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Research paper

In vitro acaricidal activity of essential oil and alcoholic extract of *Trachyspermum ammi* against *Dermanyssus gallinae*

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ABSTRACT

This study aimed to assess *in vitro* acaricidal activity of essential oil (EO) and alcoholic extracts (AE) of Ajowan against *D. gallinae*. Using contact and spraying bioassays, different doses of EO and AE were tested. Cypermethrin and thymol (positive controls), and ethanol and distilled water (negative controls) were also tested. The results indicated that effects were method and dose dependent. Statistical analysis showed a highly significant difference for contact and spraying bioassays and various doses of EO and AE ($p < 0.0001$) as, the spraying method was more effective than the contact method for acaricidal activity at 24 h post treatment (PT). In this method, Cypermethrin, EO and thymol caused over 90 % mite mortality at 50 μgcm^{-2}, while AE caused this rate at 150 μgcm^{-2}. In the contact bioassay, all tests produced low mortality rates except for Cypermethrin. Thymol was the main constituent of EO (Area = 42.26 %) and AE (Area = 45.8 %). Results of the present study indicated that Ajowan had a satisfactory acaricidal effect against *D. gallinae in vitro*. It was also found that the spraying method could be used to control the *D. gallinae* as the most appropriate method.

1. Introduction

Dermanyssus gallinae, known as poultry red mite De Geer 1778, is one of the most important ectoparasites of poultry, especially laying hens all over the world (Sparagano et al., 2013; Kim et al., 2016; Tabari et al., 2017; Kim et al., 2018). This haematophagous mite causes huge economic losses in the poultry industry due to its worldwide prevalence and abundance in poultry houses, reduced egg production and quality (poor shell integrity and blood staining of the shell surface), irritation, anemia and even death of chickens (George et al., 2009a; Sparagano et al., 2013). Moreover, *D. gallinae* plays an important role in the transmission of the rickettsial, viral and bacterial pathogens, and occasionally causes dermatitis and a nuisance to individuals working at heavily infested poultry farms (George et al., 2009b; Na et al., 2011).

Control of *D. gallinae* is commonly reliant on the continued application of conventional synthetic acaricides, however, their repeated use has often resulted in the development of mite resistance (Kim et al., 2004, 2018), chemical residues in food and undesirable environmental effects (George et al., 2009a; Na et al., 2011). Therefore, the application of synthetic acaricides is becoming restricted in poultry farms, owing to undesirable effects of synthetic acaricides and also increasing demand for the organic and safe foodstuffs (George et al., 2010a; Faghihzadeh-Gorji et al., 2014; Camarda et al., 2018; Kim et al., 2018). Hence, these emerging challenges have persuaded researchers to find new alternative strategies. The bioactive plants have been suggested as a well-received approach for mite control because of various advantages such as low non-target organism toxicity, short environment persistence, biodegradation to non-toxic products, eco-friendliness, and organic food production (Isman, 2006; Moreno et al., 2012; Zhu et al., 2013). The acaricidal properties of many plant extracts and essential oils have been reported against *D. gallinae* with promising results mainly as fumigant action (Kim et al., 2004, 2007; George et al., 2009a; Ghrabi-Gammar et al., 2009; Maurer et al., 2009; George et al., 2010b; Dehghani-Saman et al., 2015; Nechita et al., 2015; Kim et al., 2016; Camarda et al., 2018; Kim et al., 2018).

Ajowan, scientifically known as *Trachyspermun ammi L.* (synonym: *Carum copticum* Benth. & Hook), belongs to the Apiaceae or Umbelliferae family. It is an annual, aromatic, grassy plant with white flowers and small grayish brown seeds. The ripening seeds of this plant contain 2.5–5 % essential oil. This plant is one of the most well-known

