours of testing. These results suggest that essential oils could be used as control tools against the species studied in storage silos.*

KEYWORDS: *Artemisia arborescens, Rosmarinus tournefortii, essential oil, insecticidal activity.*

INTRODUCTION

Cereals are essential and vital crops in the human diet, contributing to food security and the local economy by providing an important source of essential nutrients to the population (Mughal & Fontan Sers 2020). However, the preservation of these commodities is often compromised by pest attacks, leading to significant losses in terms of the quantity, quality and nutritional value of the products (Hendrival & Aryani 2019).

Tribolium castaneum, commonly known as the red flour beetle and *Rhyzopertha dominica* known as the lesser grain borer are major pests of stored grain products that cause significant economic losses (Shafique et al. 2006, Opit et al. 2012). Controlling these pests in the context of stored commodities is a major challenge, requiring the development of integrated management strategies to ensure food security and reduce post-harvest losses (Cai et al. 2022, Tabikha 2022).

The intensive use of chemical pesticides has had negative effects on the environment and human health (Tudi et al. 2021, Lopes-Ferreira et al. 2022). Indeed, the excessive and inappropriate use of chemical insecticides in agriculture has led to the emergence of multi-resistant phytopathogenic microorganisms, creating a thorny problem for plant production (Chang et al. 2001).

Many plant species have therefore been the subject of extensive research, and their secondary metabolites have been formulated into botanical pesticides for plant protection. Among these metabolites, essential oils have been shown to be effective bioactive agents in biological control as ovicides, larvicides and repellents against various species of insect pests of stored cereals, presenting several advantages, such as their natural origin, their low toxicity for mammals and their biodegradability (Abouelatta et al. 2020, Chidambaram et al. 2022, Saada et al. 2022, Amane et al. 2023, Assadpour et al. 2023).

The genus *Artemisia* is one of the most important genera in the Asteraceae family. *Artemisia arborescens* (Vaill.) L., commonly known as mugwort or tree sagebrush, has been studied in traditional medicine for its anti-parasitic, anti-inflammatory and antioxidant properties (Koul et al. 2017, Jaradat et al. 2022). In addition, recent studies have also revealed its insecticidal potential against various crop pests (Bouzenna & Krichen 2013, Pandey & Singh 2017).

On the other hand, the species from Lamiaceae family are studied for its therapeutic properties, in particular the species *Rosmarinus tournefortii* de Noé, known as Tournefort rosemary is used in phytotherapy for its medicinal and aromatic properties (Rašković et al. 2014). Essential oils extracted from this plant contain various active compounds, including camphor and α-pinene, which have shown potential insecticidal activities (Menaceur et al. 2016).

In the present study, the insecticidal activity of *A. arborescens* and *R. tournefortii* essential oils was tested against adults of two stored wheat pest species namely *T. castaneum* and *R. dominica*. Chemical characterisation was carried out using gas chromatography coupled with mass spectrometry in order to identify the major compounds in these two essential oils. To our knowledge, no previous studies have been reported on the activity of these essential oils against these insects.

The crucial point to emphasise is that neither arborescent mugwort nor tournefort rosemary have been explored for their specific insecticidal effect on red flour beetle and lesser grain borer. This gap in research is significant, as it opens up a new and promising avenue for studying the insecticidal potential of these plants on these pests. The lack of previous studies highlights the need for this research, offering a unique opportunity to explore potential solutions through under-exploited indigenous natural resources for the control of common pests of food stocks.

MATERIALS AND METHODS

Arial parts of *A. arborescens* Land *R. tournefortii* were collected in the region of Ouled Saadi, commune of El Hachimia, wilaya of Bouira (Algeria) (36°09'24.9 "N 3°48'02.2 "E) in February 2022. These plant parts were rinsed with distilled water and air-dried in the dark for 20 days. The dried parts were ground to a powder and sieved, then stored in airtight glass jars at 4°C. These two plant species were identified in a herbarium at the National Higher School of Agronomy, El Harrach, Algiers, Algeria.

Adults of *T. castaneum* (Herbst) (Coleoptera: Tenebrionidae) and *R. dominica* (Coleoptera: Bostrichidae) were obtained from the Laboratory of the Department of Agronomic Sciences, University of Bouira (Algeria). The adults were reared in glass containers containing wheat grains and covered with a fine-mesh fabric to ensure aeration. The containers were kept in the dark in a growth chamber set at 27 ± 1°C, 70 ± 2% relative humidity.

