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Antifungal Effect of Plant Essential Oils on Controlling *Phytophthora* Species

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In this study, antifungal activity of essential oils of *Cymbopogon citratus* and *Ocimum basilicum* and two fungicides Mancozeb and Metalaxyl-Mancozeb in six different concentrations were investigated for controlling three species of *Phytophthora*, including *P. capsici*, *P. drechsleri* and *P. melonis* on pepper, cucumber and melon under *in vitro* and greenhouse conditions, respectively. Under the *in vitro* condition, the median effective concen- tration (EC_{50}) values (ppm) of plant essential oils and fungicides were measured. In greenhouse, soil infested with *Phytophthora* species was treated by adding 50 ml of essential oils and fungicides (100 ppm). Disease severity was determined after 28 days. Among two tested plant essential oils, *C. citratus* had the lowest EC_{50} values for inhibition of the mycelial growth of *P. capsici* (31.473), *P. melonis* (33.097) and *P. drechsleri* (69.112), respectively. The mean EC_{50} values for Metalaxyl-Mancozeb on these pathogens were 20.87, 20.06 and 17.70, respectively. Chemical analysis of plant essential oils by GC-MS showed that, among 42 compounds identified from *C. citratus*, two compounds β-geranial (α-citral) (39.16%) and z-citral (30.95%) were the most abundant. Under the greenhouse condition, Metalaxyl-Mancozeb caused the greatest reduction in disease severity, 84.2%, 86.8% and 92.1% on melon, cucumber, and pepper, respectively. The *C. citratus* essential oil reduced disease severity from 47.4% to 60.5% compared to the untreated control ($p \leq 0.05$). Essential oils of *O. basilicum* had the lowest effects on the pathogens under *in vitro* and greenhouse conditions. These results show that essential oils may contribute to the development of new antifungal agents to protect the crops from Phytophthora diseases.

Keywords **:** *Cymbopogon citratus*, essential oil, *Ocimum basilicum*, *Phytophthora*

Phytophthora species cause destructive diseases of a huge range of agriculturally and ornamentally important plants including those in forests and other natural ecosystems. Damping off and blight caused by the *Phytophthora* species, is one of the most devastating diseases affecting cucurbit and vegetables production in Iran (Etebarian, 2012). Some species such as *P. capsici*, *P. drechsleri* and *P. melonis* can cause strong pathogenicity on pepper, cucurbit, tomato, cantaloupe and ornamental plants that cause damping off disease and rot of crown and root (Lamour et al., 2003; Tabarrae et al., 2011). The genus *Phytophthora* is a soil borne pathogen and survives in the soils as oospores and mycelia for several years in plant debris (Jee et al., 2001). Management of soil borne pathogen in the field includes crop rotation, cultural practices, chemical control and use of resistant cultivars (Someya et al., 2000). The long-term survival of pathogen even in the absence of susceptible host limits the effectiveness of crop rotation (Shouan Zhang et al., 2010). Fungicide application does not always prove economic against soil borne pathogens and it has led to environmental pollution, pathogen resistance, and increased risk for human and animal health (Anna On et al., 2015; De Curtis et al., 2010; Hausbek and Lamour, 2004). In addition, excessive use of fungicides creates imbalance in the microbial community in soil (Anna On et al., 2015; Para and Ristaino, 2001). Therefore, in order to solve problems as those mentioned above, several research groups have sought cheap alternatives, of low toxicity, to control soil borne pathogens (Joyce Mendes Andrade Pinto et al., 2010; Stangarlin et al., 2008). Among the potentially useful practices, the use of plant products like essential oils and plant extracts against several phytopathogens has already been demonstrated (Behtoei et al., 2012; Carvalho et al., 2008;

