التركيب الكيميائي والتضاد الفطري لزيت النارنج الطيار (*Citrus aurantium* L.) تجاه ممرضات النبات الفطرية

Chemical Composition and Antifungal Activity of Bitter Orange (*Citrus aurantium* L.) Essential Oil Against Plants Pathogenic Fungi

Z. Al –Naser[1] **N. Al- Abrass[2]** **M. Said Al-Masri[3]**

(1) Dep. Plant Protection, Faculty of Agriculture, Damascus University, Syria.

(2) National Commission for Biotechnology (NCBT), Damascus University, Syria.

(3) Atomic Energy Commission of Syria, Damascus, Syria.

zinzanasera@gmail.com:

الملخّص

أُجريت الدراسة خلال عامي 2016 و2017 في قسم وقاية النبات وهيئة التقانات الحيوية في كلية الزراعة، بجامعة دمشق (سورية). بهدف دراسة التركيب الكيميائي والتضاد الفطري لزيت النارنج الطيار (*Citrus aurantium* L.) تجاه ممرضات النبات الفطرية. تم تحليل الزيت الطيار المستخلص من قشور ثمار النارنج كاملة النضج والطازجة بطريقة التقطير، بوساطة جهاز الكروماتوغرافي الغازي الملحق بوحدة الكتلة. أظهرت النتائج أن نسبة الزيت بلغت 1.87% على أساس الوزن الطازج. تم تحديد 35 مركباً تمثل 99.9 % من الزيت، وكانت المركبات الرئيسة هي Limonene (93.10 %)، تلاه Myrcene (2.46 %)، والعديد من المركبات الأوكسجنية من السيسيكوتربينات. تم تقييم الفاعلية للزيت الأساسي للنارنج (*C. aurantium*) كمضاد فطري تجاه خمسة فطريات ممرضة للنباتات:

including Alternaria alternate (Fr.) Keissler, *Botrytis cinerea* Pers., *Penicillium expansum* Link, Fusarium oxysporum Schlechtend: Fr. (emend. Snyd. and Hans.).

بطريقة تسميم الوسط المغذي عند تراكيز مختلفة. أظهرت النتائج أن التضاد الفطري لزيت النارنج كان ضعيفاً عند التركيز 250 ppm، و لكن عند التركيز 2000 ppm ثبط بمعنوية نمو الفطريات الخمسة جميعها. إضافة لذلك أشارت النتائج أيضاً إلى أن التضاد الفطري للزيت ضد الفطريات المختبرة ازداد تدريجياً مع زيادة تركيز الزيت. ولم تظهر الفطريات *S. cepivorum* و *A. alternate* و *B. cinerea* أي نمو ميسليومي عند التراكيز 1500 و1500 و2000 ppm على التوالي. في حين كان زيت النارنج فعالاً على فطر *F. oxysporum* (96.23 %)، ومتوسط الفاعلية كمضاد فطري تجاه الفطر *P. expansum* (70.56 %). وكانت قيم التركيز النصفي الفعال (EC50) كالآتي: 610 و 840 و 1050 و1170 و1460 ppm لكلٍ من *S.cepivorum* و *A. alternate* و *Botrytis cinerea* و *F. oxysporum* و *P. expansum* على التوالي.

تظهر هذه النتائج إمكانية استخدام زيت النارنج في المكافحة الحيوية لأمراض النبات كمبيدات فطرية حيوية آمنة.

الكلمات المفتاحية: *C. aurantium*، الزيت الطيار، GC–MS ، فطريات.

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Abstract

This study was conducted in 2016 -2017 at The Department of Plant Protection, and National Commission for Biotechnology (NCBT) at the Faculty of Agriculture, Damascus University, Syria. The essential oil isolated by hydro-distillation from the fresh peel of fully matured ripen fruits of bitter orange *Citrus aurantium* L. that analyzed by GC–MS. The results showed that the yield of *C. aurantium* was 1.87% (fresh peel). Thirty five different components were identified constituting approximately, 99.9% of the oil. The major components were limonene 93.10% followed by myrcene 2.46% and many oxygenated sesquiterpenes.