The essential oils of *A. arborescens* and *R. tournefortii* were obtained by hydrodistillation using a Clevenger-type apparatus (Clevenger 1928). The operation involved immersing 100g of dry raw material in one liter of distilled water. The extraction time was around 3 hours. The essential oils obtained were collected in shaded bottles and stored at 4°C until use.

Chromatographic characterisation of the essential oils of *A. arborescens* and *R. tournefortii* was carried out on a Chrompack CP 9002 electronic pressure-regulated gas chromatograph, equipped with a DB-5 type fused silica capillary column 30 m long, 0.25 mm in diameter and 0.25 µm film thickness, a flame ionisation detector set at 280°C and supplied with a H2/air gas mixture and a split/ splitless injector set at 250°C. The carrier gas is nitrogen at 1 ml/min and the injection mode is split (leak ratio 1/50, leak rate 66 ml/min). The column temperature was programmed from 50°C (3 min) to 250°C at a rate of 2°C/min, then maintained at 250°C for 10 min. Constituents were identified by co-injection with commercial standards and comparison of their mass spectra (MS), retention indices (RI) and Kováts indices (KI) with those in the databases (Adams 2007).

The toxicity of essential oils to adults of *T. castaneum* (Herbst) and *R. dominica* (F.) was assessed using the method described by Kim et al. (2003). The contact test was carried out in glass jars containing 10 mg of food, into which 20 individuals were introduced. Then, using a micropipette, the different concentrations of essential oils (1µl) were applied to the dorsal surface of each individual. The controls were treated with the diluting solvent (acetone). The control and treated batches were maintained under the same conditions described above for mass rearing. Each treatment was repeated six times, three times for each insect. The insecticide treatment was carried out at the same time for both insects. Observations were made for 72 hours and when no movement was observed, the insects were considered dead.

Mortality rates were presented as mean± standard deviation. A probit analysis was carried out to estimate the LC50s values with their 95% confidence limits using SPSS software, the results of which were considered significant when the $P<0.05$.

RESULTS AND DISCUSSION

The chemical composition of the essential oils of *A. arborescens* and *R. tournefortii* determined by gas chromatography identified 20 compounds. The results in Table 1 show that the relative concentrations of these compounds varied between the two essential oils. For example, in the essential oil of *A. arborescens*, camphor is the main constituent (46.35%) accompanied by other minority constituents which are not unimportant: terpinene-4OL (6.53%), chamazulene (5.06%), linanol (4.35%), camphene (3.99%), myrcene (3.83%) and α-pinene (3.47%). However, camphor remains the most common constituent in the essential oil of *R. tournefortii* (29.20%), followed by camphene (19.42%), α-pinene (17.17%), and β-pinene (8.90%).

Chromatographic characterization of the essential oils of *A. arborescens* and *R. tournefortii* is essential to determine their chemical composition. According to our results, camphor is the most abundant compound, followed by low percentages of terpinene -4OL and chamazulene in *A. arborescens* essential oil. These results are similar to those obtained by Erel et al. (2012) in Turkey who also reported high levels of camphor (33.39%). In contrast, the study conducted by Jaradat et al. (2022) in Palestine showed that β-thujone was the main molecule (89.64%), while camphor represented only 5.34% of the essential oil of *A. arborescens*.

Furthermore, in the case of *R. tournefortii*, the main compounds identified are camphor, camphene, α-pinene. A study by Bendeddouche et al. (2011) in Sidi bel Abbes (western Algeria) found higher concentrations of camphor (37.6%), but very low levels of camphene (0.7%) and α-pinene (1.8%). On the other hand, a study by Tahri et al. (2014) in Morocco reported even higher levels of camphor (39.27%). These variations in chemical composition can be attributed to environmental, genetic, and agronomic factors specific to each region, as well as to the extraction techniques used.