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Dikbas et al., 2008). Plant essential oils are volatile natural complex compounds characterized by a strong odor and are formed by aromatic plants as secondary metabolites, which play an important role in the protection of the plants against plant pathogens both *in vitro* and *in vivo* (Mollaei et al., 2011; Reddy et al., 1998). These compounds not only do not leave toxic residue to the environment but also have lower toxicity against mammals (Duke, 1985; Garcia et al., 2005). Essential oils activity of *C. citratus* and *Ocimum* species against plant pathogens tests have been conducted *in vitro*, with a few examples of greenhouse or field studies (Helal et al., 2006; Joyce Mendes Andrade Pinto et al., 2010; Kumar et al., 2010; Mahanta et al., 2007; Prakash et al., 2011; Paret et al., 2010; Somada et al., 2007). Few researches of essential oils have been reported in Oomycetes, such as *Phytophthora citrophthora* (Del Rio et al., 1998), *P. cactorum* (Lee et al., 2008) and *P. capsici* (Bi et al., 2012). However, in order to increase the efficacy of essential oils in *Phytophthora* species management, the products need to be studied further.

The objectives of this study were to evaluate the efficacy of *Cymbopogon citratus* (DC.) Stapf and *Ocimum basilicum* L. natural essential oils to control three species of *Phytophthora* in compared to fungicides (Mancozeb and Metalaxyl-Mancozeb) under *in vitro* and greenhouse conditions.

Materials and Methods

Culture media and organisms. The pathogens including *P. capsici*, *P. drechsleri* and *P. melonis* were isolated from infected pepper, cucumber and melon seedling respectively in Ardabil province, Iran. Infected samples of plants after surface-disinfesting in sodium hypochlorite (2%) were cultured on corn meal agar (CMA, Merk, Germany) media amended with 100 ppm PCNB (pentachloronitrobenzene), 100 ppm ampicillin, 30 ppm rifampicin and 25 ppm hymexazol (CMA-PARPH) at 25 ± 2ºC for 10 days. Pathogenicity of isolates was assessed on pepper, cucumber and melon seedlings at the two-three true leaf stage under greenhouse condition described by Tabarraei et al. (2011). The *Phytophthora* species were identified according to the morphological characteristics of their mycelia and spores as described by Ershad (1992) and Stamps et al. (1990).

Plant materials and extracting essential oils from plant. The plant species used in this research were *C. citratus* and *O. basilicum*. The leaves of *C. citratus* and *O. basilicum* were collected from growing field in their natural habit (Sanandaj, Kurdistan Province, Iran) at flowering stage on June 2013. The collected parts of plants were subsequently dried under the shade condition with proper ventilation. Approximately 300 g of air-dried leaves of both plants were subjected to hydro-distillation for 3 h using a Clevenger type apparatus at 100ºC. The oils were dried over anhydrous sodium sulphate (Na_2SO_4), due to sodium sulphate pentahydrate formation and then the oils were stored at 4ºC for further analysis (Amini et al., 2012; Dev et al., 2011).

Analysis of essential oils by gas chromatography-mass spectrometry (GC-MS). The GC-MS analysis was done at 250ºC on an Agilent 7890A gas chromatograph at 70 eV. The GC column was as follows: HP-5MS; the size of fused silica capillary was 0.25 × 3000 µm × 0.25 µm film thickness and it was used as the carrier gas (helium) with a flow rate of 0.8 ml/min. The GC column used was programmed as follows: 50ºC (5 min), 240ºC at the rate of 3ºC/min. The injection temperature was 250ºC. The mass spectrometer was operating in E1 mode at 70 eV. The compounds of essential oil were identified tentatively by comparing their relative retention times and mass spectra with those of pure authentic samples and WILEY and NBS (Ozcan et al., 2006).

Determining antifungal activity *in vitro*. The antifungal assay of essential oil and two fungicides (Mancozeb and Metalaxyl-Mancozeb) were carried out in the petri dishes (8 cm in diameter) containing CMA. Essential oils were dissolved in Tween 80 (0.5% v/v) before testing for fungal toxicity. Six concentrations of essential oil and fungicides were selected by pre-test for each pathogen (Table 1) and were mixed to CMA media at 40ºC after autoclaved. A disc of 5 mm in diameter (7-days old stock cultures) of each pathogen was placed in the center of the Petri dishes. Petri dishes containing CMA media and Tween 80 were used

Table 1. Selected concentrations (ppm) of plant essential oils and fungicides based on pre-test on *Phytophthora* spp.