The antifungal activity of *C. aurantium* essential oil was evaluate against five plants pathogenic fungi, *including Alternaria alternate* (Fr.) Keissler, *Botrytis cinerea* Pers., *Penicillium expansum* Link, Fusarium oxysporum Schlechtend: Fr. (emend. Snyd. and Hans.) and *Sclerotium cepivorum* Berk: using the poisoned food at various concentrations. Results showed that the antifungal activity of *C. aurantium* oil was weak at 250 ppm, but at 2000 ppm it significantly inhibited the growth of all five fungi. The results also, indicated that the antifungal activity of oil against the tested fungi increases parallel with raising concentrations of oil. S. cepivorum and *A. alternata* and *B. cinerea* did not show any mycelium growth in the presence of oil at concentration of 1500, 1500 and 2000 ppm, respectively. Moreover, the *C. aurantium* oil at 2000 ppm was effectiveness on F. oxysporum (96.23%), and showed moderate fungicidal activity against *P. expansum* (70.56%). The values of EC50 for the oil were, 610; 840; 1050; 1170; and 1460 ppm for S.cepivorum, *A. alternata*, *Botrytis cinerea*, *F. oxysporum* and *P. expansum*, respectively. These results show that the oil of *C. aurantium* had potential for use in the biological control of plant disease as a safe biofungicides.

Key words: *C. aurantium*, essential oil , GC–MS, Fungi.

Introduction

In world crop production, preharvest losses due to fungal disease may amount to 12% in the world. Fungal species of the genera *Alternaria alternate* (Fr.) Keissler, *Botrytis cinerea* Pers. :Fr., *Penicillium expansum* Link, *Fusarium oxysporum* Schlechtend: Fr. (emend. Snyd. and Hans.) and *Sclerotium cepivorum* Berk have been considered to be major plant pathogens world wide (Agrios, 2005). *Penicillium* and *Fusarium species* produce mycotoxins in food besides causing seedling blight, seed rot, kernel rot, stalk rot, wilt and stunt (Barkai-Golan, 2008 and Agrios, 2005). Usually, Chemicals used in the control of diseases in the open field, greenhouse and storage (Maloy, 1993).

The history of fungicide development has been instructive to us in terms of benefits derived as well as the hazards, which accompany indiscriminate use of these poisons. A 1986 National Academy of Sciences (NAS) report on pesticides residues on food indicated that fungicides pose more of a carcinogenic risk than insecticides and herbicides together besides developing resistance towards pathogens (Research Council, Board of Agriculture, 1987). Protection against phytopathogenic and saprophytic fungi encounters problems such as: increase of resistance to classical pesticides, the treatment costs, and the fact that most available antifungal drugs have only fungistatic activity (Dixit, *et al*., 1995). Also, the usual antimicrobial chemicals used in agriculture for plant disease control (Benzimidazoles, aromatic hydrocarbons and sterol biosynthesis inhibitors) are associated with series at problems. Currently, there is a strong debate about the aspects safety of chemical preservatives since they are considered responsible for many carcinogenic and teratogenic attributes as well as residual toxicity (Palou *et al*., 2008).

Agricultural studies have focused on the biocontrol of plant disease for a long time. Discovery of antifungal

compounds in plants is an efficient way to create new pollution-free pesticides. Many plants have antimicrobial activities that are related to their antimicrobial constituents, including alkaloids, terpenes, polysaccharide, esters, ketones, and quinones (Farzaei *et al*., 2015). Effective components extracted from plants have promising potential for this purpose because of their high efficacy, low toxicity, and selective characteristics (Sanei-Dehkordi *et al*., 2016). Hence, use of some safe bioactive compounds like essential oils has been proved beneficial in bringing down the physiological activities of fruits during storage and minimizing the overall qualitative and quantitative losses (Porat *et al*., 2002). In addition, there is an increasing demand for organically produced fruit, and hence, at is urgent to replace synthetic fungicides with safer and biodegradable alternatives (Wisniewski *et al*., 2001).

Essential oils are volatile oily liquids obtained from different plant parts and widely used as food flavours. In spite of having been long recognized for their antibacterial, antifungal, antiviral, insecticidal and antioxidant properties (Sacchetti *et al*., 2005; Sokovic and Griensven, 2006) and in some cases, a direct food-related application (Madsen and Bertelsen, 1995). Various biopesticides that contain nicotine, rotenone, matrine, toosendanin and other similar compounds as their main component; have been developed for example, Green Gold, Econeem, Akign™, Neem Azal™, and Saferin (Mondal *et al* ., 2007).

