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Antifungal Activity of Essential Oil and Extracts of *Piper chaba* Hunter Against Phytopathogenic Fungi

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Abstract The efficacy of the essential oil and various leaf extracts of *Piper chaba* Hunter was evaluated for controlling the growth of some important phytopathogenic fungi. The hydrodistilled essential oil was analysed by GC–MS. Fifty-three compounds representing 95.1% of the total oil were identified, of which α-humulene (16.4%), caryophyllene oxide (12.2%), viridiflorol (8.1%), globulol (7.4%), β-selinene (7.1%), spathulenol (6.2%), (*E*)-nerolidol (5.1%), linalool (4.5%) and 3-pentanol (3.5%) were the major compounds. The oil (1,000 ppm) and organic extracts (1,500 ppm) of *P. chaba* Hunter displayed a great potential of antifungal activity as a growth inhibition against the tested phytopathogenic fungi, *Fusarium oxysporum* KACC 41083, *Phytophthora capsici* KACC 40157, *Colletotrichum capsici* KACC 410978, *Fusarium solani* KACC 41092 and *Rhizoctonia solani* KACC 40111 in the range of 55.1–70.3% and minimum inhibitory concentration ranging from 125 to 500 μg/ml. The oil had also a strong detrimental effect on spore germination of all the tested fungi along with concentration as well as time-dependent kinetic inhibition of *P. capsici*. The results obtained from this study may contribute to the development of new antifungal agents to protect crops from fungal diseases.

Keywords *Piper chaba* Hunter · Piperaceae · Essential oil · α-Humulene · Antifungal activity · MIC

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Introduction

Fungi have long been recognized as causal agents of plant diseases. Rice sheath blight (*Rhizoctonia solani*), grey mold rot (*Botrytis cinerea*), fruit rot (*Fusarium solani*), vascular wilt (*F. oxysporum*), water soaked spot (*Sclerotinia sclerotiorum*) and fruit rot (*Phytophthora capsici*) are important plant diseases. Chemical fungicides are known to be highly effective for controlling the postharvest diseases in various vegetables and fruits. However, they are not considered as being long-term solutions due to the concerns associated with exposure risks, health and environmental hazards, residue persistence, and development of tolerance [1, 2]. The increasing recognition and importance of fungal infections and the difficulties encountered in their treatment have stimulated the search for synthetic chemical fungicide alternatives. Research focused on plant-derived fungicides and their possible applications in agriculture is being intensified as there is enormous potential to inspire and influence modern agro-chemical methods.

Essential oils are made up of many different volatile compounds and have been shown to possess antimicrobial and fungicidal properties [3]. Essential oils and plant extracts are arousing increasing interest because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multi-purpose functional uses [4]. So, essential oils and plant extracts are one of the most promising groups of natural compounds for the development of safer antifungal agents.

The genus *Piper* (Piperaceae) comprises 500 species, which are distributed in tropical and subtropical regions. They are mostly shrubs and rarely herbs or trees [5]. The plant *Piper chaba* Hunter is a branched, rambling shrub which is cultivated in Bangladesh, India, and Malaysia [6]. In Bangladesh, a number of *Piper* species are noted for

their ethnomedical properties, of which the reputed stimulant, carminative, diuretic and diaphoretic agents of *P. nigrum* are probably the best known. Like other plants of *Piper* genus, the plant possesses vast medicinal properties. The root of *Piper chaba* is alexiteric; useful in asthma, bronchitis, and consumption. The fruit has stimulant and carminative properties, and is used in hemorrhoidal complaints [6]. The stem is used to allay post-delivery pain in mothers and also useful in reduction of rheumatic pains and diarrhoea [7].

In previous studies, alkaloids such as piperamine 2, 4-decadienoic acid piperidide, kusunokinin and pellitorine [8], lignan [9], and a novel piperine dimer namely chabamide [10] have been isolated from the stem bark of the plant. The root has been reported to contain some alkamides such as piperine, sylvatine, piplartine and piperlonguminine besides β-sitosterol [11]. Furthermore, β-caryophyllene, caryophyllene oxide and a few monoterpene hydrocarbons, a moderate content of sesquiterpenes and high amount of aliphatic hydrocarbons have been found in the fruit oil of the plant [12]. However, there is no report available in the literature on the analyses of essential oil from leaves of *P. chaba* Hunter and the antifungal property.