Phytophthora spp.	*C. citratus*	*O. basilicum*	Mancozeb	Metalaxyl-Mancozeb
P. capsici	15, 30, 45, 60, 75, 90	50, 120, 190, 260, 330, 400	7.5, 20.5, 33.5, 46.5, 59.5, 72.5	5, 14, 23, 32, 41, 50
P. drechsleri	25, 39, 53, 67, 81, 95	50, 120, 190, 260, 330, 400	7.5, 20.5, 33.5, 46.5, 59.5, 72.5	5, 14, 23, 32, 41, 50
P. melonis	15, 29.5, 44, 58, 5, 73, 87.5	50, 120, 190, 260, 330, 400	7.5, 20.5, 33.5, 46.5, 59.5, 72.5	5, 14, 23, 32, 41, 50

as controls. All petri dishes were incubated at 25 ± 2°C. Experiments were carried out in a completely randomized design. After seven days, the colony diameter was measured and the growth inhibition percent of treatments compared to negative control was calculated by the following formula: Inhibition (%) = $[(d_c - d_t)/d_c] \times 100$: where d_c and d_t are the radial growth (mm) of pathogen in the control and treated plates, respectively (Amini et al., 2012). In order to test whether plant essential oils were fungistatic or fungicidal, fungal disc of pathogens, inhibited by essential

Table 2. Components of *C. citratus* essential oil identified by GC-MS analysis

Number	RT*	Compound	Percentage(%)
1	6.875	Camphene	0.34
2	7.796	Methyheptenone	1.15
3	7.905	β-Myrecene	0.17
4	8.980	Limonene	5.83
5	9.158	1,3,6-Octatriene, 3,7-dimethyl	0.58
6	9.444	cis-β-Ocimene	0.39
7	10.125	Nonanone	0.87
8	10.909	β-Linalool	1.38
9	12.047	1,6-Heptadiene, 2,3,6-trimethyl	0.183
10	12.345	Trans-Chrysanthemal	0.32
11	12.408	6- Octenal, 3,7-dimethyl-, (R)	0.75
12	12.728	2-Cyclopenten-1-one, 3,4,4-trimetnyl	0.72
13	13.032	Cyclopentanecarboxylic acid, 2-methyl-3-methylene-, methyl ester	0.16
14	13.243	Cyclohexane, ethenyl	1.43
15	13.484	Cyclohexane, ethenyl	0.24
16	13.867	Decanal	0.25
17	13.959	2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, cis	0.17
18	14.725	D-Citral	0.58
19	15.172	Z-Citral	30.95
20	15.292	Geraniol	0.47
21	16.081	β-Geranial (α-Citral)	39.16
22	16.167	2,7-Octadiene, 4-methyl	0.47
23	16.270	Acetic acid, 1,7,7-trimethyl bicycle(2.2.1) hept-2-yl ester	0.20
24	17.157	Cyclooctanemethanol	0.18
25	17.546	n-Heptadecylcyclohexane	0.16
26	18.050	Eugenol	0.35
27	18.353	(+)-Cycloisosativene	0.17
28	18.485	Cyclobutane, (1-methylethylidene)	0.19
29	18.725	Geranyl acetate	3.10
30	19.000	β-Elemene	0.29
31	19.784	Caryophyllene	3.44
32	20.081	α-Bergamotene	0.39
33	20.390	Iso-Eugenol	0.43
34	20.608	α-Caryophyllene	0.42
35	21.586	1h-Cyclopropa(a)naphthalene,1a,23,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-)	0.18
36	21.884	Benzene,1-methyl-4-(1,2,2-trimethylcyclopentyl)-, (R)	0.30
37	22.067	Naphtalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)	0.46
38	22.261	Naphtalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)	0.33
39	22.393	Vinyldimethyl(1,3,3-triboromoprophyl)silane	0.22
40	22.879	Cyclohexanemethanol, 4-ethenyl-α,α,4-trimethyl-3-(1-methylethenyl)	0.17
41	23.772	Caryophyllene oxide	2.02
42	24.355	3,5-Dimethylcyclohex-1-ene-4-carboxaldehyde	0.17
Total	–		99.063

