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Chemical composition and fungitoxic activities of *Lavandula officinalis* L. oil and comparison with synthetic fungicide on the growth some fungi *in vitro*.

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Abstract: This study were conducted in 2013 - 2014 at Department of Plant Protection, and National Commission for Biotechnology (NCBT) in Faculty of Agriculture, Damascus University. Components of essential oils from the *Lavandula officinalis* L. were determined using Gas Chromatography- Mass Spectrometry (GC-MS). The results showed that yield of lavender oils province were 2.71% (fresh flowers) and 0.52 % (fresh leaves). Thirty- eight and Thirty -one compounds were identified in the two essential oils, respectively. The important components were Linalool (33.16% & 9.34%), Borneol (18.89% & 6.75%), Eucalyptol (8.85% & 22.40%) and Champhor (7.15% & 23.34%) in fresh flowers and leaves, respectively. The fungistatic activity of the essential oils was tested against *Fusarium solani* (Mart.) Sacc, *F. oxysporum* Schltdl, *Aspergillus niger* Van., *Botrytis cinerea* (Pers. :Fr.) and *Penicillium digitatum* (Pers.: Fr.) on PDA medium by the poisoned food technique. The results showed that essential oil from fresh flowers of *L. officinalis* L. at 500, 1000 and 1500 ppm exhibited strong antifungal activity than oil obtained from fresh leaves on the tested fungi. Large percentage antifungal activities of lavender oil are related with Linalool and Borneol of terpenes as the important compounds. Benomyl fungicide at 120 ppm had completely inhibitory effect on tested fungi.
The essential oil from fresh flowers showed the highest fungistatic activity with significant differences in dose. *Fusarium solani*, *F. oxysporum* and *A. niger* were the most sensitive fungi *P. Digitatum* and *B. cinerea* the most resistant of two the essential oils. Therefore, essential oils from fresh flowers of *L. officinalis* L. could be used to control the fungal *Fusarium solani*, *F. oxysporum* and *A. niger*.
Key word: *Lavandula officinalis* L., Essential oil, Benomyl, Fungi.

Introduction

Synthetic fungicides are currently used as the primary means for the control of plant diseases. Historically, chemical fungicides have proved to be non-specific and therefore can act on organisms other than the target fungus, including other naturally occurring beneficial agents. Because of their chemical nature, they may also be toxic and non-biodegradable. Chemical residues can build up in the soil and throughout the food chain[1]. However, the alternative control methods are needed because of the negative public perceptions about the use of synthetic chemicals, resistance to fungicides among fungal pathogens, and high development cost of new chemical[2] .

Pathogenic fungi alone nearly 20% reduction in yield of major food and cash crops. Fungal species of the genera *Aspergillus*, *Fusarium* and *Alternaria* have been considered to be major plant pathogens[3].

Various plant materials are believed to have antifungal activity and many essential oils have been reported to have antifungal activities with no side effects on humans and animals[4,5]. Plants have an almost limitless

ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives[6]. The mechanisms thought to be responsible for phenolic toxicity to microorganisms include enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more nonspecific interactions with proteins[7]. The antifungal activity of essential oils can be attributed to the presence of some components such as carvacrol, α-terpinly acetate, cymene, thymol, pinene, linalool which are already known to exhibit antimicrobial activity[8,9]

Lamiaceae family has cosmopolitan distribution. Many members of this family are useful economically for medicinal, culinary, ornamental and various commercial utilizations. Previous studies on the essential oils of many Lamiaceae show that, these plants have a broad range of biological activities, notably their antimicrobial potency[10]**.** Lavender (*Lavandula officinalis* L.), a member of the Lamiaceae (Labiatae) family, native to southern Europe and the Mediterranean area, grows in full sun on dry, well-drained, stony calcareous soils[11]. Lavender oils contain more than 100 compounds, with the two major constituents being linalool and linalylacetate[12,13]. Lavender oil was also reported to be an effective antifungal agent against *Aspergillus nidulans* and *Trichophyton mentagrophytes*[14].

The aim of this study:

The purpose of this study was to investigate the essential oils composition of lavender (*Lavandula officinalis* L.) produced in Syria. Also antifungal activity of essential oils of *L. officinalis* L. have been compared with synthetic fungicide (benomyl) on culture of some fungi. To develop environment-friendly alternatives to synthetic fungicides for the control of fungal plant diseases.

