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Chemical composition of rocket, thyme and parsley essential oils and their effect on some fungi and aflatoxin production

Abstract

Aims: Determination the chemical compositions of essential oils from parsley, rocket seeds and thyme leaves and evaluate their effect at different concentrations on some dominant fungi on wheat grains and aflatoxin production.

Methods and results: The chemical compositions of the oils were determined by using Gas Chromatography Mass Spectrometry (GC-MS). The antimicrobial activities of the tested essential oils were examined using the agar well diffusion method. Determination of aflatoxins was carried out using (HPLC) after extraction of aflatoxins from YES culture. The three EOs significantly inhibited the three fungal growth "*Aspergillus flavus, Alternaria alternata* and *Fusarium oxysporum*" and aflatoxin production by Aspergillus flavus. The extent inhibition of fungal growth and aflatoxin production was dependent on the type, concentration and the main active compounds of essential oil content that have an impact inhibitor against fungi.

Conclusion: The studied essential oils could inhibit all mentioned fungi moreover, rocket and thyme were more effective than parsley oil against studied fungi.

Significance and impact of the study: The use an efficient biocontrol method to reduce mycotoxigenic fungi and mycotoxins is very important to avoid the aflatoxin health hazards using safe way.

Keywords: chemical compositions, essential oils, fungi, *aspergillus flavus*, aflatoxins

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Introduction

Fungal contamination from the main causes of wheat grains induce the great economic losses and a high risk for human and animal health through the synthesis of mycotoxin.[1,2]

Aflatoxins are produced by many species of *Aspergillus*, a fungus, most notably *Aspergillus flavus* and *Aspergillus parasiticus*.[3] The International Agency for Research on Cancer (IARC, 1993)[4] has classified aflatoxin B_1 as a group 1 carcinogen.

Essential oils have a good source of biologically active compounds.[5] In the last years essential oils from the different plants were used in the prevention of fungal growth and mycotoxins production in cereals.[6,7]

In our current study, plant essential oils of rocket, parsley seeds and thyme leaves were tested as antifungal activity against fungal growth and aflatoxin production *in vitro*.

Materials and methods

The Parsley, rocket seeds and thyme leaves were purchased from a shop of selling herbs and medicinal plants, Cairo, Egypt, while *Aspergillus flavus*, *Alternaria alternata* and *Fusarium oxysporum* isolated from wheat grain samples.

Extraction of essential oils

These plant materials were milled to a fine powder in an electrical mill and extracted with hexane at room temperature and stored at 4°C as described by Kim et al.[8]

Chemical composition of essential oils using GC-MS

The chemical compositions of the oils were analyzed using GC-MS (Agilent; GC: 6850 Series II; MS: 5975C VL MSD) using an Agilent 19091S-433E colonna at the laboratory of Food and Feed Central Lab., Agric. Research Centre, ministry of Agric., Giza, Egypt.

The sample was injected through the auto sampler and analyzed with HP5MS column. The oven temperature was programmed as follows; 50°C for 4min, then 3°C/min to 280°C for 10min and 5min solvent delays. Helium gas was used as a carrier gas at a flow rate of 10ml/min. The mass spectrometry detector temperature was 280°C and recorded at 70ev with scanning from 50 to 550amu at 3.35sec. The identification of the compounds was based on the kovats retention index and retention time. The chemical structures of isolated the compounds were elucidated using mass spectroscopy (MS) data as reported by Adams.[9]

Preparation of potato dextrose agar (PDA)

Thirty one g of PDA powder was transferred to a bottle and dissolved in 1L distilled water by brining to boil and autoclaved for 15min at 121°C. The medium was allowed to cool and then supplemented with sterile 0.001% streptomycin.

Antifungal activity Evaluation of plant essential oils in *vitro*

The antifungal activities of the tested essential oils were examined using the agar well diffusion method as described by Kacaniova et al.[10] Petri dishes containing 10mL PDA were seeded with a fixed count of fungal spores. Wells of 5mm diameter were done by a sterilized cork borer and the essential oils were applied at 5µl, 7.5µl and 10µl from each tested essential oils.

The dishes were allowed to stand for at least 1hr for diffusion and then incubated for 7–10days at 28°C for *Aspergillus flavus* and *Alternaria alternata* and for 4-7days at 25°C for *Fusarium oxysporum*.

Preparation of spore suspension

The fungal culture was grown on PDA slants at 28°C for about 14days or until good sporulation to make a fungal spore suspension.