RT*: Retention time

Table 3. Components of *O. basilicum* essential oil identified by GC-MS analysis

Number	RT*	Compound	Percentage(%)
1	7.562	β-Pinene	0.52
2	9.038	β-Myrcene	3.57
3	9.444	Limonene	1.26
4	10.617	Eucalyptol	1.10
5	11.029	β-Ocimene	10.51
6	12.271	Fenchone	3.07
7	12.820	Linalool	0.53
8	13.150	Camphor	0.64
9	14.067	Borenol	60.06
10	14.909	Terpineol-4	0.57
11	15.698	Estragole	0.76
12	16.511	β-Citral	1.49
14	19.223	Carvacrole	0.93
15	19.738	β-Elemene	1.04
16	20.087	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)	2.06
17	20.539	Isomethyl-Eugenol	0.49
18	20.596	α-Bergamotene	0.51
19	21.277	β-Farensene	1.38
20	21.655	Homulene	0.72
21	21.855	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)	0.63
22	22.067	γ-Elemene	1.077
23	23.606	Azulene, 1,2,3,5,6,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)	0.68
24	23.755	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)	0.72
25	24.453	(−)-Spathulenole	0.58
26	25.065	Caryophyllen oxide	3.98
27	41.699	(+)-Epi-bicyclosesquiphellenderene	0.59
Total			99.47

RT*: Retention time

oils in treated plates, were re-inoculated into fresh CMA media without essential oil. Pathogen growth was observed at 25 ± 2°C for 10 days. If renewed mycelial growth was observed, the inhibition was qualified as fungistatic. On the other hand, if the contrary was the case and pathogens did not grow, fungicidal effect was assumed.

Finally, median effective inhibitory concentration (EC_{50}) values for essential oils and fungicides (ppm) on three *Phytophthora* species were measured. All tests were repeated twice. The Probit analysis was used to measure EC_{50} with POLO-PC software (2002).

Effect of the plant essential oils and fungicides on *Phytophthora* diseases in greenhouse conditions. Seeds of pepper (*Capsicum annum* L.), cucumber (*Cucumis* sativus L.) and Cantaloupe (*Cucumis mello* L.) were disinfected with 5% sodium hypochlorite for 5 min, rinsed with sterile distilled water and sown in pots (15 cm diameter and 20 cm height) containing steam-sterilized soil (loam clay soil). After one month at the 2–3 true-leaf stage, three mycelia discs (5 mm diameter) of each pathogen were placed in the soil around the bush (at a depth of 8 cm). Plant not received mycelia discs was as control (Tabarraei et al., 2011). Then, plant essential oils or fungicide at 100 ppm were applied at 50 ml/bush and was mixed with soil around the bush. Sterile distilled water was used as control. All pots were kept in a greenhouse at 23–27°C, 60–70% relative humidity, 16 h light, 8 h darkness. Plants were watered twice a week and fertilizer solution added to pots once a week (NPK 1:1:1) at the rate of 3 g/l. After four weeks, disease severity was determined using a scale of 0 to 5, where 0 represents healthy and symptomless of disease; 1, small brownish lesion at the base of stem; 2, stem lesions extend to cotyledons or the lesion has girdled the stem causing plant collapse; 3, plant has collapsed with all leaves wilted or turned yellow except for the young leaves; 4, plant has

completely collapsed; 5, plant is dead (Shouan Zhang et al., 2010).