Material and Methods

Plant materials:

This investigation carried out in 2012-2013, in Department of Plant Protection, and National Commission for Biotechnology (NCBT) in Faculty of Agriculture, Damascus University.

Flowers of *L. officinalis* L. were collected at morning from plants that were grown in Al—Nabk gardens – Damascus town, Syria at the full flowering stage in 21 June 2013 . The leaves were obtained from the same gardens after flowers stage in 28 October 2013. The samples were cleaned in shade condition to prevent hydrolysis of the existing materials.

Essential oil extraction:

The oils were taken from 150 g of the samples in hydro distillation method with the help of Clevenger set for three hours[15]. Following the sample oils were dried with anhydrous sodium sulfate. The oils yields were calculated on a fresh weight basis (W/W). The oil extracts were stored in sterile dark vials at 4°C for future uses.

Oils Analysis:

Analysis of oils were carried out by GC-MS chromatography (GC-agilent 7986, indictor: inert-MS) in Atomic Energy Commission (AECS)- Damascus, Syria. This instrument was fitted with HP-5MS capillary column (30cm×0.25mm i.d., film thickness 0.25µm). The temperature injector and indictor 250 °C .The oven temperature program was 60-270°C (2.5°C per min.).. The identity of components was ascertained based on the spectra and compared with library and literature data. Also, the identification of each compound was confirmed by comparison of its retention index with those of authentic compounds[12].

Fungicide used :

Benomyl: methyl 1-(butylcarbamoyl)benzimidazol-2-ylcarbamate (IUPAC).

Mode of action : Systemic fungicide with protective and curative action. Uses effective against a wide range of Ascomycetes, and Fungi Imperfecti and some Basidiomycetes in cereals, grapes, pome and stone fruit, rice and vegetables. Also used as pre-harvest sprays or dips for the control of storage rots of fruit and vegetables. Formulation type WP. [16]. 'Benlate' WP. 50%

Antifungal Assays:

Five fungi (*Fusarium solani* (Mart.) Sacc, *F. oxysporum* Schltdl, *Aspergillus niger* Van., *Botrytis cinerea* (Pers. :Fr.) and *Penicillium digitatum* (Pers.: Fr.)) used were provided from the collection of the Department of Plant Protection, Faculty of Agriculture, Damascus University.

The toxicity of the essential oils of *L. officinalis* L. against the five fungi were tested by using the poisoned food technique[17]. The oils were diluted in 95% ethanol and were then mixed and homogenized by ultrasonication with 200 mL of culture medium potato dextrose Agar (PDA), after autoclaving to achieve final concentrations of 25, 50, 100, 150, 200, 250, 500, 1000 and 1500 ppm. of medium. Control growth medium contained equivalent amounts of 95% ethanol. The fungicide (benomyl) was used at 0.0, 0.5, 1, 5, 10, 20, 40, 60, 80, 100 and 120 ppm. Aqueous suspension of fungicide was freshly prepared by dissolving the chemical in sterile distilled water. Amount of fungicide suspension containing the appropriate concentration were added to 200 ml of molten PDA (approximately 50 ˚C) after autoclaving. The amended medium was poured into sterilized petri dishes (9 mm) and allowed to gel. Three plates were used as replicates for each treatment. After solidification, each plate was inoculated with 5-mm disk of mycelia from the growing edge of a 7-day old colony. Inoculated plates were incubated at 25 ˚C with 12-h light. Measurements of colony size were taken on seventh day by measuring two diameters of the growing colony and the average was calculated. The percentage of fungal growth inhibition was calculated as formula:

Growth inhibition% = [(growth in control – growth in sample)/growth in control] × 100.

The ED_{50} value for each fungus, which was defined as the concentration of oil or fungicide causing 50 % inhibition of mycelial growth, was determined.

Statistical analysis:

The experiment was conducted using a completely randomized design. All statistical analyses were carried out using spss 20 software was used for data analysis. A *p*-value <0.05 was considered statistically significant.

Table 1. Main components of the essential oils from *L. officinalis* L., by GC/MA chromatography.