The genus Citrus (family: Rutaceae) includes about 17 species distributed throughout the tropical and temperate regions. Citrus constitutes an important group of fruits in the world, which includes fruits such as oranges, mandarins, grapefruits, pummelos, tangerines, tangor, citranges. In each group, several varieties have been developed in the world (Shaw, 1977, Davies and Albrigo, 1994). *Citrus aurantium* L. is popularly known as “bitter orange” or “sour orange” and its fruit extracts are being marketed and traditionally used as herbal weight-loss products and as appetite suppressants, although in Traditional Chinese Medicine they are prescribed in concert with other support herbs. (Arias *et al*., 2005 and Stohs *et al*. 2011). *C. aurantium* is a tree up to 6 m height, with leathery leaves and white aromatic flowers (Azadi, 2012). Orange essential oil is a natural flavoring material obtained by the removal of water in orange juice in the process of making frozen orange juice concentrate. It is usually condensed in the first stage of an evaporator and separated from the aqueous portion by centrifugation. The composition and flavor quality of this product varies considerably depending on the orange cultivar, maturity, and processing (Sato *et al*., 1996 and Boussaada, 2006). Several researches evoked the antifungal activity of essential oil extracted from citrus (Sharma and Tripathi, 2006 and Espina *et al*., 2011). Boelens et al., (1989) studied the chemical composition of bitter orange (*C. aurantium*) peel oils from fully developed, living, unripe and ripe fruits. Fifty constituents were quantified, comprising about 99% of the oils. Lower aliphatic constituents are formed during ripening. Ripe bitter orange peels contained higher concentrations of aliphatic aldehydes, oxygen-containing monoterpenes and sesquiterpenes than the peels of fully developed unripe fruits. Changes were found in the concentrations of linalol and linalyl acetate (together 0.3–3.2%) and in those of limonene (92–95%) in the peel oils from living bitter oranges. Hognadottir and Rouseff (2003). Investigated that using GC–MS, 95 volatile components were detected in orange essence oil, of which 55 were aroma active. the most abundant compounds were: limonene, 94.5%; myrcene, 1%; valencene, 0.8%; linalool, 0.7%, and octanal, decanal, and ethyl butyrate, 0.3% each. Boussaada (2006) studied the peel oils of *Citrus aurantium* L. var. *amara* from Tunisia (Nabeul) by GC/MS. The major constituent of the peel oils was found to be limonene (90.6%). Chutia *et al.*, (2009) showed that the essential oil isolated by hydro-distillation from the peel of fully matured ripen fruits of Citrus reticulata (Blanco) was analyzed by GC–MS.

Thirty seven different components were identified constituting approximately >99% of the oil. The major components were limonene (46.7%), geranial (19.0%), neral (14.5%), geranyl acetate (3.9%), geraniol (3.5%), b-caryophyllene (2.6%) and nerol (2.3%). The antifungal activity of the oil was tested by poisoned food technique against some plant pathogenic fungi. The minimum inhibitory concentration for *A. alternata*, *R. solani*, *C. lunata* was 0.2 ml/100 ml, whereas >0.2 ml/100 ml for *F. oxysporum* and H. oryzae. In other study, Abderrezak *et al*., (2014) were analyzed peel hydrodistilled essential oils of *Citrus aurantium* from Constantine (Algeria) by GC/MS. The major compounds of the peel essential oil were linalool (12%), cis-linalool oxide (8.1%), trans-carveol (11.9%), endo-fenchyl acetate (5.5%) and carvone (5.8%).

The objectives:

The aim of this study was to determine the chemical composition of the essential oil of the fresh *peel Citrus aurantium* by GC-MS, evaluate antifungal activity of the oil against five plant pathogenic fungi viz: *Alternaria alternata*, *Botrytis cinerea*, *Penicillium expansum*, *Fusarium oxysporum* and *Sclerotium cepivorum* by poisoned food technique

Material and Methods

The present study was carried out in the Department of Plant Protection of Damascus University, and National Commission for Biotechnology (NCBT), Damascus, Syria during the years 2016- 2017.