Therefore, we undertook investigations of the chemical composition of the essential oil and the antifungal properties of the essential oil and extracts from *P. chaba* Hunter occurring in Bangladesh and the results are reported in this communication.

Materials and Methods

Plant Material

The leaves of *P. chaba* Hunter were collected from Jessore area of Bangladesh in June 2007 and identified by a senior taxonomist Dr. M. Oliur Rahman, Bangladesh National Herbarium, Dhaka, Bangladesh, where a voucher specimen (DACB 32565) has been deposited.

Isolation of the Essential Oil

The air-dried leaves (250 g) of *P. chaba* Hunter were subjected to hydrodistillation for 3 h using a Clevenger type apparatus. The oil was dried over anhydrous sodium sulfate and preserved in a sealed vial at 4 °C until further analysis.

Preparation of Organic Extracts

The air-dried leaves (50 g) of *P. chaba* Hunter was extracted with hexane, chloroform, ethyl acetate and 95.0% ethanol separately at room temperature and the solvents were evaporated by vacuum rotary evaporator. Solvents (analytical grade) for extraction were obtained from commercial sources (Sigma–Aldrich, St. Louis, MO, USA).

Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

The GC–MS analysis of the essential oil was performed using a Shimadzu GC–MS (GC-17 A) equipped with a FID detector and ZB-1 MS fused silica capillary column (30 m × 0.25 i.d., film thickness 0.25 μm). For GC–MS detection, an electron ionization system with an ionization energy of 70 eV was used. Helium gas was used as the carrier gas at a constant flow rate of 1 ml/min. Injector and mass transfer line temperatures were set at 220 and 290 °C, respectively. The oven temperature was programmed from 50 to 150 °C at 3 °C/min, then held isothermally for 10 min and finally raised to 250 °C at 10 °C/min. Diluted samples (1/100, v/v, in methanol) of 1 μl was manually injected in the splitless mode. The relative percentage of the oil constituents was expressed as a percentage by peak area normalization.

The identity of the components of the volatile oil was assigned by comparison of their retention indices (RI), relative to a series *n*-alkane indices on the ZB-1 capillary column and GC–MS spectra from the Wiley 6.0 MS data and literature data [13]. The relative amounts (RAs) of individual components of the oil were expressed as percentages of the peak area relative to the total peak area.

Microorganisms

The plant-pathogenic fungi were obtained from the Korean Agricultural Culture Collection (KACC), Suwon, Republic of Korea. Cultures of each fungal species were maintained on potato-dextrose agar (PDA) slants and stored at 4 °C. The fungal species used in the experiment were *Fusarium oxysporum* KACC 41083, *Phytophthora capsici* KACC 40157, *Colletotrichum capsici* KACC 410978, *Fusarium solani* KACC 41092, *Rhizoctonia solani* KACC 40111, *Sclerotinia sclerotiorum* KACC 41065 and *Botrytis cinerea* KACC 40573.

Preparation of Spore Suspension and Test Samples

The spore suspension of *F. oxysporum*, *P. capsici*, *C. capsici*, *F. solani*, *S. sclerotiorum* and *B. cinerea* were obtained from their respective 10 days old cultures, mixed with sterile distilled water to obtain a homogenous spore suspension of 1×10^5 spore/ml. Essential oil and plant extracts were dissolved in dimethyl sulfoxide (DMSO) separately to prepare the stock solutions with their respective known weights, which were further diluted to prepare test samples.

Determination of Antifungal Activity of Essential Oil and Organic Extracts

The essential oil and extracts were bio-assayed by the poisoned food technique [14]. Essential oil was mixed with dimethyl sulfoxide (DMSO) so as to ease its incorporation into the agar medium in the proportion 1 volume oil to 9 volumes DMSO. The essential oil was tested at 1,000 ppm (i.e., μl/l). Organic extracts (diluted in DMSO) were tested at 1,500 ppm. The oil and organic extracts were incorporated into the autoclaved and cooled (50 °C) PDA medium through 0.45 μm sterile Millipore filters. The medium amended with oil or extracts was then poured into sterilized petri dishes. A mycelial disc of 5 mm diameter of the test pathogens taken from a 10-day-old culture, with the help of a sterilized cork borer was placed at the center of the medium. Some plates prepared as controls without the oil or extracts but only DMSO. The plates were then sealed with parafilm and incubated at 28 °C for 5–7 days, the time by which the growth of control would have reached the edges of the plates. Growth inhibition of each of the fungal strains was calculated as the percentage of inhibition of radial growth relative to the control along with the antifungal effect on the fungal mycelium. The plates were used in triplicates for each treatment.