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medicinal plants naturally growing in Afghanistan, Egypt, India, Iraq, Iran (locally named Zenian) and Pakistan. Several investigations have demonstrated insecticidal activity of Ajowan (Shojaaddini et al., 2008; Seo et al., 2009, 2012; Mahmoodi and Valizadegan, 2014; Al-Mekhlafi, 2018; Chaubey, 2018). Miscellaneous therapeutic potentials of Ajowan can be attributed to its active components including carbohydrates, proteins, amino acids, fat, vitamins, carotene, tannins, glycosides, alkaloids, steroids, saponins, flavonoids and minerals (Sonar et al., 2016; Dhaiwal et al., 2017). Although, up to 47 compounds have been identified in Ajowan, thymol is known as the major constituent of this medicinal plant (Dhaiwal et al., 2017). Other important components include ρ-cymene, γ-terpinene, α-terpinene, carvacrol, α-pinene, β-pinene and β-myrcene (Sonar et al., 2016; Dhaiwal et al., 2017). On the best our knowledge, the acaricidal activity of Ajowan has not been investigated against *D. gallinae*. Therefore, this study was aimed to evaluate the acaricidal activity of essential oil (EO) and alcoholic extracts (AE) from Ajowan against *D. gallinae* based on contact and spraying toxicity bioassay.

2. Materials and methods

2.1. Mite source

Between February 2017 and February 2018, mites were collected over three to four days of each week from a laying poultry farm in Meshkinshahr, Ardebil province, Iran. Samples were transferred immediately to the Parasitology Laboratory, Faculty of Veterinary Medicine, University of Tabriz, Iran and stored in sealed plastic bags at 22 °C under a 16:8 h light: dark cycle (George et al., 2010c).

2.2. Preparation of Ajowan seeds

Fresh Ajowan seeds were purchased from the local market in Tabriz, East Azerbaijan province, Iran. Then, the seeds were identified and authenticated at the Agriculture and Natural Recourses Research Center (ANRRC) of the East Azerbaijan Province (Iran). A sample of plant seeds was preserved in the herbarium of ANRRC under a specified voucher specimen number (ANRRC-1443; *T. ammi* seeds).

2.3. Isolation of essential oil and alcoholic extract

The Ajowan seeds were ground mechanically using a commercial electric mill. To provide EO, 100 g of seed powder was submitted to hydro-distillation in a Clevenger-type apparatus at 100 °C for 3 h. Subsequently, the extracted brownish matter was dried over anhydrous sodium sulfate (Moazeni et al., 2012). To provide AE, 200 g of seed powder was macerated with 400 ml of ethanol 95 % (Merck, Germany) in a Soxhlet apparatus, and then subjected to hot percolation for 4 days. Subsequently, the extract was filtered and the solvent was evaporated using a rotary evaporator (Heidolph, Germany), and the acquired extract was dried in a desiccator (Sonar et al., 2016). Finally, the dried EO and AE were stored in dark glass bottles at 4 °C until required.

2.4. Contact and spraying bioassays of EO and AE

For contact toxicity bioassays, under optimal conditions (temperature = 25 ± 1 °C, humidity = 55 ± 5 %), the circular filter papers of 4.8 cm diameter (approximate area of 18 cm^2) were treated with the provided concentrations of EO and AE (50, 100, 150, 200, 250 and 300 $\mu g cm^{-2}$) in 50 μl ethanol. After drying for 2 − 3 min under a fume hood, the dried filter papers were put into Petri dishes. 50 live newly fed adult mites were transferred on treated filter papers, water-soaked cotton was placed in the Petri dishes to supply humidity, and finally Petri dishes were covered with their lids and sealed with parafilm (Kim et al., 2007).

For spraying toxicity bioassay, firstly the filter papers without any treatment were placed into Petri dishes and groups of 50 mites were transferred on filter papers, after which similar concentrations of EO and AE mixed with 500 μl of 5 % ethanol (v/v) were sprayed directly onto the mites and finally the dishes were immediately covered and sealed tightly. In the same manner, Cypermethrin (EC Cypermethrin 10 %, Hacker, Iran) and thymol (Merck, Germany) at 500 μl of distilled water were used as the positive control, and ethanol and distilled water were used as negative control. Three replications were considered for each dilution for the two methods. Subsequently, all Petri dishes were left for a period of 24 h to monitor the acaricidal activity of Ajowan preparations. After 24 h, the legs of mites were agitated with an entomological pin under a loop, if the legs did not move, the mite was considered to be dead (Kim et al., 2016).