Table 1. Chemical composition of essential oils from the aerial parts of *A.arborescens* and *R. tournefortii*

Constituents	Pic N°	Tr (mn)	*A. arborescens*	Pic N°	Tr (mn)	*R. tournefortii*
a-thujène	01	11.96	0.25 %	01	11.73	0.69 %
α-pinène	02	12.32	3.47 %	02	12.50	17.17 %
Camphène	03	13.13	3.99 %	03	13.34	19.42 %
Sabinene	04	14.62	0.13 %	--	--	--
β-pinène	05	14.77	0.22 %	04	14.94	8.90 %
Myrcene	06	15.77	3.83 %	05	15.83	0.68 %
a.Phellandrène	--	--	--	06	16.59	0.23 %
a.terpinène	07	17.33	0.96 %	07	17.40	0.33 %
P.Cymène	08	17.83	1.09 %	08	17.94	2.02 %
limonène	09	18.13	1.04 %	09	18.27	4.46 %
1.8 Cinèole	10	18.48	0.14 %	10	18.60	3.27 %
gamma-terpinène	11	20.19	2.34 %	11	20.25	0.63 %
Terpinolene	12	22.22	0.54 %	12	22.29	0.37 %
linalol	13	23.34	4.35 %	13	23.47	0.12 %
Camphor	14	26.52	46.35 %	14	26.70	29.20 %
Bornéol	15	27.88	0.33 %	15	27.97	1.11 %
Terpinene -4OL	16	28.75	6.53 %	16	28.76	0.75 %
Alpha.Terpéneol	17	29.25	0.25 %	17	29.76	0.55 %
Verbenone	--	--	--	18	36.39	1.22 %
Béta.Cariophyllène	18	45.19	1.19 %	19	45.23	1.91 %
Chamazulène	19	49.21	5.06 %	--	--	--
a.Humulène	20	51.24	1.15 %	20	45.23	0.97 %

Pic : Peak, represent the retention time or intensity of detected substances in analytical techniques. Tr : Trace (mn)

According to tables 2 and 3, it should be noted that contact tests showed that the essential oils of *A. arborescens* and *R. tournefortii* exhibited insecticidal activity against *T. castaneum* and *R. dominica*. Insect mortality rates increased as the concentration of essential oil increased. These results also showed that the insecticidal effect was time-dependent, with a progressive increase in insect mortality over time.

Table 2. Mortality rates obtained in *Tribolium castaneum* by contact test

Essential oils	Concentration (µl/ml)	Time (h)		
		24h	48h	72h
A. arborescens	1	0 ± 0	0 ± 0	5.0 ± 7.07
	2	0 ± 0	15 ± 7.07	20 ± 5.77
	3	13.33 ± 23.09	60 ± 14.14	65 ± 7.07
	4	36.66 ± 15.27	86.66 ± 5.77	96.66 ± 5.77
	5	90 ± 10	100 ± 0	100 ± 0
	6	93.33 ± 5.77	100 ± 0	100 ± 0
	Indicator	0 ± 0	0 ± 0	0 ± 0
R. tournefortii	1	3.33 ± 5.77	6.66 ± 5.77	10.0 ± 0.0
	2	16.66 ± 5.77	20.0 ± 0.0	30.0 ± 0.0
	3	33.33 ± 5.77	36.66 ± 11.54	43.33 ± 11.54
	4	53.33 ± 5.77	63.33 ± 5.77	63.33 ± 5.77
	5	60.0 ± 0.0	73.33 ± 5.77	80.0 ± 10.0
	6	80.0± 10	80 ± 7.07	93.33 ± 11.54
	Indicator	0 ± 0	0 ± 0	0 ± 0

The essential oils showed considerable mortality rates in the insects treated. Various studies have demonstrated the insecticidal properties of several essential oils extracted from various aromatic plants belonging to the Asteraceae and Lamiaceae families against several insect pests of stored cereal products (Boussaada et al. 2008, Hernández-Cruz et al. 2019, Basile et al. 2022).

Contact tests confirmed the insecticidal effect of the essential oils used, showing a significant increase in insect mortality as the concentration of essential oil increased. In addition, efficacy was time-dependent, with a progressive increase in insect mortality over the hours following treatment. These observations correlate with those of Zouirech et al. (2022) and El Abdali et al. (2022).