The growth inhibition of treatment compared to the control was calculated as a percentage, using the following formula:

$$\text{Inhibition}\,(\%) = [(\mathrm{C} - \mathrm{T})/\mathrm{C}] \times 100,$$ where C and T are the radial growth (mm) of fungus in the control and treated plates, respectively.

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the essential oil and various organic extracts against fungal pathogens was determined by the agar dilution method as described before [15]. Briefly, 10-ml aliquots of potato dextrose broth (PDB) were prepared in 25-ml Erlenmeyer flasks. The oil and plant extracts were dissolved with DMSO, sterile filtered (0.45 μm) and then added to the different flasks in order to obtain concentrations of 62.5–2,000 μg/ml of culture medium. The final concentrations of DMSO in the assay did not exceed 2%. In addition, one flask with uninoculated oil or extract-free medium, was included as a sterile control. Using a micropipette, an inoculum of 5 μl (10^5 spore/ml) of the spore suspension was inserted into each flask of medium containing a known concentration of samples, as well as a sample-free medium. All cultures were incubated in a shaking incubator at 150 rpm for 3–8 days at 27 ± 2 °C or until good growth was apparent in the oil or extract-free control. The growth in all flasks was visually compared with that of the control in order to determine % inhibition. The growth was scored in the following manner: 4+, growth comparable to that of the sample-free control; 3+, growth approximately 75% that of the control; 2+, growth approximately 50% that of the control; 1+, growth 25% or less that of the control; and 0, no visible growth (data not shown). The minimum concentration at which no visible growth was observed was defined as the MIC, which was expressed in μg/ml.

Spore Germination and Growth Kinetics Assay

Six concentrations of the essential oil (31.25, 62.5, 125, 250 and 500 μg/ml) and one control (0.5% DMSO) were separately tested for spore germination of different fungi including *P. capsici, F. oxysporum, C. capsici* and *F. solani* [16]. The samples were inoculated with spore suspension of each fungal pathogen containing 10^5 spore/ml. From this, aliquots of 10 μl spore suspension from each were placed on separate glass slides in triplicate. Slides containing the spores were incubated in a moisture chamber at 25 °C for 24 h. Each slide was then fixed in lactophenol-cotton blue and observed under the microscope for spore germination. The spores that had generated germ tubes were counted and the percentage of spore germination was calculated. The control (0.5% DMSO) was tested separately for spore germination of different fungi. All experiments were conducted in triplicate.

P. capsici which appeared to be more susceptible to the essential oil in the spore germination study was chosen as the test fungus for a kinetic study and evaluation of antifungal activity of the essential oil. A 10-μl spore suspension of this fungal species was inoculated to different concentrations of the oil (31.25, 62.5 and 125 μg/ml) in a test tube and a homogenous suspension (about 2×10^5 spore/ml) was made by inverting the test tubes 3–4 times. After the specific intervals i.e., 30, 60, 90, 120 and 150 min, the reaction mixtures were filtered through Whatman No. 1 filter paper and the retained spores were washed two or three times with sterile distilled water. The filter was then removed and spores were washed off into 10 ml of sterile distilled water. From this 100 μl of spore suspension was taken onto the glass slide and incubated at 24 ± 2 °C for 24 h. About 200 spores were counted and the percentage spore germination was calculated. Control sets were prepared in 0.5% DMSO. All experiments were conducted in triplicate.

Statistical Analysis

The essential oil and various extracts were assayed for antifungal activity. Each experiment was run in triplicate, and mean values were calculated. A *t*-test was computed for the statistical significance of the results.

Results

Chemical Composition of the Essential Oil

The hydrodistillation of the air-dried leaves of *P. chaba* Hunter gave a dark yellowish oil with a yield of 0.31% (w/w). GC–MS analyses of the oil led to the identification of 53 different compounds, representing 95.1% of the total oil (Table 1). The oil contains a complex mixture consisting of mainly oxygenated mono- and sesquiterpenes, and mono- and sesquiterpene hydrocarbons. The major compounds of the oil were α-humulene (16.4%), caryophyllene oxide (12.2%), viridiflorol (8.1%), globulol (7.4%), β-selinene (7.1%), spathulenol (6.2%), (*E*)-nerolidol (5.1%), linalool (4.5%), and 3-pentanol (3.5%).