Statistical analysis. Experiments were designed as completely randomized design with four replicates. All data analyses were conducted using the SPSS (Statistical Package for the Social Science, Version 18). The means were compared by Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$. Experiments were repeated two times.

Results and Discussion

Chemical composition of the essential oils. The percentages and the retention indices of the identified components in two plants by the GC-MS analysis are listed in Table 2 and 3. Chemical analysis of plant essential oils by GC-MS indicated z-citral (30.95%), β-geranial (α-citral) (39.16%) and caryophyllene (3.44%) as the main components in *C. citratus* oils (Table 2). Gupta et al. (2011) reported that citral (77.8%), limonene (4%) and geraniol (2.7%) were the main components in *C. citratus* oils. Also, borenol (60.06%), caryophyllen oxide (3.98%), β-myrcene (3.57%) and fenchone (3.07%) were identified as the main chemical compounds in *O. basilicum* oils (Table 3). Dev et al. (2011) indicated eugenol (61.7%), isopropyl palpitate (11.3%) and 2,3-dihydroxy propyl elaidate (5.1%) as major chemical compounds among *O. basilicum* oils. Differences in composition of essential oils might have been derived by harvest time, as well as local, climatic and seasonal factors (Rahimi-Nasrabadi et al., 2013).

Antifungal test. Damping off and blight disease caused by the *Phytophthora* species can cause severe losses in cucurbit and vegetables plants in various parts of the world including Iran (Hausbek and lamour, 2004; Etebarian, 2012). In this study, we evaluated the control efficacy of two plant essential oils against three *Phytophthora* species under *in vitro* and greenhouse conditions compared to fungicides.

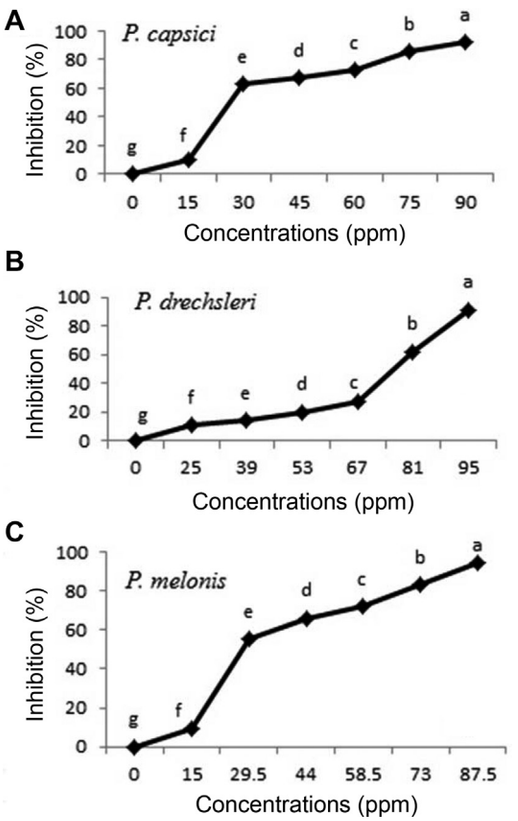

Fig. 1. Effect of *C. citratus* essential oil on growth of *Phytophthora* species *in vitro* (A, *P. capsici*; B, *P. drechsleri*; C, *P. melonis*). Mean by different letters indicate significant differences among treatment ($P \leq 0.05$) according to DMRT. Data are mean of four replicates.