2.5. GC–MS and HPLC analyses of EO and AE

All chemicals, reagents and solvents were purchased from Merck and Sigma-Aldrich companies and used without further purification. In order to characterize the chemical composition of EO and AE of Ajowan seeds, an Agilent GC 6890 N coupled with MS 5973 N (USA) was used to analyze by gas chromatography–mass spectroscopy. The system has a column of HP 5 MS (length 30 m × internal diameter 0.25 mm × film thickness 0.25 μm). GC conditions were: injection split 1:20, injector temperature 274 °C, and temperature program: from 50 °C (3 min) to 120 °C at 3 °C/min, then to 150 °C (5 min) at 3 °C/min, then to 274 °C (20 min) at 7 °C/min; flow rate was 1 ml/min; helium as carrier gas at 25 kPa, with flow rate 1 ml/min. In order to quality and quantity thymol, HPLC analysis was performed using a Knauer HPLC system (Berlin; Germany) equipped with analytical column C18 (4.6 × 250 mm), a LC-20AT Solvent Delivery Unit, a LC-20AT pump, DAD, a SPD-20A UV–vis Detector, and a Hamilton 702SNR. Flow rate was 1 ml/min. The mobile phase was deionized water/acetonitrile (40/60; v/v). By scanning the entire wavelength ranging from 200 to 700 nm in the UV–vis light area, the marker (Thymol: Merck, Germany) with dilution of 25 % was scanned using the UV–vis Detector.

2.6. Data analysis

The mortality rates of mites were analyzed using a two-way ANOVA (SPSS 16.0 for Windows, Inc., Chicago, IL, USA). The normal distribution and equality of variances were tested before the analysis of variance of data. Significant differences were specified between treatments using Tukey test as Mean ± SE (%); and $p < 0.05$ was considered to be significant. Furthermore, values of LC_{50}, LC_{90} and LC_{99} were measured using the Probit analysis (SPSS 16.0 for Windows, Inc., Chicago, IL, USA) to determine the appropriate dose.

3. Results

3.1. Acaricidal effects of AE and EO, thymol and cypermethrin

Generally, the results indicated that the responses to treatments were method and dose dependent. In the spraying method, Cypermethrin, EO and thymol exhibited over 90 % mite mortality at 50 $\mu g cm^{-2}$, whereas AE caused this mortality rate at 150 $\mu g cm^{-2}$. In the contact method, EO and AE exhibited lower mortality rates compared with positive controls. Cypermethrin revealed the highest acaricidal activity followed by thymol, EO and AE, in both spraying and contact bioassays (Table 1). *D. gallinae* mortality rates indicated that there were differences for contact and spraying methods among various concentrations of EO and AE ($p < 0.0001$). The LC_{50}, LC_{90} and LC_{99} values for different concentrations of EO and AE indicated differences for contact and spraying methods ($p < 0.0001$).

In overall, there were significant differences between the LC values obtained for EO and AE. According to Table 2, in spraying method the LC_{50} value for AE was higher than that for EO (AE/EO = 66.6/44.12, $P < 0.01$), while there was no difference between LC_{50} values for EO

Table 1
The mortality rates of *Dermanyssus gallinae* (Mean ± SE %) with different concentrations of treatments in contact and spraying bioassays.