Antifungal Activity of Essential Oil and Organic Extracts

The essential oil of *P. chaba* Hunter exhibited a moderate to high antifungal activity against all the tested fungi except *S. sclerotiorum* and *B. cinerea*. As shown in Table 2, 1,000 ppm concentration of the oil showed potent inhibitory effect on the growth of five phytopathogens, *R. solani* (70.3%), *P. capsici* (68.2%), *F. oxysporum* (66.1%), *F. solani* (56.6%), and *C. capsici* (55.1%). The solvent extraction process of the air-dried leaves of *P. chaba* Hunter yielded in hexane (7.5 g), chloroform (6.6 g), ethyl acetate (5.4 g) and ethanol (6.3 g) extracts. Hexane, chloroform, ethyl acetate and ethanol extracts (1,500 ppm) also showed growth inhibition against some of the phytopathogens but not for all (Table 3). Ethyl acetate extract of *P. chaba* Hunter showed a great potential antifungal activity against *R. solani* (70.0%), *P. capsici* (65.5%), *F. solani* (64.0%), *C. capsici* (58.1%) and *F. oxysporum* (56.3%). Ethanol extract showed inhibition (54.4–68.2%) against *R. solani, P. capsici, F. solani, F. oxysporum* and *C. capsici*. Chloroform extract had good antifungal activity against *F. solani, P. capsici*, *R. solani, F. oxysporum* and *C. capsici* with growth inhibition ranged from 54.5 to 63.5%, whereas hexane extract inhibited 54.5–56.4% growth of *P. capsici*, *R. solani* and *F. oxysporum*.

Minimum Inhibitory Concentration (MIC)

According to the results given in Table 2, MIC of essential oil was found more effective against *P. capsici* and *F. oxysporum* (125–250 μg/ml) as compared to those of *C. capsici* and *F. solani* (500 μg/ml for each). On the other hand, the chloroform, ethyl acetate and ethanol extracts were found more susceptible than the hexane extract against the tested fungi (Table 4). The ethyl acetate extract

Table 1 Chemical composition of the essential leaves oil of *Piper chaba* Hunter

No	RT[a]	Components	(%)[b]
1	780	Hexanal	0.4
2	858	Hexanol	0.3
3	920	Tricyclene	2.2
4	932	α-Pinene	0.5
5	940	Camphene	0.3
6	963	β-Pinene	0.9
7	966	3-Octanol	1.1
8	1,000	δ-3-Carene	0.4
9	1,001	1,4-Cineole	0.6
10	1,003	*p*-Cymene	1.6
11	1,005	1,8-Cineole	0.8
12	1,025	3-Octen-2-one	0.2
13	1,075	Linalool	4.5
14	1,082	Terpinolene	0.4
15	1,102	3-Pentanol	3.5
16	1,136	Phenyl ethyl alcohol	0.5
17	1,163	Citronellyl acetate	1.0
18	1,248	Piperitone	1.2
19	1,252	Linalyl acetate	1.1
20	1,278	Isobornyl acetate	0.3
21	1,320	Dihydrocarvyl acetate	0.2
22	1,342	α-Terpineol acetate	0.4
23	1,368	Geranyl acetate	0.4
24	1,375	α-Copaene	0.6
25	1,381	β-Bourbonene	0.3
26	1,390	β-Elemene	0.7
27	1,404	α-Cedrene	0.2
28	1,428	β-Copaene	0.2
29	1,438	Aromadendrene	0.3
30	1,447	α-Humulene	16.4
31	1,455	*trans*-β-Farnesene	0.4
32	1,470	β-Chamigrene	0.2
33	1,486	β-Selinene	7.1
34	1,490	Germacrene D	0.9
35	1,493	α-Selinene	1.1
36	1,494	Ledene	0.3
37	1,513	β-Bisabolene	0.4
38	1,518	Myristicin	0.2
39	1,532	Elemol	0.8
40	1,548	Germacrene B	0.2
41	1,549	(*E*)-Nerolidol	5.1
42	1,551	Spathulenol	6.2
43	1,554	Elemicin	0.2
44	1,561	Caryophyllene oxide	12.2
45	1,566	Globulol	7.4
46	1,569	Viridiflorol	8.1
47	1,574	Epiglobulol	0.5