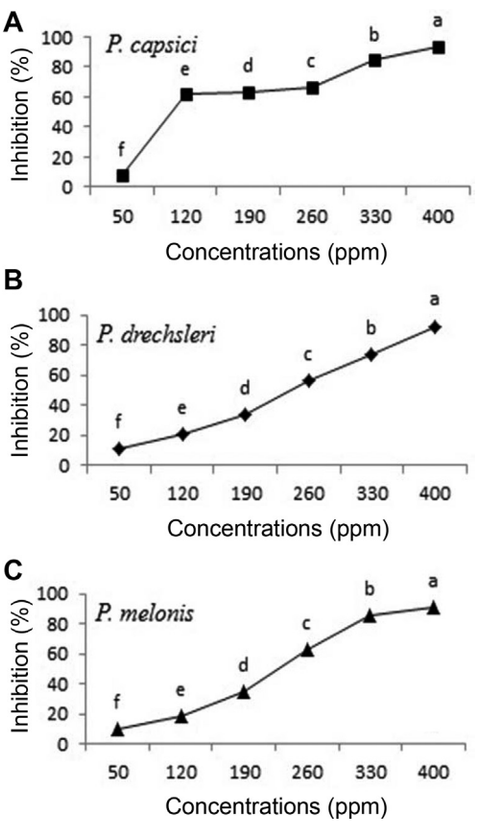

Fig. 2. Effect of *O. basilicum* essential oil on growth of *Phytophthora* species *in vitro* (A, *P. capsici*; B, *P. drechsleri*; C, *P. melonis*). Mean by different letters indicate significant differences among treatment ($P \leq 0.05$) according to DMRT. Data are mean of four replicates.

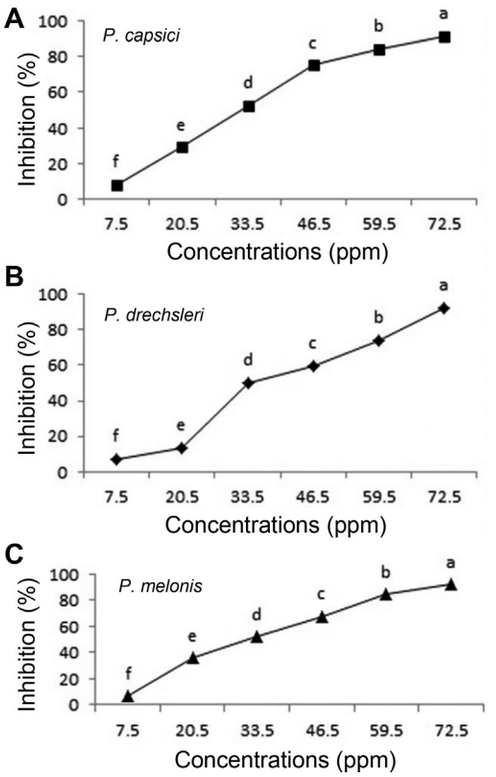

Fig. 3. Effect of Mancozeb on growth of *Phytophthora* species *in vitro* (A, *P. capsici*; B, *P. drechsleri*; C, *P. melonis*). Mean by different letters indicate significant differences among treatment ($P \leq 0.05$) according to DMRT. Data are mean of four replicates.

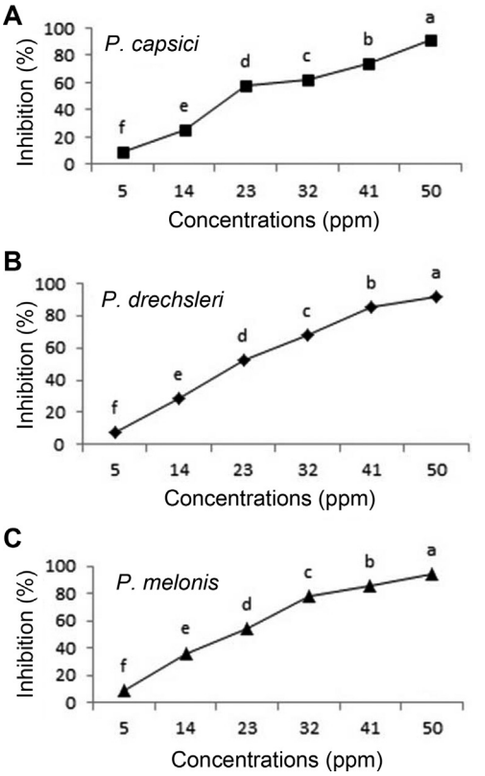

Fig. 4. Effect of Metalaxyl - Mancozeb on growth of *Phytophthora* species *in vitro* (A, *P. capsici*; B, *P. drechsleri*; C, *P. melonis*). Mean by different letters indicate significant differences among treatment ($P \leq 0.05$) according to DMRT. Data are mean of four replicates.