No	Component	Retention time (min.)	Percentage %	
			Flowers	Leaves
1	1-Hexano	5.41	0.36	-
2	Alpha-Pinene	6.33	0.54	0.55
3	Camphene	7.43	0.61	0.26
4	Bête-Pinene	9.18	1.43	0.87
5	Beta-Myrcene	9.89	0.51	-
6	3-Carene	10.99	0.95	0.76
7	Limonene	12.91	1.64	1.94
8	Beta-Phellandrene	13.49	0.85	-
9	Eucalyptol	14.39	8.85	22.40
10	Cis-Linaloloxide	17.93	0.74	-
11	Linalool	22.15	33.16	9.34
12	Cryptone	23.11	-	4.36
13	Isobutric acid	23.94	0.39	-
14	Champhor	25.51	7.15	23.34
15	3-Cyclohexen-1-ol	29.46	4.25	0.34
16	Borneol	30.13	18.89	6.75
17	P-menth-1-en-8-ol	31.10	0.87	0.27
18	Butanoic-acid	31.34	0.60	0.10
19	Hexyl n-valerate	32.07	0.33	0.07
20	3,7-Octadiene-2,6-diol	32.42	0.45	0.11
21	P-Cymen-8-ol	32.81	0.82	0.43
22	3-Ethoxyacrylonitrile	33.33	0.74	-

Table (continued)

No	Component	Retention time (min.)	Percentage %	
			Flowers	Leaves
23	Linalyl anthranilate	34.05	4.03	1.13
24	Crypton	34.40	0.83	0.27
25	2,6-Octadien-1-ol	35.87	0.47	0.11
26	2-Methylundecane	37.44	0.36	-
27	Malonic acide	37.82	1.83	0.75
28	Propanal	38.50	0.54	0.23
29	Pyrazole-4-carboxylic acid	40.60	0.27	-
30	1,3-Dioxolane	41.73	0.38	0.17
31	Benzenemethanol	42.70	0.36	0.12
32	Propanoate	44.93	0.53	0.27
33	Caryophyllene	46.12	0.36	2.76
34	Acetic acid	47.02	0.57	0.21
35	1,5,7-Octatrien-3-ol	47.65	0.31	0.56
36	1,6,10-Dodecatriene	49.11	0.72	0.30
37	4-Hexen-1-ol	51.69	0.25	-
38	1,6-Octadien-3-ol	54.55	0.61	0.21
39	Caryophllene oxide	63.72	0.57	13.49
40	Alph-bisapolol	71.13		2.21

Results and Discussion

Results of GC- MS analysis oil extraction of *Lavandula officinalis* L. presented in Table 1. The oil yields of fresh plant (W/fresh weight) obtained by hydrodistillation were (2.71% and 0.52%) from fresh flowers and leaves of *L. officinalis* L., respectively, this showed an increment of (5.21%) in the oil of the fresh flowers in compared with that obtained from fresh leaves. Similar results to study were obtained with[12,13] are reported that lavender oils contain more than 100 compounds, with the two major constituents being linalool and linalylacetate. The essential oil content in the leaves of lavender cultivated in Northwest Iran was found to be 0.64% based on dry weight[18]. Also, the essential oils isolated by steam distillation from the fresh and dry flowers of *Lavandula officinalis* L., the oil yields were (1.35% , 3.8%) respectively[19].

We identified total 38 and 31 compounds in essential oils in fresh flowers and leaves of *L. officinalis* L. respectively. The main compound of lavender oil in fresh flowers identified, Linalool (33.16%), Borneol (18.89%), Eucalyptol (8.85%), Champhor (7.15%), 3-Cyclohexan-1-ol (4.25%), Linalyl anthranilate (4.03 %), Malonic acid (1.83%), Limonene (1.64%) and beta-Pinene (1.43), and important constituents of the oil obtained from fresh leaves were Champhor (23.34%), Eucalyptol (22.40%), Caryophyllene oxide (13.49%), Linalool (9.34%), Cryptone (4.36%), Caryophyllene (2.76%), alpha-Bisapolol (2.21%), Limonene (1.94%) and beta-Pinene (0.87%). This is supported by the observation of several investigations[18-20].

The essential oils of various lavandula species showed that linalool was the most important compounds of these plants. Therefore, we investigated linalool in lavender. Results showed that maximum linalool percentage (33.16%) was obtained in fresh flower, and minimum linalool percentage (9.34%) was shown in the fresh leaves. The essential oil content in the inflorescence of lavender (*Lavandula angustifolia* Mill.) cultivated in the mid hills of Uttarak hand was found to be 2.8 % based on the fresh weight. Thirty seven constituents, representing 97.81 % of the oil were identified. The major components of the oil were linalyl acetate (47.56 %), linalool (28.06 %), lavandulyl acetate (4.34 %) and α-terpineol (3.75 %)[21]. Also, identified 17 compounds in lavender from Xinjiang, China, with linalool (44.54%), geraniol (11.02%), lavandul acetate (10.78%), 3, 7-dimethyl-2, 6-octadien-1-ol (10.35%), and isoterpineol (6.75%) as the main components[22]. The compounds lavender from china are included 1,5-Dimethyl-1-vinyl-4-hexenyl butyrate as the most abundant component (43.73%), followed by 1,3,7-Octatriene, 3,7-dimethyl- (25.10%), Eucalyptol (7.32%), and Camphor (3.79%)[23].