Table 1 continued

No	RT[a]	Components	(%)[b]
48	1,580	Ledol	0.2
49	1,602	Humulene oxide	0.3
50	1,660	Juniper camphor	0.2
51	1,697	(*Z*,*E*)-Farnesol	1.1
52	1,761	Aristolone	0.2
53	1,768	Tetradecanoic acid	0.3
		Total	95.1

[a] Retention index relative to *n*-alkanes on ZB-1 capillary column

[b] Relative area (peak area relative to the total peak area)

Table 2 Antifungal activity of essential oil (1,000 ppm) of *Piper chaba* Hunter against phytopathogenic fungi

Fungal strains	Essential oil[a]		MIC (μg/ml)[d]
	RG (mm)[b]	I (%)[c]	
P. capsici KACC 40157	15.0 ± 0.4	68.2 ± 0.5	125
F. oxysporum KACC 41083	16.1 ± 0.5	66.1 ± 0.6	250
F. solani KACC 41092	19.5 ± 0.5	56.6 ± 0.6	500
C. capsici KACC 410978	20.2 ± 0.6	55.1 ± 1.4	500
R. solani KACC 40111	13.4 ± 0.7	70.3 ± 1.1	na
S. sclerotiorum KACC 41065	nd	nd	nd
B. cinerea KACC 40573	nd	nd	nd

nd no detection of antifungal activity, *na* not applicable

[a] Values are represented as the means ± SD of three experiments

[b] Radial growth of fungus pathogens

[c] Inhibition percentage

[d] Minimum inhibitory concentration

displayed potent antifungal activity against *F. oxysporum*, *P. capsici*, *C. capsici* and *F. solani* with MIC values of 125–500 μg/ml. The MIC values of chloroform and ethanol extract against *P. capsici*, *F. oxysporum*, *C. capsici* and *F. solani* were found within the range 250–500 μg/ml, whereas no inhibition was observed against *S. sclerotiorum* and *B. cinerea*. However, the hexane extract did not show activity against all the phytopathogens tested, except *P. capsici* and *F. oxysporum* (MIC: 500 μg/ml for each).

Table 4 Minimum inhibitory concentration of various organic extracts of *Piper chaba* Hunter against phytopathogenic fungi

Fungal strains	MIC (μg/ml)			
	HAE	CHE	EAE	ETE
P. capsici KACC 40157	500	250	125	250
F. oxysporum KACC 41083	500	500	500	500
F. solani KACC 41092	nd	250	250	250
C. capsici KACC 410978	nd	500	500	500
R. solani KACC 40111	na	na	na	na
S. sclerotiorum KACC 41065	nd	nd	nd	nd
B. cinerea KACC 40573	nd	nd	nd	nd

HAE hexane extract, *CHE* chloroform extract, *EAE* ethyl acetate extract, *ETE* ethanol extract, *nd* no detection of antifungal activity, *na* not applicable

Spore Germination and Growth Kinetics Assay

The results obtained for essential oil from the spore germination assay of each of the test fungi are shown in Fig. 1. DMSO (0.5%, v/v) as a control did not inhibit the spore germination of any of the plant pathogens tested. There was a significant inhibition of fungal spore germination by different concentrations of essential oil. A 100% inhibition of fungal spore germination was observed in *P. capsici* and *F. oxysporum* at 125 and 250 μg/ml concentrations of essential oil, respectively. Essential oil also exhibited

Table 3 Antifungal activity of various organic extracts (1,500 ppm) of *Piper chaba* Hunter against phytopathogenic fungi