Sample preparation

The fresh fully matured ripe fruits were harvested from *Citrus aurantium* trees grown in garden of faculty of Agriculture – Damascus university, Syria

Extraction of essential oil

The fruits were washed with water to remove dirt, and then peeled with a sharp knife manually. 100 g fruit peels were placed in a 1000 ml round-bottom distillation flask, and the plant material was wetted with 500ml distilled water. The essential oil were obtained by hydro-distillation using clevenger-type apparatus for 3 h. The oil were dried over anhydrous sodium sulphate and then stored in sealed glass vials at 4 to 5°C prior to analysis (Handa, 2005).

GC-MS analysis

Analysis of oils was 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). 0.5 µl of essential oils were injected for analysis, and Helium was used as carrier gas at 1 ml/min. The temperature injector and indicator 250 °C .The oven temperature program was 60270°-C (2.5°C per min.). The compounds of essential oils were identified according to GC-MS Retention time, and based on the spectra and compared with library and literature data.

-Fungal pathogens

Alternaria alternata, *Botrytis cinerea*, *Penicillium expansum*, *Fusarium oxysporum* and *Sclerotium cepivorum* fungi were used. They were provided by the laboratory of Plant Protection Department, Damascus University, Syria. The fungi were cultured on potato dextrose agar (PDA) at 251°±C.

-Antifungal activity measurements

The fungi-toxicity of the oil was evaluated against the tested fungi by the poisoned food technique of (Falck, 1907). Potato Dextrose Agar (PDA) was autoclaved (20 min at 1.03 kh cm2, at 121°C) then different concentrations of essential oils (0, 250,500, 750, 1000, 1250, 1500 and 2000 ppm) were added

aseptically to sterile molten PDA medium (45°C) containing Tween 20 (0.5%, v/v). The resulting media were immediately dispensed (20 ml) into sterilized Petri plates (9 cm). A mycelial disk of 5 mm diameter of the tested pathogens was taken from the 7 day old cultures, with the help of a sterilized needle, and was placed at the center of the Petri plates. In the controls, water and Tween 20 were used instead of essential oils. Inoculated Petri plates were incubated at 25± 2°C . The colony diameter of tested pathogen in each treatment was recorded on the 10 day when the control plates were full with mycelium of the test pathogen. Fungitoxicity was expressed in terms of percentage of mycelia growth inhibition and calculated as per formula of Pandey et al. (1982).

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

The effective concentrations at which 50% pathogen inhibition (EC_{50}) value for each fungus, which was defined as the concentration of oil causing 50% inhibition of mycelial growth, was determined.

Statistical analysis

The experiment was conducted using a Completely Randomized Design (CRD). All statistical analysis were carried out using SPSS, 20 software was used for data analysis. LSD (0.01) was considered statistically significant.

Results and Discussion

- Yield of the essential oil

The essential oil obtained by hydro-distillation from fresh peel of *citrus aurantium* had a light yellow color and pleasant soft odor. The extraction yield of *citrus aurantium* ripe fruit fresh peel essential oil was 1.87% (basis on the weight fresh) . The results are also in accordance with the found by Sarrow *et al*., (2013) as a clear yellow volatile oil with a fresh sweet odor was obtained through the hydro-distillation of peel of *C. aurantium* at 1.67% (mL/100 g of fresh tissue). Nevertheless, Berrabeh *et al*., (2016) found that the essential oils were isolated by steam-distillation from fresh peel of *Citrus aurantium* grown in Eastern Morocco and harvested in February (EO1) or December (EO2). Yields of EO1, EO2 were 1.01%, 1.02%, respectively. These variations could be explained, according to Burt (2004) by the difference of harvest period, type of soil, climate of the region and relative humidity of air on the day of the collect. According to Minh *et al*., (2003) the species yield of Citrus genus varied from 0.2 to 2%.

- Chemical composition of *C. aurantium* (bitter orange) essential oil:

The GC-MS analysis results of *C. aurantium* (bitter orange) oil are summarized in Table 1. Based on Table (1), 35 constituents (99.9% of the total oil) were identified from the oil of bitter orange ripe fruit peels. The major components of the peel essential oil was limonene 93.10%, followed by myrcene 2.46%. While, the other components were lower than 1% as, α- pinene (0.56%), linalool (0.44%), carvone (0.38%), N-Desanal (0.33%) and linalyl (0.17%). These results are in accordance with those of Ladaniya (2008), who reported the existence of more than 150 compounds found in essential oils of Citrus genus.