Treatment	Concentrationμgcm^{-2}	Mortality rates (Mean ± SE (%))	
		Spraying	Contact
Cypermethrin	300	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
	250	100.0 ± 0.0^{a}	98.7 ± 2.3^{a}
	200	100.0 ± 0.0^{a}	94.7 ± 6.5^{a}
	150	100.0 ± 0.0^{a}	94.0 ± 7.6^{a}
	100	100.0 ± 0.0^{a}	73.3 ± 6.0^{b}
	50	95.3 ± 4.1^{ab}	38.0 ± 7.2^{e}
Thymol	300	100.0 ± 0.0^{a}	73.3 ± 7.0^{b}
	250	99.3 ± 1.1^{a}	68.0 ± 6.0^{bc}
	200	99.3 ± 1.1^{a}	58.7 ± 4.1^{c}
	150	99.3 ± 1.1^{a}	49.3 ± 4.1^{d}
	100	96.7 ± 3.1^{ab}	40.0 ± 5.3^{e}
	50	90.0 ± 2.0^{b}	29.3 ± 4.1^{f}
EO	300	100.0 ± 0.0^{a}	58.7 ± 9.9^{c}
	250	100.0 ± 0.0^{a}	50.0 ± 8.0^{d}
	200	99.3 ± 1.1^{a}	41.3 ± 8.1^{e}
	150	98.7 ± 2.3^{a}	26.0 ± 9.1^{f}
	100	96.7 ± 4.1^{ab}	15.3 ± 5.8^{gh}
	50	94.7 ± 1.1^{b}	9.3 ± 5.0^{h}
AE	300	100.0 ± 0.0^{a}	48.0 ± 4.0^{d}
	250	98.7 ± 1.2^{a}	41.3 ± 4.1^{e}
	200	94.7 ± 2.3^{b}	38.7 ± 6.1^{e}
	150	94.0 ± 0.0^{b}	28.7 ± 8.1^{f}
	100	70.7 ± 6.1^{c}	16.0 ± 3.4^{gh}
	50	40.0 ± 10.1^{d}	9.3 ± 3.1^{h}
Control		1.3 ± 0.7^{e}	0.0 ± 0.0^{i}

Different letters indicate significant difference between treatments in each bioassay using Tukey test ($p < 0.05$).

and AE (AE/EO = 311.25/298.81, $p > 0.05$). Similarly, LC_{90} and LC_{99} values for EO and AE indicated differences in both methods ($p < 0.01$). Table 2 shows all treatments ranked by mortality of *D. gallinae*. Totally, spraying method had better efficacy than contact method in terms of LC values.

3.2. GC–MS analyses of EO and AE

Tables 3 and 4 and Fig. 1A and B present the chemical components and the spectra of AE and EO. The main components in AE were thymol (45.08 %), benzene (18.91 %) and γ-terpinene (12.45 %); the figures for EO were thymol (42.26 %), benzene (23.11 %), γ-terpinene (19.69 %) and β-pinene (6.41 %).

3.3. HPLC analyses of EO and AE

According to the HPLC analyses of AE and EO of Ajowan and the marker (thymol), the spectra of thymol in AE (Fig. 1C) and EO (Fig. 1D) completely overlapped with the thymol spectrum at retention time of 2.85 and 2.95 min, respectively. Therefore, HPLC analyses supported the presence of thymol in EO (Area = 42.26 %) and AE (Area = 45.08 %) as a major constituent in GC–MS analyses.

4. Discussion

Ajowan is a well-known traditional plant with anti-parasitic and pesticide properties. The insecticidal activity of this plant against *Culex pipiens* (Al-Mekhlafi, 2018), *Trialeurodes vaporariorum* (Mahmoodi and Valizadegan, 2014), *Aedes aegypti* (Seo et al., 2012), *Anopheles stephensi* (Pandey et al., 2009; Torabi-Pour et al., 2017) and *Sitophilus zeamais* (Chaubey, 2018) has been demonstrated. Despite the extensive reports concerning excellent insecticidal activity of Ajowan, there was no study to evaluate its acaricidal activity on *D. gallinae* and this work seems to be the first acaricidal study of Ajowan against *D. gallinae in vitro*. The findings of the current study indicated that the effectiveness is extraction method and dose dependent. In our study it is clear that EO gave results as good as a single component such as thymol and Cypermethrin, while AE was behind in terms of efficacy. This could be due to the volatility of extracted compound with some having a very short-term effect.