Fungal strains	Organic extracts[a]							
	HAE		CHE		EAE		ETE	
	RG (mm)[b]	I (%)[c]	RG (mm)[b]	I (%)[c]	RG (mm)[b]	I (%)[c]	RG (mm)[b]	I (%)[c]
P. capsici KACC 40157	19.3 ± 0.5	56.4 ± 0.6	16.5 ± 0.4	63.5 ± 0.6	15.4 ± 0.5	65.5 ± 0.5	17.1 ± 0.5	63.3 ± 0.7
F. oxysporum KACC 41083	20.3 ± 0.5	54.5 ± 1.3	20.2 ± 0.6	54.6 ± 1.1	19.0 ± 0.5	56.3 ± 0.6	19.2 ± 0.6	56.4 ± 1.1
F. solani KACC 41092	nd	nd	16.5 ± 0.5	63.5 ± 1.2	16.0 ± 0.4	64.0 ± 0.8	16.3 ± 0.7	63.3 ± 0.6
C. capsici KACC 410978	nd	nd	20.2 ± 0.6	54.5 ± 0.7	19.0 ± 0.5	58.1 ± 1.0	20.3 ± 0.5	54.4 ± 1.1
R. solani KACC 40111	20.3 ± 0.6	55.1 ± 1.1	17.1 ± 0.3	63.0 ± 0.5	13.4 ± 0.4	70.0 ± 1.1	15.0 ± 0.4	68.2 ± 0.5
S. sclerotiorum KACC 41065	nd	nd	nd	nd	nd	nd	nd	nd
B. cinerea KACC 40573	nd	nd	nd	nd	nd	nd	nd	nd

nd no detection of antifungal activity, *HAE* hexane extract, *CHE* chloroform extract, *EAE* ethyl acetate extract, *ETE* ethanol extract

[a] Values are represented as the means ± SD of three experiments

[b] Radial growth of fungus pathogens

[c] Inhibition percentage

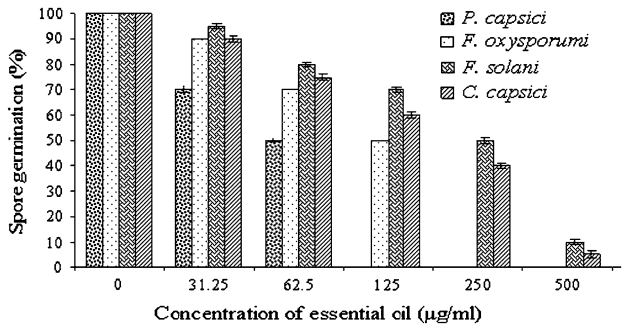

Fig. 1 Effect of different concentrations (µg/ml) of the essential oil of *Piper chaba* Hunter on spore germination of tested fungi

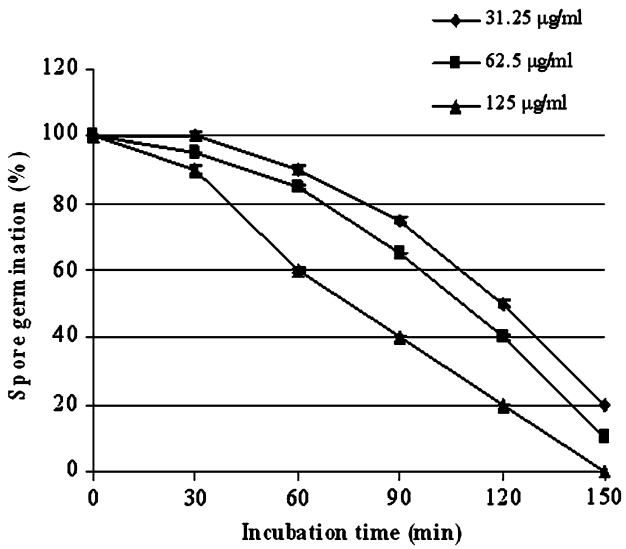

Fig. 2 Kinetics of inhibition of *P. capsici* spores by the essential oil of *Piper chaba* Hunter

a potent inhibitory effect on the spore germination of *F. solani* and *C. capsici* in the range of 50–80% at concentrations ranging from 250 to 500 µg/ml.

The antifungal kinetics of the essential oil against *P. capsici* is shown in Fig. 2. Exposure of *P. capsici* spores to different concentrations of the essential oil for a period of 30–150 min caused varying degree of inhibition of spore germination. An increase in fungicidal activity was observed with increase in exposure time and concentration. The essential oil at 31.25 µg/ml showed antifungal activity but not rapid killing and about 50% inhibition was observed at exposure time of 120 min. However, there was a marked increase in the killing rate at 62.5 and 125 µg/ml after 30 min of exposure, and 95% and 100% inhibition of spore germination was observed on 150 min exposure, respectively. At low concentration, significant rate of inhibition was the characteristic feature of the essential oil.