Preparation of yeast extract

Yeast extract was prepared by adding 20g yeast extract powder, 150g sucrose adjusted to 1L distilled water, heated to 100°C. One hundred ml of liquid media were put in conical flasks and sterilized at 121°C for 15min. The flasks were cooled and 100µl Tween 40 was added for each. Inoculation was carried out by adding 1ml of a spore suspension from a toxigenic *Aspergillus flavus* with 50µl, 75µl and 100µl/100ml media for each parsley, rocket and thyme oils and then incubated at 28°C for 15days.

Extraction and determination of aflatoxin from cultures media

Extraction of aflatoxins from YES culture was carried out according to the method of Munimbazi and Bullerman.[11] Where, the mycelium of each flask contained YES medium was harvested by filtration through filter paper, and then extracted by 100ml chloroform. The aflatoxins determination was done using (HPLC) according to AOAC (2007).[12]

Statistical analysis

All data were statistically analyzed using the General Linear Models Procedure of the Statistical Analysis System.[13] All statements of significance were based on probability of $P<0.05$.

Results

Chemical composition of essential oils

Chemical composition of rocket seeds essential oil

From the data presented in Table 1 the rocket essential oil contained 19 compounds, representing 99.78% of the total oil components.

Moreover, high amounts of erucic acid 45.17%, followed by oleic 13.18%, cis-11-eicosenoic 10.87%, linoleic 4.84%, 4-hydroxyphenylpyruvic 4.47%, palmitic 1.43% and linolenic acid 0.99% as well asminor quantities of other essential and non-essential fatty acids.

Chemical composition of thyme leaves essential oil

Twenty-nine compounds, representing 100% of the total chemical composition, were identified in the thyme essential oil (Table 2). The main constituents of essential oil were thymol 40.98%, p-cymene 15.32%, γ-terpinene 12.01%, carvacrol 2.63%, α-pinene 1.12% and α-terpineol 0.33% and traces components such as, linalool 0.7, cineole 0.41%, camphor 0.3% and bornyl acetate 0.29%.

Table 1 Chemical composition of rocket seeds essential oil

No.	RT (min)	Components	Percentage composition (%)
1	3.561	Cis-Verbenol	0.46
2	4.26	Cumene,p-ethyl	0.45
3	10.073	Ascorbic acid, permethyl -	4.21
4	11.01	Cyanidin cation	0.83
5	11.625	4-Hydroxyphenyl pyruvic acid	4.47
6	15.854	9-Cis-Retinal	1.24
7	13.51	Butylated hydroxytoluene	0.34
8	16.24	4-Hydroxy-2-methoxybenzladehyde	1.1
9	16.323	Terpineol	3.53
10	16.817	Palmitic acid	1.43
11	17.314	Linoleic acid	4.84
12	18.985	Oleic Acid	13.18
13	19.104	Linolenic acid	0.99
14	19.621	Rhodopin	1.51
15	20.876	Colchicine	2.2
16	21.156	Phytol	1.83
17	22.03	Vanillic acid	1.13
18	22.587	Cis-11-Eicosenoic acid	10.87
19	23.226	Erucic acid	45.17

Table 2 Chemical composition of thyme leaves essential oil

No.	RT (min)	Components	Percentage composition (%)
1	3.6	P-Cymene	15.32
2	4.28	Cuminaldehyde	0.31
3	5.821	Gentisic acid	0.27
4	6.27	α-Terpineol,(-)-	0.33
5	6.404	Trans-β-Ocimene	1.45
6	6.585	Pseudolimonen	0.76
7	6.89	α-Pinene	1.12
8	7.662	Cineole	0.41
9	8.161	γ-terpinene	12.01
10	8.729	Linalool	0.7
11	9.232	Camphor	0.3
12	9.657	Terpinen-4-ol	0.68
13	10.171	Bornyl acetate	0.29
14	10.668	Caryophyllene	2.39
15	12.274	Thymol	40.98
16	12.785	Carvacrol	2.63
17	13.799	Ascorbic acid 6-palmitate	1.43
18	14.68	Farnesol	0.44

Table Continued

No.	RT (min)	Components	Percentage composition (%)
19	14.787	3-Octanone	0.35
20	16.309	Isolongifolol	1.98
21	17.386	Trans-Geranylgeraniol	2.02
22	18.073	β-Citronellol	2.18
23	18.483	Caryophyllene oxide	0.45
24	21.463	3-Hydroxy-4-methoxycinnamic acid	0.13
25	21.992	24,25-Dihydroxyvitamin D3	0.68
26	22.48	Retinol	0.84
27	22.911	β-Carotene	4.64
28	23.637	α-Cadinol	0.89
29	23.991	Rhodopin	4.02

Chemical composition of parsley seeds essential oil

The obtained data from GC: MS revealed the presence of 19 identified components, which represented 99.9% of the total amount as shown in Table 3. The main constituents of the essential oil were myristicin 34.18%, α- pinene 16.14%, apiol 15.69% and sabinene 0.68%. Also, 1-allyl-2,3,4,5-tetramethoxy-benzene 9.93%, β-pinene 7.3%, β-phellandrene 4.11%, carotol 2.44%, β-myrcene 2.38%, camphene 2.07% and α-thujene 0.97% were identified.