Data of table (1) showed that the major component of the essential oil was the limonene. Previous studies have shown that the amount of limonene in bitter orange peel essential oils is in majority (Boelens *et al*., 1989, Boussaada, 2006, Ben Hsoun, 2013). Moreover, Sarrow *et al*., (2013) reported that limonene (94.67%), myrcene (2%) and linalool (0.67%) were the main components of bitter orange ripe peel essential oil, grown in Greece. Also, Sanei-Dehkordi *et al*., (2016) reported that the major components of the peel essential oil of *C. aurantium* was limonene 94.81% , followed by myrcene 1%. Berrabeh *et al*., (2016)

found that the major components of the peel essential oil of C. aurantium were limonene (92.62%) , linalool (1.98%) and myrcene (1.34%). On the other hand, These compositions of *C. aurantium* vary significantly from the other studies (Chutia *et al*., 2009 and Abderrezak *et al*., 2014). These variety in constituents may be due to variety in cultivar and maturity and processing. The composition and flavor quality of this product varies considerably depending on the orange cultivar, maturity, and processing (Sato *et al*., 1996; Burt, 2004; and Boussaada, 2006).

Table 1. Main Compounds of fresh peel *Citrus aurantium* L. essential oil, by GC-MA chromatography.

No.	Chemical compounds	Retention time (min.)	Percentage (%)	No.	Chemical compounds	Retention time (min.)	Percentage (%)
1	α-Pinene	6.808	0.56	18	N-Decanal	24.233	0.33
2	Sabinene	8.883	0.14	19	Trans- Carveol	25.117	0.16
3	β -Pinene	9.142	0.06	20	Cis- Carveol	25.842	0.09
4	Myrcene	10.058	2.46	21	Carvone	26.300	0.38
5	N-Octanal	11.158	0.23	22	Linalyl acetate	26.617	0.17
6	Limonene	13.383	93.10	23	Geraniol	26.900	0.06
7	Trans-β- Ocimene	14.167	0.06	24	Geranial	27.700	0.06
8	Linalool oxide	15.733	0.04	25	Perillaldehyde	27.938	0.07
9	Octanol	16.117	0.07	26	Neryl acetate	32.283	0.04
10	Linalool	17.858	0.44	27	Geranyl acetate	33.258	0.20
11	Nonanal	18.058	0.07	28	1-Dodccanol(Gas) n-Dodccanol	34.767	0.09
12	Trans- p-Mentha-2,8-dienol	19.158	0.21	29	Trans-Caryophyllene	34.967	0.16
13	Cis-Limonene oxide	19.650	0.13	30	Limonen-10-yl-acetate	35.900	0.02
14	Trans- Limonene oxide	19.942	0.07	31	Trans-Farnesol	36.567	0.02
				32	α- Caryophyllene	36.642	0.02
15	Trans- p-2,8-Menthadien-1-ol	20.100	0.17	33	2-Dodecenal	37.492	0.05
				34	Germacrene	37.867	0.06
16	Nerol	22.633	0.02	35	Trans-Nerolidol	41.625	0.10
17	α- Terpineol	23.633	0.12		**Total**	**-**	**99.90**

- Antifungal effect of the essential oil of Citrus aurantium (bitter orange)

The antifungal activity of the essential oil of *Citrus aurantium* (bitter orange) was determined against five fungi including *Alternaria alternata* , *Penicillium expansum*, *Botrytis cinerea* , *Fusarium oxysporum*, and *Sclerotium cepivorum*) by poisoned food technique at different concentration. The results indicated that essential oil of *C. aurantium* significantly inhibited the growth (colony diameter) of pathogens over untreated PDA plates (table. 2 and fig. 1).. Percentage of growth inhibition was significantly influenced by essential oil

concentration. The rate of growth reduction was directly proportional to the concentration of tested material in the medium., in other words, an dependent effect dose was obtained in relation with the tested treatment. On the other hand, the oil completely inhibited the growth of *S. cepivorum*, *A. alternata* and *B. cinerea* fungi at 2000 ppm. Whereas, the oil showed moderate antifungal activity against *F. oxysporum* (53.26%) and *P. excpencium* (40.12%) at 1250ppm (Table 1). However, oil showed significantly greater suppression of the growth of S. cepivorum and A. alternate for all tested concentrations comparing with the other tested fungi .Contrarily, the oil at 250 ppm showed the lowest effects against the tested fungi.