Discussion

Many essential oils and their constituents are found to exhibit antifungal properties, but the high cost of production of essential oils and the low concentration of active principles often prevent their direct use in the control of fungal diseases of plants and animals. In spite of this limitation, chemical investigation of antifungal compounds present in essential oils is considered important because of the possibility of synthesizing these compounds or their analogues which may be used in the control of fungal diseases.

The hydrodistillation of the leaves of *P. chaba* Hunter gave dark yellowish oil with the major components of the oil having oxygenated mono- and sesquiterpenes, and mono- and sesquiterpene hydrocarbons. In recent years, several researchers have reported that mono- and sesquiterpene hydrocarbons and their oxygenated derivatives are the major components of essential oils of plant origin, which have enormous potential to inhibit microbial pathogens [17]. In the present study, the essential oil of *P. chaba* Hunter showed remarkable antifungal effect against all the fungi tested except *B. cinerea* and *S. sclerotiorum*. This activity could be attributed to presence major components (e.g., α-humulene, caryophyllene oxide, viridiflorol, globulol, *β*-selinene, spathulenol, (*E*)-nerolidol, linalool, 3-pentanol and *p*-cymene), and/or other minor components present in the oil.

Some earlier papers on the analysis and antifungal properties of the essential oil of some species of various genera have shown that they have a varying degree of growth inhibition effects against some *Fusarium*, *Botrytis*, and *Rhizoctonia* species due to their different chemical composition [18, 19]. Bouchra et al. reported that the oils of seven Moroccan Labiatae, which consisted mainly of carvacrol, linalyl acetate, and thymol as major components, exhibited a complete mycelial inhibition effect on the growth of *B. cinerea* [20]. On the other hand, the oils of *Pistacia vera*, *P. terebinthus* and *P. lentiscus* had moderate activities at 750 ppm doses against *R. solani* [18]. In spite of this, most of these oils are available for purchase as whole or as a part of pharmaceutical or cosmetic products, indicating that toxic properties do not prohibit their use. However, the ongoing investigation of toxic or irritant properties is imperative, especially when considering any new products for human use, whether medicinal or otherwise.

In this study, the essential oil and the different extracts showed varying antifungal activity against some important plant pathogenic fungi. This research work also described the complex effect of volatile oil on fungal spore germination and exhibited a wide range of anti-fungal activity. During the kinetic study of *P. capsici*, it appeared that exposure time of the volatile oil had a little effect on the fungicidal activity at lower concentration but at the

concentration of 62.5 and 125 μg/ml, the fungicidal action was very rapid and showed 100% spore germination inhibition of *P. capsici*. This activity could be attributed to the presence of phenolic compound and oxygenated mono- and sesquiterpene, and sesquiterpene hydrocarbons, and these finding are in agreement with a previous report [21]. Volatile compounds, such as α-humulene, caryophyllene oxide, viridiflorol, globulol, β-selinene, spathulenol, (*E*)-nerolidol, linalool, 3-pentanol and *p*-cymene have been claimed to contain the antifungal properties [22–24]. Those claims are further supported by our findings; indicating high contents of α-humulene, caryophyllene oxide, viridiflorol, globulol, β-selinene, spathulenol, (*E*)-nerolidol, linalool, 3-pentanol and *p*-cymene; comprising 70.3% of the leaves essential oil (Table 1). Also, the antifungal activity of individual components of essential oils, such as α-humulene and caryophyllene oxide has been reported previously [17]. On the other hand, the components present in lower amounts, such as α-selinene, β-pinene, 1, 8-cineole, α-pinene and camphene also contributed to antifungal activity of the oil [22, 25]. It is also possible that the minor components might be involved in some type of synergism with the other active compounds [26]. Therefore, it would also be interesting to study the effects of essential oil and crude extracts of *P. chaba* Hunter against other important fungi for developing new antifungal agents to control serious fungal diseases in plant, animal and human beings.

Thus, *P. chaba* Hunter mediated oil and organic extracts could become an alternative to synthetic fungicides for using in agro-industries and also to screen and develop such novel types of selective and natural fungicides in the treatment of many plant pathogens causing severe destruction to crops and vegetables.

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