Table 3 Chemical composition of parsley seeds essential oil

No.	RT (min)	Components	Percentage composition (%)
1	3.389	P-Cymene	0.26
2	4.124	Limonene	0.13
3	6.981	α-Pinene	16.14
4	7.242	Camphene	2.07
5	7.739	Sabinene	0.68
6	9.761	β-pinene	7.3
7	10.219	β –Myrcene	2.38
8	12.05	β-Phellandrene	4.11
9	13.584	α. Thujene	0.97
10	14.155	Butylated hydroxytoluene	0.32
11	14.39	Carophyllene	0.08
12	14.708	γ-terpinene	0.29
13	14.997	P-Cymenene	0.1
14	15.306	Vitamin A acid methyl ester	0.52
15	15.603	Elemicin	2.31
16	15.877	Myristicin	34.18
17	16.9	1-Allyl-2,3,4,5-tetramethoxy-benzene	9.93
18	19.585	Carotol	2.44
19	20.903	Apiol	15.69

Antifungal activity of the three essential oils on the fungal growth (in vitro study)

In our current study, the essential oils of parsley, rocket seeds and thyme leaves were tested at three various concentration 5µl, 7.5µl and 10µl/10ml for their inhibitory effect on fungal growth of *Aspergillus flavus*, *Alternaria alternata* and *Fusarium oxysporum* (Table 4-6).

Table 4 Antifungal activity of rocket seeds oil against *Aspergillus flavus*, *Alternaria alternata* and *Fusarium*

Fungi spp	Inhibition zones		
	5µl/10ml	7.5µl/10ml	10µl/10ml
Aspergillus flavus	20.0±5.1	21.0±9.3	23.0±1.45
Alternaria alternate	5.8±2.60	6.3±5.80	7.0±1.93
Fusarium oxysporum	2.5±0.88	2.8±1.89	3.0±1.80

Each value represents the mean± standard deviation of triplicates.

Table 5 Antifungal activity of thyme leaves oil against *Aspergillus flavus, Alternaria alternata* and *Fusarium oxysporum*

Fungi spp	Inhibition zones		
	5µl/10ml	7.5µl/10ml	10µl/10ml
Aspergillus flavus	16.5±3.6	20.0±1.5	22.0±3.08
Alternaria alternate	15.0±4.1	15.4±1.75	16.2±2.5
Fusarium oxysporum	16.0±2.08	18.0±1.32	19.0±0.63

Each value represents the mean±standard deviation of triplicates.

Table 6 Antifungal activity of parsley seeds oil against *Aspergillus flavus*, *Alternaria alternata* and *Fusarium oxysporum*

Fungi spp	Inhibition zones		
	5µl/10ml	7.5µl/10ml	10µl/10ml
Aspergillus flavus	8.0±1.5	10.0±0.76	10.0±1.55
Alternaria alternate	6.0±4.9	8.0±2.7	8.5±4.6
Fusarium oxysporum	13.5±1.04	15.0±1.3	17.0±0.63

Each value represents the mean± standard deviation of triplicates.

Antifungal activity of rocket seeds oil on fungal growth

The present study was conducted to assess the effect of rocket seeds oil *in vitro* as an antifungal agent. Antifungal activity of rocket oil was evaluated against the growth of three fungal species; *Aspergillus flavus*, *Alternaria alternata* and *Fusarium oxysporum*.

The data tabulated in Table 4 showed an antifungal activity of rocket oil against *Aspergillus flavus*, *Alternaria alternata* and *Fusarium oxysporum*. From the obtained results it could be noted that rocket essential oil at a concentration of 7.5 and 10µl/10ml showed higher antifungal activity more than the concentration of 5µl/10ml.

The influence of rocket essential oil against *A. flavus* was examined and the inhibition zones were 21 and 23mm for both concentrations 7.5 and 10µl/10ml, respectively. However, its inhibition zones for *Alternaria alternata* were 5.8, 6.3 and 7mm at the three tested concentrations 5, 7.5 and 10µl/10ml, respectively. Concerning its inhibition zones for *Fusarium oxysporum*, they were 2.5, 2.8 and 3mm

at the three tested concentrations 5, 7.5 and 10µl/10ml, respectively.