After 72h of treatment, the essential oil of *A. arborescens* caused total mortality rates (100.0 ± 0.0%) in *T. castaneum* and *R. dominica* at doses of 5µl/ml and 6µl/ml. On the other hand, the essential oil of *R. tournefortii* showed maximum mortalities of 80.0±10.0% with the 5µl/ml concentration and 93.33 ± 11.54% with the 6µl/ml concentration in *T. castaneum*. On the other hand, the same oil caused 90.0 ± 10.0% mortality with the 5µl/ml concentration and total mortality (100.0 ± 0.0%) with the 6µl/ml concentration in *R. dominica*.

Table 3. Mortality rates obtained in *Rhyzopertha dominica* by contact test

Essential oils	Concentration (µl/ml)	Time (h)		
		24h	48h	72h
A. arborescens	1	3.33 ± 5.77	50 ± 0	55 ± 7.07
	2	10 ± 0	60 ± 0	60 ± 0
	3	20 ± 0	63.33 ± 5.77	65 ± 7.07
	4	63.33 ± 5.77	65 ± 7.07	76.66 ± 15.27
	5	80 ± 0	90 ± 10	100 ± 0
	6	100 ± 0	100 ± 0	100 ± 0
	Indicator	0 ± 0	0 ± 0	0 ± 0
R. tournefortii	1	3.33 ± 5.77	13.33 ± 11.54	16.66 ± 5.77
	2	30 ± 0	30 ± 0	40 ± 0
	3	50 ± 0	50 ± 0	63.33 ± 5.77
	4	63.33 ± 5.77	63.33 ± 5.77	76.66 ± 15.27
	5	66.66 ± 5.77	73.33 ± 5.77	90.0 ± 10
	6	100 ± 0	100 ± 0	100 ± 0
	Indicator	0 ± 0	0 ± 0	0 ± 0

The results obtained show that the essential oils of *A. arborescens* and *R. tournefortii* have promising insecticidal effects against *T. castaneum* and *R. dominica*, which could be attributed to their chemical composition. This potential efficacy stems from the presence of certain components previously evaluated for their therapeutic properties. The camphor is the component that has a lethal effect on insects; it acts by disrupting the insect's nervous system. Camphor is a monoterpene widely present in essential oils and has antiseptic and anti-inflammatory antioxidant and antifungal properties (Chang et al. 2001, Zuccarini 2009, Ehrnhöfer-Ressler et al. 2013, Silva & Faria 2014). Terpinene is known for its antimicrobial and antifungal properties (Zhang et al. 2018).

In order to determine the concentrations of the essential oils studied necessary to cause 50% mortality, LC50 values were estimated in *T. castaneum* and *R. dominica*.

According to Table 4, the lowest LC50 value (1,210 µl/ml) was raised by the essential oil of *A. arborescens* in *R. dominica*.

Looking at the LC50 values obtained in this study, it is clear that *R.dominica* was the more sensitive species to essential oils than *T.*

castaneum. These results are in agreement with those found by Ncibi et al. (2019) who showed that *R. dominica* (LC50= 150µl/l of air) was more sensitive to Armoise essential oil compared to *T. Castaneum* (LC50 ≥ 150 µl/l of air) in the inhalation test. On the other hand, the results found by Abouelatta et al. (2020) confirmed that *R. dominica* species showed lower LC50s than *T. castaneum* in the *Pelargonium graveolens* essential oil inhalation test.

Table 4. Lethal concentration and chi-square values for essential oils of *A. arborescens* and *R. tournefortii*

Plant species	Insect	CL_{50} (µl/ml)	CL_{90} (µl/ml)	Slope	Intercept	Chi-square
A. arborescens	*T. castaneum*	2.425	3.859	5.41	-1.95	37.979
	R. dominica	1.210	5.088	0.87	0.07	47.513
R. tournefortii	*T. castaneum*	2.878	7.008	3.32	-1.47	11.602

CONCLUSIONS

In conclusion, the insecticidal effect against *T. castan*eum and *R. dominica* was recorded using the essential oils of arborescent mugwort and that of tournefort rosemary at all concentrations and at different exposure times. This effect is related to the chemical composition of the essential oil of mugwort and rosemary containing camphor, terpinene-4OL, chamazulene, camphene, α-pinene and β-pinene. These results suggest that essential oils could be used as potential alternatives to chemical pesticides for the control of agricultural pests. Their use could help reduce reliance on chemicals and promote more sustainable and environmentally friendly approaches to pest control.

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