The GC–MS analyses indicated that thymol was the main constituent of both AE (45.08 %) and EO (42.26 %). Previous studies have also reported thymol as the main compound of Ajowan (Park et al., 2007; Mathew et al., 2008; Pandey et al., 2009; Goudarzi et al., 2011; Moazeni et al., 2012; Seo et al., 2012; Mahmoodi and Valizadegan, 2014). The insecticidal activity of Ajowan has been mainly attributed to its thymol content (Pandey et al., 2009; Seo et al., 2012). The acaricidal/insecticidal properties of thymol have been demonstrated against *Rhipicephalus sanguineus* (Matos et al., 2019) *Culex pipiens* (Youssefi et al., 2019), *D. gallinae* (Tabari et al., 2015), *Rhipicephalus microplus* (Araújo et al., 2015), *Ae. aegypti* (Seo et al., 2012), *Alphitobius diaperinus* (Szczepanik et al., 2012)*, A. stephensi* (Pandey et al., 2009), *Drosophila melanogaster* (Enan, 2005a), and *Varroa jacobsoni* (Calderone et al., 1997). In both cases the top three compounds are the same (thymol, γ-terpinene and benzene) highlighting that the other compounds in EO worked better than those in AE. Generally, the major components of plant materials play the main role to determine the biological properties, but this point should not be ignored that the potential of major compositions may be regulated by other minor components and the biological activities of plant materials are on account of synergistic/antagonistic interactions of all constitutes (Szczepanik et al., 2012).

Plant EOs and other plant derivatives contain highly complex volatile compounds, and the acaricidal effects of these molecules pertain partially to their higher vapor pressure and lipophilic properties (Na et al., 2011; Kim et al., 2016) and act mainly as a fumigant on *D. gallinae* (George et al., 2010b). The concurrent application of fumigation and contact methods can also cause synergistic acaricidal effects of plant materials, and double the fumigant and contact effects of EOs and their derivatives have been demonstrated on some species of house dust mites (Ahn et al., 2006). Kim et al. (2004, 2007) studied the effects of 56 plant EOs and 40 extracts of oriental plants on *D. gallinae* using

Table 2
The concentrations of different treatments for LC_{50}, LC_{90} and LC_{99} (Mean ± SE) against *Dermanyssus gallinae* in spraying and contact bioassays.

		LC (μgcm^{-2})			Rank order			
Bioassay	Treatment	LC_{50}	LC_{90}	LC_{99}	LC_{50}	LC_{90}	LC_{99}	Overall
Spraying	Cypermethrin	16.60 ± 7.55^{a}	34.48 ± 14.73^{a}	51.93 ± 19.71^{a}	1	1	1	1
	Thymol	23.83 ± 7.70^{a}	56.21 ± 4.74^{a}	208.13 ± 44.09^{b}	2	2	2	2
	EO	44.12 ± 6.72^{b}	129.77 ± 19.79^{b}	214.77 ± 19.80^{b}	3	3	3	2
	AE	66.60 ± 12.92^{c}	154.65 ± 2.11^{c}	312.54 ± 25.57^{d}	4	5	5	3
Contact	Cypermethrin	78.29 ± 9.73^{c}	152.71 ± 13.85^{c}	283.13 ± 80.32^{c}	5	4	4	3
	Thymol	216.19 ± 16.17^{d}	545.50 ± 32.47^{d}	1220.98 ± 83.69^{e}	6	6	6	6
	EO	298.81 ± 56.31^{e}	742.76 ± 91.37^{e}	1445.53 ± 57.62^{f}	7	7	7	7
	AE	311.25 ± 4.32^{e}	1442.3 ± 40.42^{f}	1536.39 ± 331.46^{g}	8	8	8	8

Different letters indicate significant difference between treatments in each column using Tukey test ($p < 0.01$).

Table 3
The chemical composition (%) of Ajowan alcoholic extract analyzed by Gas chromatography-mass spectrometry.

Area	Rt	Composition	Row	Area	Rt[a]	Composition	Row
0.56	10.49	**α-hydroxyfriedelan-29-oic acid**	7	0.63	4.12	**Benzeneacetamide**	1
0.98	10.61	**Indanol**	8	1.56	5.13	**β-Pinene**	2
45.08	11.64	**Thymol**	9	2.79	5.59	**η-Decane**	3
18.91	11.71	**Benzene**	10	8.87	6.05	**Methanamine**	4
1.09	21.13	**Thymyl acetate**	11	12.45	6.70	**γ-Terpinene**	5
0.38	26.40	**Hexagermane**	12	0.41	7.41	**Hexahydronaphthlene**	6

[a]Retention time.

Table 4
The chemical composition (%) of Ajowan essential oil analyzed by Gas chromatography-mass spectrometry.