These variations with our results and other authors could be due to differences in location, elevation, genetic makeup of the plant or due to an adaptive process to particular ecological conditions. Lawrence also

observed a wide variation in the quantitative composition of lavender oil depending on plant genotype and cultivation area, and the composition of the oil from lavenders were recognized to vary significantly according to altitude, microclimate and region[24].

Our results showed that harvesting time had significant effect on essential oil content in two stages. The minimum essential oil percentage was obtained in leaves compared with in full flowers in *L. officinalis* L. There fore the harvesting time have a great importance in production of essential oil and influenced on the quantity and quality of essential oil. Among *L. officinalis* L. main components are 1,8-cineole, camphor, linalool and its acetate (both accounting for 38.78% of the total oil), and lavandulyl acetate[24].

In vitro Fungi toxicity Assay:

Table 2: Inhibition percent of mycelia growth of tested fungi by different concentrations of *L. officinalis* L. oils on PDA.

% Inhibition										Concen. (ppm)
F. solani		*F. oxysporum*		*A. niger*		*B. cinerea*		*P. digitatum*		
Flowers	Leaves	Flowers	Leaves	Flowers	Leaves	Flowers	Leaves	Flowers	Leaves	
18.25	11.75	14.50	8.12	11.12	5.12	7.45	3.12	5.10	2.52	25
34.45	16.15	28.33	11.45	16.18	8.35	14.25	6.89	8.12	5.11	50
53.75	25.88	42.67	21.13	32.13	13.12	19.10	8.11	12.40	6.28	100
74.33	35.33	68.97	29.16	47.14	17.56	27.75	9.98	16.50	8.33	150
97.14	48.17	80.14	31.19	61.12	22.45	41.10	13.12	22.70	11.12	200
100	61.89	97.50	43.67	81.67	29.18	60.50	17.34	31.20	14.12	250
100	73.16	100	55.12	89.25	36.13	76.45	20.13	36.60	17.15	500
100	85.12	100	67.19	100	41.11	88.10	26.11	38.90	21.12	1000
100	92.15	100	80.45	100	49.12	93.30	32.89	41.20	25.18	1500
90	**210**	**125**	**330**	**173**	**1505**	**215**	**>1500**	**>1500**	**>1500**	**[1]ED_{50} (ppm)**

L.S.D (p≤0.05) : Between concentrates : 1.14, L.S.D (p≤0.05) : Between oils : 1.87 L.S.D (p≤0.05):Between interactions : 2.12

1:The median effective dose (ED_{50}) was detrmined as the concentration of the oils in PDA which causes 50% reduction in linear growth of fungus as compared with growth on PDA alone.

Different doses (25, 50, 100,150, 200, 250,500,1000 and 1500 ppm) of the essential oils from fresh flowers and leaves of *L. officinalis* L. were tested against *F. solani*, *F. oxysporum, A. niger, B. cinerea and P. digitatum* to determine their antifungal activity *in vitro* tests. The results presented in Tables 2 show that the essential oils exhibited variable degrees of antifungal activity against the tested fungi. The essential oils from fresh flowers and leaves of *L. officinalis* L. inhibited all of the fungi tested by the inhibition rates of (41.20% & 25.18 %) for *P. digitatum*, (93.30% & 32.89%) for *B. cinerea*, (100% & 49.12%) for *A. niger*, (100% & 80.45%) for *F. oxysporum*, and (100% & 92.15%) for *F. solani*, respectively, at 1500 ppm concentration. Biological activities of essential oils depends on the qualitative and quantitative characteristics of their components, which is affected by the plant genotype, plant chemo-type, organ of plant, geographical origin, season, environmental, agronomic conditions, extraction method and storage condition of plant and essential oils[25]. The effect of 20 essential oil constituents on *Aspergillus flavus* growth and aflatoxin production was tested at the level of 1000 ppm. Five oils, namely geraniol, nerol and citronellol (aliphatic oils), cinnamaldehyde (aromatic aldehyde) and thymol (phenolic ketone), completely suppressed growth and aflatoxin synthesis. Trials for determining the minimum inhibitory concentration (MIC) of these oils revealed that geraniol, nerol and citronellol were effective at 500 ppm, while thymol and cinnamaldehyde were highly effective at doses as low as 250 and 200 ppm, respectively[26].