Different species of fungi have different reaction to the essential oil, from the investigated 5 fungi species the most resistant for essential oil was *P. excpencium*, and the sensitive for the influence of oil oils were *S. cepivorum* and *A. alternate*. Furthermore, data analysis showed significant differences between fungi and between doses (LSD 0.01). This antifungal activity of *C. aurantium* may be due to his compounds. The chemical compounds of essential oils like linalool, caryophyllene oxide, a-pinene, a-terpineol (monoterpenes) have antifungal and antibacterial activity (Matasyoh *et al*., 2007) which are found in appreciable amounts in *C. aurantium* (Table,1). These compounds diffuse into and damage cell membrane structures. Sokovic and Griensven (2006) observed antifungal activity of limonene and a-pinene (MIC 4.0–9.0 ml/ml) against Verticillium fungicola and Trichoderma harzianum which are found at different amount in different plant essential oils. The essential oils and its related substances act to make the cell membrane of the fungus permeable, causing leakage (Piper *et al*., 2001). Our results are in accordance with the ones obtained by other authors, such as Viuda-Martos *et al*., (2008) who studied the essential oil of lemon, orange, pummelo and mandarin on the fungi associated with the deterioration of foods, they verified a huge potential antimicrobial. Similar studies realized by Sharma and Tripathi (2006) reported that the essential oil of Citrus genus is a mixture of volatile compounds with huge potential antifungal, totally reducing or inhibiting its growth, being dependent-dose.

The effective concentrations at which 50% pathogen inhibition (EC50) resulted from the use of *C. aurantium* oil were calculated (Table 2). The values of EC_{50} for the oil were, 610; 840; 1050; 1170; and 1460 ppm for *S.cepivorum*, *A. alternata*, *Botrytis cinerea*, *F. oxysporum* and *P. expansum*, respectively.

The effective concentration for inhibition of the mycelial growth in 50% was also estimated by Caccionia *et al*., (1998), who verifyied the fungicide activity of the essential oils of the peels of several species of Citrus genus, and found some results in a similar way. For the six cultivars analyzed of the species *C. sinensis*, they obser-ved the values of EC_{50}, on the fungus Penicillium digitatum, and the values of oxygenated monoterpenes quantities were inversely proportional. As the value of EC_{50} decreased, the quantity of oxygenated monoterpenes increased. In this study, for the essential oil of *C. aurantium*, it was observed the highest the content of limonene (93.10), the most efficient was against the pathogen. These results corroborated with Sonboli *et al*., (2006) who attributed this activity to the presence of limonene and linalool in the essential oil from Salvia. Essential oils are made up of many different volatile compounds and their production by plants is believed to be predominantly a defense mechanism against pathogens and pests, and they have been shown to possess antimicrobial and antifungal properties (Znini *et al*., 2011). They are considered as non-phytotoxic compounds, less environmental effects, and wide public acceptance (Znini *et al*., 2011; Gumus *et al*., 2010).

Table.2. Inhibition percentage of mycelia growth of tested fungi by different concentrations of *C. aurantium* L. oils on PDA.

Concentration of oil (ppm)	The tested fungi				
	S. cepivorum	*A. alternata*	*B. cinerea*	*F. oxysporum*	*P. expansum*
	(%) inhibition in mycelial growth				
250	32.25Fa	25.56Fb	20.24Gb	14.58Gc	8.56Gd
500	43.56Ea	33.89Eb	26.23Fc	19.56Fd	14.78Fd
750	56.23Da	44.89Db	39.25Ec	32.25Ed	24.12Ee
1000	75.78Ca	58.56Cb	47.28Dc	41.25Dd	31.75De
1250	90.23Ba	85.12Bb	60.58Cc	53.26Cd	40.12Ce
1500	100Aa	100Aa	85.23Bb	75.89Bc	51.25Bd
2000	100Aa	100Aa	100Aa	96.23Aa	70.56Ab
[1]EC_{50} (ppm)	610	840	1050	1170	1460

L.S.D $_{0.01}$: Between concentrates : 4.25, L.S.D$_{0.01}$: Between Fungi: 5.46

The values followed by the same letter indicate no significant difference between treatments.
(The small letters refer to difference between fungi. The letters capital refer to difference between concentrates).
1.The median effective concentration (EC_{50}) was determined 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 percentage inhibition (%) In control= 0.