It could be concluded that the antifungal effect of rocket essential oil on the three fungal spp. were arranged as follow: *A. flavus>Alternaria alternata>Fusarium oxysporum* and the inhibitory effect of the rocket essential oil increased in proportional to its concentrations.

Antifungal activity of thyme leaves oil on fungal growth

Thyme (Thymus vulgaris) essential oil was tested at three concentrations of 5, 7.5 and 10µl/10ml to study its effect on fungal growth. The obtained results are shown in Table 5. From these results it could be noted that thyme leaves oil had an antifungal activity against the three tested fungi; *Aspergillus flavus*, Alternaria alternata and *Fusarium oxysporum*. The largest antifungal activity of thyme essential oil was observed for *Aspergillus flavus* at concentration of 10µl/10ml. The essential oil of thyme showed remarkable inhibition zones arranged as follow: *Aspergillus flavus>Fusarium oxysporum>Alternaria alternata* for all tested concentrations 10>7.5>5µl/10ml, respectively. The fungal growth decreased with an increase of essential oil concentration.

The inhibition zones of thyme essential oil for *A. flavus* were 16.5, 20 and 22mm and for *Alternaria alternata* were 15, 15.4 and 16.2mm, while the inhibition zones for *Fusarium oxysporum* were 16, 18 and 19mm at the three tested concentrations 5, 7.5 and 10µl/10ml, respectively.

Antifungal activity of parsley seeds oil on fungal growth

Parsley essential oil at three concentrations 5, 7.5 and 10µl/10ml were tested as an antifungal agent and the results in Table 6 showed that parsley oil had antifungal activity against the three tested fungi; *Aspergillus flavus*, *Alternaria alternata* and *Fusarium oxysporum* and the best inhibition was observed against *Aspergillus flavus* at concentration of 10µl/10ml.

Essential oil of parsley showed remarkable inhibition zone againest *Fusarium oxysporum>Aspergillus flavus>Alternaria alternata* at all tested concentrations 10>7.5>5µl/10ml, respectively.

The inhibition zone caused by parsley oil against *Aspergillus flavus* were 8, 10 and 10. However, while against *Alternaria alternata* were 6, 8 and 8.5mm. Concerning, *Fusarium oxysporum*, they were 13.5, 15 and 17 at three concentrations 5, 7.5 and 10µl/10ml, respectively.

Effect of the tested essential oils on aflatoxin production by A. flavus

This part of our current study was conducted to reduce aflatoxin production by *A. flavus in vitro* using mentioned essential oils.

The results of aflatoxins B_1, B_2, G_1 and G_2 shows that the control sample has the highest value of total aflatoxins compared with the treated samples with tested essential oils (Table 7) and the results revealed the effect of the three essential oils at different concentrations 50, 75 and 100µl/100ml on aflatoxin production by *Aspergillus flavus*.

Table 7 Effect of essential oils at different concentration on aflatoxin production by A. flavus

Reduction%	Total	Reduction%				Con.	Essential oils types
		G2	G1	B2	B1		
93.7	0.1	74.47	97.12	94	94.01	50	
95.8	0.067	78.79	100	96.73	94.2	75	Rocket
97.25	0.0439	82.8	100	100	96.37	100	
74.12	0.413	71.39	68.142	92.74	77.36	50	
93.77	0.0994	77.23	100	100	87.59	75	Thyme
96.74	0.053	84.46	100	100	94.45	100	
43.26	0.905	46.79	41.05	100	28.14	50	
52.3	0.761	50.34	55.66	100	33.67	75	Parsley
54.17	0.731	56.73	55.79	100	37.35	100	
-	1.59508	0.14096	0.74644	0.16497	0.54271	-	Control

Reduction effect of rocket seeds oil on aflatoxin production by A. flavus

The results presented in Table 7 showed that the reduction by rocket essential oil for aflatoxins B_1, B_2, G_1 and G_2 produced by *A. flavus* were 94.01, 94, 97.12 and 74.47% at 50µl/100ml, respectively, 94.2, 96.73, 100 and 78.79% at 75µl/100ml, respectively, and 96.37, 100, 100 and 82.8% at 100µl/100ml, respectively.

The influence of rocket essential oil against total AFs production by *A. flavus* was evaluated and the reduction values were 93.7, 95.8 and 97.25% at the three tested concentrations, while AFB_1 were reduced to reach 94.01, 94.2 and 96.37% at the three tested concentrations, respectively.

Rocket essential oil caused a 100% reduction for AFB_2 at concentration of 100µl/100ml, and it caused a 100% reduction for AFG_1 at both concentrations of 75 and 100µl/100ml.