Percentage of growth inhibition was significantly ($P < 0.05$) influenced by essential oil concentration. Mycelia growth was considerably reduced with increasing concentration of essential oil. Observed severe hyphae collapsing, plasmatic membrane rupture and destruction of mitochondria in *Aspergillus niger* treated with essential oils of *Thymus eriocalyx* and *T. xporlock*[27] Lavender oil was also reported to be an effective antifungal agent against *Aspergillus nidulans* and *Trichophyton mentagrophytes*[14].

Our results showed that all treatment concentrations of oil significantly reduced of fungi growth in PDA medium compared with control. The result reveled that the oil extracted from fresh flowers caused significant inhibition of mycelia growth of the tested fungi compared with the oil extracted from fresh leaves. On the other hand, the essential oil extracted from fresh leaves of *L. officinalis* L. exhibited lower antifungal activities against all tested fungi. Furthermore, data analysis showed the differences between oils, between doses, as well as between their interactions are significant ($p< 0.05$). The comparison of means showed maximum inhibition of *F. solani*, *F. oxysporum* and *A. niger* growth were found at highest doses, 500, 1000 and 1500 ppm. On the other hand, The results indicated that essential oil extracted from fresh leaves of *L. officinalis* L. at the higher concentrations 1000 and 1500 ppm against the mycelia growth of tested fungi didn't gave the completely inhibit growth. This result agreement with several investigations, the essential oils of 12 medicinal plants were tested for inhibitory activity against *Aspergillus flavus, A. parasiticus, A. ochraceus* and *Fusarium moniliforme*. The oils of thyme and cinnamon (< or = 500 ppm), marigold (<or = 2000 ppm), spearmint, basil, quyssum (3000 ppm) completely inhibit all the test fungi. Caraway was inhibitory at 2000 ppm against *A. flavus, A. parasiticus* and 3000 ppm against *A. ochraceaus* and *F. moniliforme*. *A. flavus, A. ochraceus, A. parasiticus* and *F. moniliforme* were completely inhibited by anise at< or = 500 ppm. The extent of inhibition of fungal growth and mycotoxin production was dependent on the concentration of essential oils used[28].

Our results proved that the oil extracted from flowers of *L. officinalis* L. had fungicidal properties against *F. solani*, *F. oxysporu* and *A. niger* than *P. digitatum* and *B. cinerea*. The bioactivity of the essential oil may be due to the presence of some highly fungitoxic components in the oil. Indeed *L. officinalis* L. essential oils have terpenes alcohol as the major components. Also, Linalool, Borneol and Eucalyptol and other compounds in oil obtained from fresh flowers. The essential oil compounds of the aerial parts of *Rosmarinus officinalis* (44.02% ρ-cymene, 20.5% linalool as main components) inhibited *in vitro* growth of *Alternaria alternata, Botrytis cinerea* and *Fusarium oxysporum*[29]. The inhibitory effect of limonene, the constituent of *Cymbopogon citrates* oil, against *A. flavus* and *A. parasiticus*, and they confirmed that limonene caused membrane injury on membrane of susceptible organism[30].

Essential oil obtained from fresh leaves showed moderately inhibitory effect on *F. solani*, *F. oxysporum* and *A. niger*. However, was lower inhibitory effect on *B. cinerea and P. digitatum*. The efficacy of essential oils of lavender and sage, can be explained by the high content of 1,8-cineole, which is capable of changing the structure and moisture of mucous membranes of fungal cells, interfering with the respiratory processes, and therefore comes to the elimination of pathogens[31].

Finally, it is difficult to compare the data with the literature because several variables influence the results, such as the environmental and climatic conditions of the plant and the choice of the isolation method and antimicrobial test. Moreover, the standard criteria for the evaluation of the plant activity are lacking and therefore the results obtained by different authors are widely different .The most active oils were *Origanum vulgare* L., *Thymus serpyllum* L., *Thymus vulgaris, Lavandula latifolia* Medik., *L. angustifolia*. *T. vulgaris* inhibited the tested fungal growth due to the presence of phenolic compounds, namely thymol and carvacrol [32].