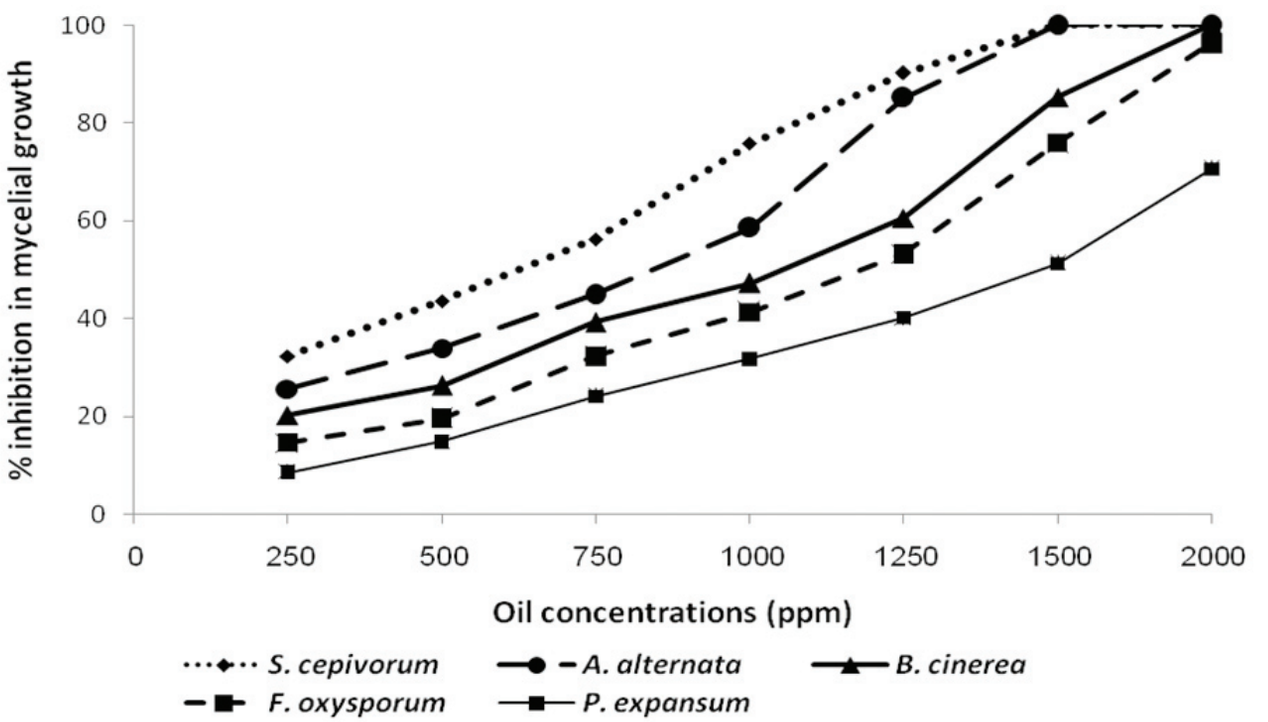

Fig. 1. Effect of *C. aurantium* L. oil on fungal growth at different concentrations on PDA.

- Conclusion

It is clear that the essential oil of *C. aurantium* had broad-spectrum inhibitory effects to tested fungi. We observed a higher inhibition rate of serious plant pathogenic fungi like *S. cepivorum*, *A. alternata*, *Botrytis cinerea*, *F. oxysporum* and *Penicillium expansum* comforting with the control. Based on these results, we conclude that the oil of *C. aurantium* may be used as an alternative of synthetic fungicides or preservatives. Further studies will be undertaken for the individual components and their antifungal activity against these pathogens for their possible application in the field or as natural preservatives.

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