The reduction effect of thyme leaves oil on aflatoxin production by *A. flavus*

The results presented in Table 7 showed the reduction effect of thyme essential oil on aflatoxins B_1, B_2, G_1 and G_2 produced by *A. flavus* and the reduction values were 77.36, 92.74, 68.142 and 71.39% at 50µl, while at 75µl they were 87.59, 100, 100 and 77.23% and at 100µl they were 94.45, 100, 100 and 84.46%, for the four mentioned AFs types, respectively. Also, the results clearing that thyme essential oil at the three tested concentrations reduced the total aflatoxin production to 74.12, 93.77and 96.74%, respectively. The

largest reduction percent was 96.74% for thyme essential oil at 100µl concentration.

The influence of thyme essential oil against AFB_1 production by *A. flavus* was evaluated and the percents of inhibition were 77.36, 87.59 and 94.45% at the three tested concentrations, respectively. It is worthy to mention that thyme essential oil reduced AFB_2, AFG_1 to 100% at concentrations of 75 and 100µl/100ml.

Effect of parsley seeds oil on aflatoxin production by A. flavus

Parsley essential oil at three tested concentrations 50, 75 and 100µl/100ml was used against aflatoxins production by *A. flavus*. The results presented in Table 7 showed that parsley has a remarkable effect on aflatoxins production and the reduction percents were 43.26, 52.3 and 54.17% at the three tested concentrations, respectively.

The effect of parsley essential oil on aflatoxins B_1, B_2, G_1 and G_2 production by *A. flavus* was studied and the reduction percent of AFs were 28.14, 100, 41.05 and 46.79% at 50µl, represented 33.67, 100, 55.66 and 50.34% at 75µl, 37.35, 100, 55.79 and 56.73% at 100µl, respectively.

Discussion

The chemical analysis of rocket oil was studied by Ugur et al.,[14] who found that the rocket oil contain high amount of erucic acid from 46.64 to 54.79%, followed by oleic 17.86-19.95%, palmitic 7.25-10.97%, linoleic 4.23-9.72%, and linolenic acid 1.98-3.01%. While Gulfraz et al.,[15] found a high concentration of erucic acid 51.2% followed by oleic acid 15.1% and cis-11-eicosenoic acid 12.5%.

Our obtained results are agreed with Shabnum et al.,[16–19] they reported that thyme oil contained thymol, γ terpinene, p-cymene, linalool, myrcene, α-Pinene, α-thujene and carvacrol at different levels.

The parsley essential oil studied by Zhang et al.,[20] who found five substance called myristicin 32.75%, apiol 17.54%, α-pinene 16.64 %, β-pinene 11.54 % and 1-allyl-2,3,4,5-tetramethoxy-benzene 10.0%.

Many researchers believed that the antimicrobial activity of *Eruca* oil is mainly due to higher concentration of erucic acid, which was present in both free and triglyceride form Khoobchandani et al.[21] The antifungal activities of plant extracts are most likely due to the presence of chemical compounds with antifungal properties. Particularly worth noting is erucin, which accounted for approximately 78.69% of the rocket extract and play an important role as an antifungal agent against *A. flavus*.[22]

Thyme essential oils are apparently amongst the best inhibitors of fungal pathogens because of the presence of the phenolic compounds such as carvacrol and thymol as main constituents which might disrupt the fungal cell membrane. Thyme oil was found to have active compounds, such as thymol and carvacrol, against a large number of microorganisms.[6,16,17,23–25]

The obtained results are agreed with Farah et al.,[26] who found that a potent effect of parsley seed extract at different concentration against *Aspergillus flavus*, *Mucor* spp. and the highest reduction was against *A. flavus*.

In our respect study Sabry[22] found that inhibition percent of *A. flavus* ability to produce aflatoxin B_1 ranged from 68.42 to 100% with different concentrations of *Eruca sativa* ethanolic extract of 0.04 to 0.1%, respectively.

El Habib[27] studied the effect of the natural food additives obtained from thyme, and other medicinal plants on AFB_1 produced by *A. flavus* and concluded that the inhibitory effect of the oils increased in proportion to their increased concentrations.

Sabry[22] indicated the ability of parsley oil on the reduction aflatoxin B_1, As well as Abdel-Khalek[28] evaluated the essential oils of some Egyptian plants for novel aflatoxin (AFB_1) and ochratoxin (OTA) inhibitors, as a potent inhibitor of fungal growth.

From the obtained date could be concluded that rocket, thyme and parsley essential oils can be used against a wide range of toxiginic fungi and aflatoxin production as a safe and natural way.

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Conflict of interest

No conflict of interest is declared.

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