The antifungal activity of two plant essential oils and two fungicides (Mancozeb and Metalaxyl-Mancozeb) was tested against species of *Phytophthora in vitro*. The results indicated that plant essential oils and fungicides reduced mycelial growth of pathogen in the culture. Fungicides of Metalaxyl-Mancozeb and Mancozeb proved to be the first and the second most effective in inhibiting mycelial radial growth of the pathogens respectively, followed by plant essential oils of *C. citratus* (Figs. 1, 3, 4 and Table 4).

The plant essential oils of *O. basilicum* showed the lowest inhibitory effects against pathogens (Fig. 2 and Table 4). Both essential oils exhibited varying degrees of antifungal activities against *Phytophthora* species. The present data showed that *C. citratus* essential oil appear to be more toxic than *O. basilicum* essential oil against *Phytophthora* species (Table 4), which is in agreement with the results of other authors (Helal et al., 2006; Shadab et al., 1992).

Table 4. EC_{50} (Lower-Upper value) of essential oils and fungicides on *Phytophthora* species (at CL* 95%)

Phytophthora spp.	Essential oils		Fungicides	
	C. citratus	*O. basilicum*	Mancozeb	Metalaxyl + Mancozeb
P. capsici	31.473 (20.633–40.780)	135.393 (75.892–189.535)	28.252 (25.494–31.034)	20.869 (15.543–26.722)
P. drechsleri	69.612 (44.157–414.629)	200.816 (124.708–313.053)	34.737 (23.493–48.378)	20.055 (16.244–23.957)
P. melonis	33.097 (24.646–40.748)	191.275 (115.835–288.030)	28.242 (25.416–31.093)	17.702 (14.180–21.219)

*Confidence limit

Table 5. Effect of plant essential oils and fungicides applied as soil drench on *Phytophthora* species after 28 days

Treatments[a]	*P. capsici*[b]		*P. drechsleri*		*P. melonis*	
	Disease[c] severity	Reduction %	Disease severity	Reduction %	Disease severity	Reduction %
C. citratus	1.5[c]	60.5	2.0[b]	47.4	1.7[b]	55.3
O. basilicum	1.9[b]	50	2.3[b]	36.8	2.2[b]	44.7
Mancozeb	1.2[d]	68.4	1.5[c]	60.5	1.2[c]	68.4
Metalaxyl + Mancozeb	0.6[e]	84.2	0.5[d]	86.8	0.3[d]	92.1
Nontreated control	3.8[a]	–	3.8[a]	–	3.8[a]	–

[a]Fifty ml of plant essential oils and fungicide (100 ppm) were applied as a root drench into a soilless potting medium at the 2–3 true-leaf stage. [b]Plants were inoculated with *Phytophthora* spp. by applying three mycelia disc (5-mm-diameter) of each pathogen into soil per plant. [c]Phytophthora disease was rated based on a scale of 0–5 as described material and methods. Mean in the column followed by different letters indicate significant differences among treatment ($p < 0.05$) according to DMRT. Data are mean of five replicates.

Essential oil of *C. citratus* showed potent inhibitory effect on the radial growth of *P. capsici* (91.9%), *P. drechsleri* (91.2%) and *P. melonis* (94.6%), as shown in Fig. 1. Mycelial growth of *Aspergillus* spp., *Alternaria alternate*, *Penicillum citrinum* and *Curvularia lunata* was inhibited by *C. citratus* essential oil (Mahanta et al., 2007). It was effective in inhibiting fungal viability and spore germination, so morphological changes of fungal hyphae were observed under light microscope by essential oil of *C. citratus* (Sulaiman Ali Al Yousef, 2013). The essential oils of *C. citratus* and *O. basilicum* were found to be highly effective against *A. niger*, *A. flavus* and *Saccharomyces cerevisiae*, whereas *C. citratus* essential oil showed the strongest activity against these fungi (Helal et al., 2006).