The influence of standard fungicide (benomyl) on the percentage inhibitory against *F. solani*, *F. oxysporum, A. niger, B. cinerea and P. digitatum*, given in Table 3, The results showed that benomyl was highly effective against *Penicillium* and *B. cinerea*. A completely inhibition of growth of the two fungi occurred at 80 and 100 ppm, respectively. However, this fungicide proved lowest efficiency on mycilial growth of *A. niger*, *F. oxysporum* and *F. solani*,, Where, the concentration of 100 ppm completely inhibited the fungal growth. Data also, indicate that the concentration of the fungicide increased, the reduction in mycelia growth of tested fungi was significantly increased. Observed that Benlate 50 % proved to be effective on growth and spore production of *Fusarium oxysporum* f.sp. *lupini* at concentration of 25 ppm[33].The *Fusarium* sp. growth was completely inhibited at 10 ppm of Benlate 50 % [34]. In *vitro* tests, linear growth and sporulation of *Botrytis allii* was inhibited by 0.5 ppm benomyl [35].

Table 3: Inhibition percent of mycelia growth of tested fungi by different concentrations of fungicide (benomyl) on PDA.

% Inhibition					Concen. (ppm)
F. solani	***F. oxysporum***	***A. niger***	***B. cinerea***	***P. digitatum***	
17.24	14.67	20.14	24.12	27.12	0.5
33.12	27.12	32.15	36.23	38.14	1
38.19	35.16	41.49	44.56	47.89	5
46.16	41.17	48.67	50.23	56.13	10
52.45	47.12	57.34	58.13	64.25	20
63.89	52.13	68.14	79.16	83.15	40
72.23	68.19	75.67	86.11	94.34	60
85.45	77.56	89.14	95.12	100	80
96.12	91.17	98.43	100	100	100
100	100	100	100	100	120
19.15	36.16	12.45	9.87	8.12	**[1]ED_{50}(ppm**

L.S.D (p≤0.05) : Between concentrates : 1.16, L.S.D (p≤0.05) : Between oils : 1.54 L.S.D (p≤0.05):Between interactions : 1.65

1:The median effective dose (ED_{50}) was detrmined as the concentration of the oils in PDA which causes 50% reduction in linear growth of fungus as compared with growth on PDA alone.

The median effective concentration (ED_{50}) values for inhibition of radial mycelial growth of *F. solani*, *F. oxysporum, A. niger, B. cinerea and P. digitatum*.on PDA medium, were 90, 125, 173 , 215 and > 1500 ppm (oil from fresh flowers) 210, 330 , 1505 , >1500 and >1500 ppm (oil from fresh leaves), and 19.15 , 36.16 , 12.45 , 9.87 and 8.12 ppm (benomyl), respectively. This indicate that *P. digitatum* and *B. cinerea* were highly sensitive to benomyl. The essential oil of white wood (*Melaleuca cajeputi*) at 1.5625% (v/v) and of cinnamon (*Cinnamomum cassia*) and lavender (*Lavandula officinalis*) at 50% (v/v) were the optimum concentrations for fungal growth inhibition of *A. flavus* IMI 242684. The essential oil of white wood at 25% (v/v) completely inhibited the growth of *A. flavus* IMI 242684 on PDA for 28 days[36]. The modern fungicides have indeed distinct spectra of activity. However, found the median effective concentration (ED_{50}) values for inhibition of radial mycelial growth of *Fusarium oxysporum, Penicillium chrysogenum, Botrytis cinerea* and *Rhizoctonia solani* on malt agar medium, were 70, 50, 3, & 200 mg a.i./l (thiophanate-methyl) , respectively[37].

Conclusion

Reviewing the above mentioned results, it could be concluded that the result demonstrated that the best harvesting time was full flowering stage in *L. officinalis* L. because of the maximum essential oil percentage and the important compounds of oil are Linalool, Broneol, Eucalyptol and Champhor founded in the flowers.

Also, this study demonstrated the *in vitro* antifungal activities of essential oils of *L. officinalis* L. against *F. solani, F. oxysporum* and *A. niger* fungi. Moreover, for the development of essential oils as alternatives of synthetic fungicides, further studies are required to evaluate phytotoxicity of essential oils for application on plants .and sensory quality of treated fruits and vegetables.

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