Area	Rt	Composition	Row	Area	Rt[a]	Composition	Row
0.12	14.63	**2(3 H)-Furanone**	13	6.41	5.79	**β-Pinene**	1
0.23	14.88	**γ-himachalene**	14	1.34	5.99	**Decane**	2
0.1	16.26	**Spathulenol**	15	23.11	6.72	**Benzene**	3
0.36	16.35	**Caryophyllene oxide**	16	19.69	7.30	**γ-Terpinene**	4
0.19	16.77	**Dillapiole**	17	0.17	7.73	**Cis-Sabinene Hydrate**	5
0.31	20.95	**Thymyl acetate**	18	0.24	8.84	**1-Hydroxylinalool**	6
0.4	21.33	**Carvacrol**	19	0.55	9.42	**Dodecane**	7
0.15	22.65	**Hexadecane**	20	1.67	10.26	**Ethanone**	8
0.23	23.67	**Heneicosane**	21	42.26	12.13	**Thymol**	9
0.24	24.84	**Tetracosane**	22	0.19	12.78	**Ether**	10
0.19	26.06	**Pentacosane**	23	0.25	13.49	**Tetradecane**	11
0.12	27.41	**Octacosane**	24	0.16	14.01	**Trans-Caryophyllene**	12

[a]Retention time.

fumigation and contact methods, and found that the effects of these products were mainly due to the action of their volatile compounds in the vapor phase. In our study it is clear that the 24 compounds obtained in EO worked synergistically better than the 12 compounds obtained in AE even if the top three compounds are the same for both treatments (thymol, γ-terpinene and benzene). However, Ali et al. (2012) also demonstrated that the environmental conditions during these tests such as humidity and dust levels at the time of spraying would affect the EO efficacy. Other authors have also shown that the timing of plant harvest for EO production or the plant parts being used would influence its efficacy against *D. gallinae* (George et al., 2010b).

In the same study the authors examined six lavender EOs at two different exposure times on *D. gallinae* and found that the strongest acaricidal effect had occurred when filter papers impregnated with EOs were immediately used after three minutes, but the mortality rate considerably decreased when the same filtered papers were used after 24 h (George et al., 2008). Moreover, George et al. (2009b) found that mites in a closed system with only the vapor phase and without direct contact with EOs, exhibited higher mortality rates than those in an open system with the same conditions. Furthermore, mites in direct contact with EOs in an open system exhibited lower mortality than those in a closed system (George et al., 2009b). These two studies indicated that the acaricidal effects of plant products, especially EOs, were mainly reliant on the fumigant action. Such fumigant effect could explain our results. In the spraying bioassay, we used EO and AE immediately after transferring mites into Petri dishes, whereas, in the contact bioassay at least three minutes was considered to dry the papers impregnated with treatments. Surely, this minimum time could play a role in the escape of volatile components and negatively affects the acaricidal effect in this method. In accordance with our findings, the acaricidal activity of *C. officinale* extract on *D. gallinae* was investigated using spray, fumigation and contact bioassays and the spraying bioassay was the most effective method with over 97 % mortality rates. In addition to fumigant action, this may be linked to cuticle layer alteration and an increased permeability of the material due to the solvent added during spraying (Kim et al., 2018). Indeed, the spraying bioassay could be considered as a contact-fumigation bioassay, which implements contact and fumigant effects concurrently.