The results of EC_{50} are presented in Table 4. Between two tested plant essential oils on *Phytophthora* spp., essential oils of *C. citratus* had the lowest EC_{50} values (ppm) for inhibition the mycelial growth of *P. capsici* (31.473 ppm) and *P. melonis* (33.097 ppm), respectively. The EC_{50} of *C. citratus* seemed almost equal to EC_{50} of Metalaxyl + Mancozeb (17.702 to 20.869) (Table 4). Essential oils of oregano, palmarosa and red thyme had the lowest EC_{50} values and inhibited production and germination of sporangia, zoospores and mycelial growth of *P. capsici* (Bi et al., 2012). The highest EC_{50} value (200.816) was observed for *O. basilicum* against *P. drechsleri* (Table 4).

The results indicated that essential oils have fungistatic effect on *Phytophthora* species and cause inhibition of fungal growth, while essential oil of *C. citratus* caused the death of *Phytophthora* species as fungicide effect, in 10 days when used in concentration above 102 ppm.

Greenhouse assessment of plant essential oils and fungicides. The fungicides and plant essential oils applied as a soil drench significantly ($p < 0.05$) reduced disease severity of *Phytophthora* diseases on pepper, cucumber and melon, compared to the nontreated control in greenhouse assays (Table 5).

Both fungicides applied as a soil drench consistently suppressed Phytophthora diseases, which is in agreement with the results of other authors (Bi et al., 2012). Metalaxyl-Mancozeb had the most significant effect against the pathogens and caused the highest decreases in the severity of the disease, which were 84.2%, 86.8%, 92.1% in relation to the negative control on melon, cucumber and pepper, respectively (Table 5). The essential oil of *C. citratus* had good effect in reducing the severity of the disease by *P. capsici*, *P. drechsleri* and *P. melonis*, which were 60.5%, 47.4% and 55.3%, respectively (Table 5). Our results are in accordance with the work by Paret et al. (2010), Mahanta et al. (2007) and Helal et al. (2006), who observed the effects of essential oils of *C. citratus* as bactericidal and fungicidal properties against wide range of pathogens such as *Ralstonia solanacearum*, *Alternaria alternata*, *Penicilliu citrinum* and *Aspergillus*. But essential oils of *O. basilicum* presented the lowest reduction on the severity of the disease, which correspond to 36.8% on *P. drechsleri* (Table 5). Results indicated that formulations containing clove oils, neem oil, pepper extract and mustard oil reduced the population of *Phytophthora nicotians* in the soil in greenhouse (Bowers and Locke, 2004). Bi et al. (2012) have reported that *P. capsici* population in soil was reduced by the essential oils of red thyme, oregano and palmarosa. *Cucumber pepo* fruits were protected against *P. capsici* infection when they were sprayed with red thyme essential oils (Bi et al., 2012). Application of clove and cassia extract reduced the population density of *Phytophthora nicotianae* 99.6 and 99.2%, respectively (Bowers and Locke, 1999).

In conclusion, our results indicated that application of two essential oils of *C. citratus* and *O. basilicum* provides

significant protection against the soil-borne oomycete pathogens such as *P. capsici*, *P. drechsleri* and *P. melonis* in pepper, melon and cucumber plant respectively under *in vitro* and greenhouse conditions. Essential oils of two plants exhibited different degrees of antifungal activity against *Phytophthora* species, so that these essential oils may be used as alternatives for synthetic chemicals for integrated management of diseases caused by *Phytophthora* species in crop, vegetable and ornamental plants after the proper clinical trials. Hence, further studies are required to develop strategies for practical application in order to control the Phytophthora diseases.

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