In the present study, although thymol content was higher in AE, EO exhibited higher mite mortality compared with AE, even at lower doses which would in turn have a positive impact of the cost of such treatment if high mortality can be achieved at lower doses. Probably, the higher acaricidal effect of EO was linked to the higher content of γ-terpinene, β-pinene and partially carvacrol in EO. Erler (2007) reported that thymol and γ-terpinene derived from Ajowan EO were the most active derivatives on rice weevil adults, *Sitaphilus oryzae* (Erler, 2007). Moreover, in a previous study the larvicidal activities of β-pinene and γ-terpinene have been reported (Park et al., 2011). In addition, the elimination of Ajowan EO derived thymol and γ-terpinene from the artificial mixture indicated a significant decrease in the toxicity of the mixture. Therefore, this result showed a synergistic larvicidal action of thymol and γ-terpinene on *Ae. aegypti* (Seo et al., 2012). On the other hand, the results of the acaricidal activity of thymol compared with those of EO and AE, revealed that thymol was overall more effective against *D. gallinae*. This may be caused by the antagonistic interaction between EO/AE and other constituents and/or due to the lesser content of thymol and its isomers and precursors. It has been suggested that the chemical compositions of plant extracts can exhibit synergic and/or suppressive effects (Sparagano et al., 2013; Kim et al., 2018). Our study did not assess the repellency effect of Ajowan derived EO and AE, which could also open more potential for some compounds to be either as toxic or repellent against arthropod pests (Stefanidesova et al., 2017). Furthermore, our study shows a plateau effect when the dose is increased highlighting some potential receptor saturation while a slow release on a longer term could affect the mites' survival. Therefore, the mode of action of plant derivatives, Octopaminergic and γ-Aminobutyric acid (GABA) receptors have been suggested as new target sites for monoterpenoids in invertebrates (Kostyukovsky et al., 2002; Priestley et al., 2003; Tong and Coats, 2010). Moreover, studies have found that plant EOs destroy the binding of the nervous system proteins, in particular ^{3}H-octopamine, in invertebrates (Enan, 2005b). In addition, the high insecticidal effect of thymol was demonstrated by the lack of its effect on a tyramine receptor (TyrR) mutant fruit fly (*D. melanogaster*, $TyrR^{neo30}$), indicated that TyrR modulates the insecticidal effect of thymol (Enan, 2005a). Interestingly, it has been proposed that thymol has an insecticidal mechanism similar to the ivermectin which interacts with glutamate receptors related to chloride (GluCl), increasing chloride ion entry to the cell leading to hyperpolarization and causing the parasite death with different manners (Ferreira et al., 2016). Thymol has a low molecular weight (150.24 g/mol) (André et al., 2017), due to their high lipophilicity, such terpenoids easily penetrate into the cuticle and are dissolved in haemolymph and bound to receptor proteins, disrupting normal neurotransmission leading to insect paralysis and death (Chaubey, 2018). More recently, the acaricidal action of thymol on *R. sanguineus* was evidenced with damaged synganglia as apoptotic process, and with severe structural alterations in acini cells

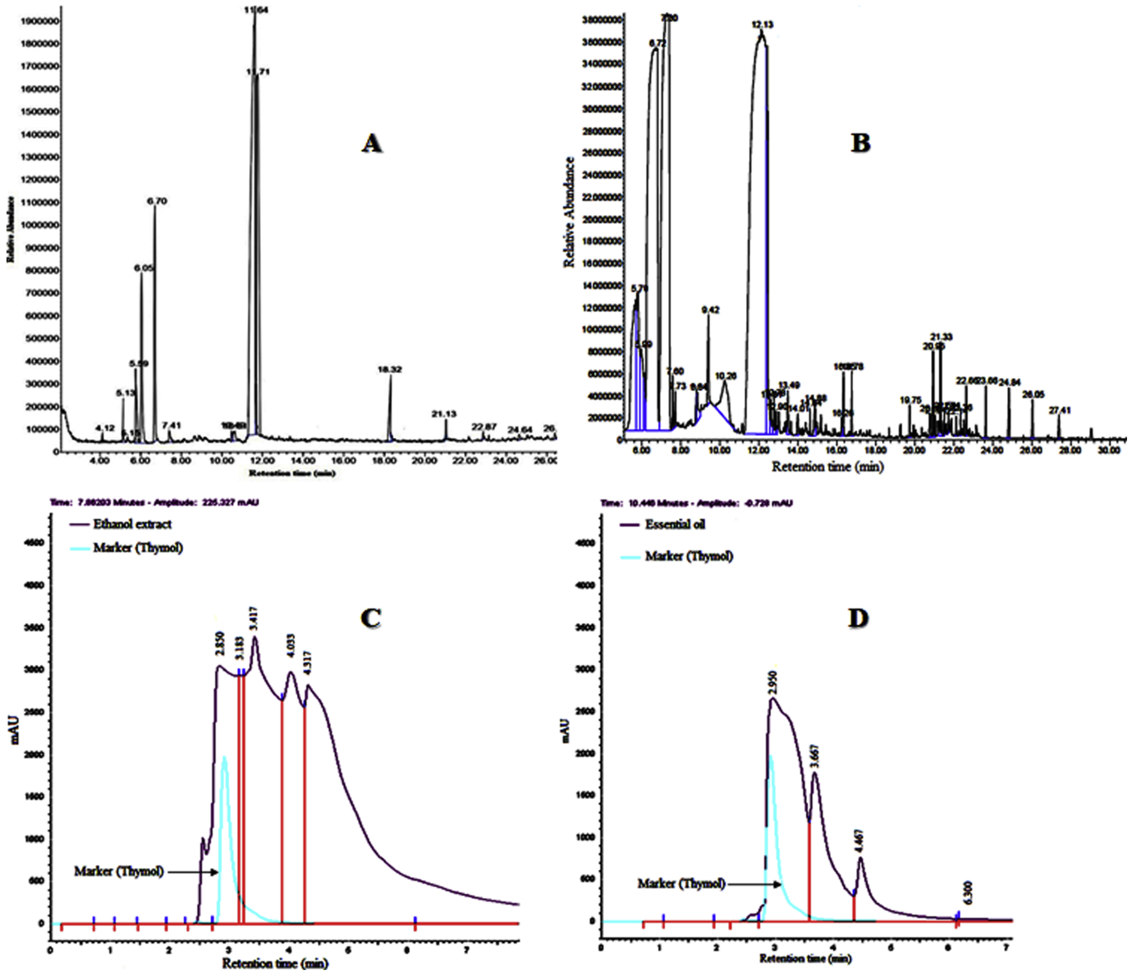

Fig. 1. Gas chromatography-mass spectrometry spectra characterization of the major principles of AE (**A**) and EO (**B**) from Ajowan, overlaid high-performance liquid chromatography chromatograms of AE with thymol (**C**) and EO with thymol (**D**): **A** and **B** exhibited that thymol was the main component of Ajowan, **C** and **D** showed the thymol spectrum in AE and EO at retention times of 2.85 and 2.95 min, respectively.

and tissue of salivary glands particularly calcium deposits in type II acini. It has been suggested that intracellular overload calcium is linked to the alterations caused by thymol at the membrane receptor location of the target cells. Increased calcium may also be related to necrosis, apoptosis and autophagy (Matos et al., 2019).

In the current study, LC_{50} values for EO and AE were respectively 44 μgcm^{-2} and 66 μgcm^{-2} in the spraying method. In a similar study (Kim et al., 2007), the values of LD_{50} for 10 more effective EOs ranged from 5.1 μgcm^{-2} to 150 μgcm^{-2}. Moreover, the values of LC_{50} for methanolic extract and stem distillate of *Asarum heterotropoids* root were 84.57 μgcm^{-2} and 34.02 μgcm^{-2}, respectively (Kim et al., 2016). Chaubey (2018) also reported LC_{50} = 0.385 μlcm^{-3} and LC_{50} = 0.317 μlcm^{-2} of *T. ammi* EO respectively for fumigation and contact bioassays on *S. zeamais* (Chaubey, 2018). In the evaluation of the fumigation effect of Ajowan EO on *T. vaporariorum* the value of LC_{50} was reported as 1.03μLL^{-1} in air (Mahmoodi and Valizadegan, 2014).

5. Conclusion

Results of the current study indicated that Ajowan had a satisfactory acaricidal effect on *D. gallinae in vitro*, due to active components like thymol, γ-terpinene and carvacrol. Efficiently, it was found that the spraying method was more effective than the contact method to exhibit acaricidal activity of Ajowan. This difference implies that herbal compounds are mostly volatile which are more effective in the vapor phase. Therefore, the spraying method, which is partially a combination of contact and fumigation methods, can be the most appropriate method to control *D. gallinae*. Future studies should seek to improve the ratio and delivery methods of such active compounds to further increase their efficacy and long-lasting effects. Finally, integrated pest management (IPM) (Harrington et al., 2011) and complementary control methods based on biosafety on farms (Sylejmani et al., 2016) and thorough cleaning between flocks and using rotational methods to again minimize potential resistance.

Declaration of Competing Interest

The authors have no conflict of interest.

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