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Inhibition of plant pathogens *in vitro* and *in vivo* with essential oil and organic extracts of *Cestrum nocturnum* L.

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ABSTRACT

The efficacy of the essential oil and various organic extracts from flowers of *Cestrum nocturnum* L. was evaluated for controlling the growth of some important phytopathogenic fungi. The oil (1000 ppm) and the organic extracts (1500 μg/disc) revealed antifungal effects against *Botrytis cinerea*, *Colletotrichum capsici*, *Fusarium oxysporum*, *Fusarium solani*, *Phytophthora capsici*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* in the growth inhibition range of 59.2–80.6% and 46.6–78.9%, respectively, and their MIC values were ranged from 62.5 to 500 and 125 to 1000 μg/mL. The essential oil had a remarkable effect on spore germination of all the plant pathogens with concentration and time-dependent kinetic inhibition of *P. capsici*. Further, the oil displayed remarkable *in vivo* antifungal effect up to 82.4–100% disease suppression efficacy on greenhouse-grown pepper plants. The results obtained from this study may contribute to the development of new antifungal agents to protect the crops from fungal diseases.

1. Introduction

The pre- and postharvest losses in world crops due to fungal disease may amount to more than 12% in developing countries [1]. Many pathogens including *Botrytis cinerea* (gray mold rot), *Fusarium oxysporum* (vascular wilt), *Colletotrichum capsici* (fruit rot), *Sclerotinia sclerotiorum* (water soaked spot) and *Fusarium solani* (fruit rot) reduce the shelf life and market values of food commodities and render them unfit for human consumption and cause undesirable effects on human health. Besides, the mycotoxins produced by fungi cause a serious health problem, and about 4.5 billion people in underdeveloped countries are exposed to the deleterious effects of these pathogens such as *Fusarium* and *Aspergillus* spp. [2]. Widespread use of pesticides has significant drawbacks including cost, handling hazards, pesticide residues, and threats to human health and environment [3]. For many years, a variety of different synthetic chemicals (benzimidazoles, aromatic hydrocarbons, and sterol biosynthesis inhibitors) have been used as antifungal agents to inhibit the growth of plant pathogenic fungi. However, there is a series of problems against the effective use of these chemicals in areas where the fungi have developed resistance [4]. In order to overcome this problem, higher concentrations of these chemicals were used, but this increases the risk of high-level toxic residues in the products and increased resistance. Therefore, public awareness of these factors has increased interest in finding safer alternative protectants to replace synthetic chemical pesticides. Some synthetic pesticides can also cause environmental pollution owing to their slow biodegradation in the environment [5]. This has also increased the need for the development of new safe and biodegradable alternatives as natural fungicides. Thus, there is a growing interest on the research of the possible use of natural products such as plant-based essential oils and extracts, which may be less damaging for pest and disease control [6].

Cestrum nocturnum L. is a garden shrub from the family Solanaceae, the flowers of which exude a special sweet fragrance at night, the main reason for its folk names night cestrum, lady of the night, night-blooming jessamine, and night-blooming jasmine [7]. It is widely naturalized in tropical and subtropical regions throughout the world, including Australia, southern China and the southernmost United States. It is also cultivated in Bangladesh in home yards and gardens. Several phytochemical studies have demonstrated the presence of important bioactive compounds in different parts of the plant: alkaloids, flavonol glycosides, steroidal saponins, fatty acids, essential oils, phenols, and others [8].

Practitioners use the plant externally for skin disorders, but several scientific reports demonstrate that it exhibits a wide spectrum of pharmacological activity when administered systemically or in isolated organ preparations. For example, it is used to treat arterial hypotension and as an analgesic, abortive, diuretic, antispasmodic, dyspeptic, antiviral, and smooth muscle relaxant; it also has negative inotropic and chronotropic actions [9].

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Most of the species of *Cestrum* have found several applications in folk medicine. *Cestrum parqui* is used in Chilean folk medicine as antifebrile and for the treatment of fever and inflammation [10]. Chinese people use leaves of *C. nocturnum* for their pharmacological significance in burns and swellings. It is also used for treating epilepsy and as stupefying charm medicine in West Indian Islands. The volatile oil of the species is known to be mosquito repellent and hence *C. nocturnum* and *Cestrum diurnum* are used to prevent malaria in several African Nations [11]. The plants of the genus have further found use in perfumery, as ornamental plants, floral scent production, etc. However, there is no report available in the literature on the analyses of essential oil from flower parts of *C. nocturnum* and its antifungal property.

Therefore, we have undertaken to investigate the antifungal activity of the essential oil and organic extracts from flowers of *C. nocturnum* growing in Bangladesh and the results are reported in this communication.

2. Materials and methods

2.1. Plant material

The flowers of *C. nocturnum* were collected from Islamic University Campus, Kushtia, Bangladesh, in December 2007. The taxonomic identification of plant materials was confirmed by a senior plant taxonomist Md. Habibur Rahman, Bangladesh National Herbarium, Dhaka, where a voucher specimen (DACB 32562) has been deposited.

2.2. Isolation of the essential oil

The air-dried flowers (200 g) of *C. nocturnum* were subjected to hydrodistillation for 3 h using a Clevenger type apparatus. The oil was dried over anhydrous Na_2SO_4 and preserved in a sealed vial at 4 °C until further analysis.

2.3. Preparation of crude extracts

The air-dried powdered material (50 g) of flowers from *C. nocturnum* was extracted with hexane, chloroform, ethyl acetate and methanol separately at room temperature and the solvents were evaporated by vacuum rotary evaporator. The extraction process yielded in hexane (7.5 g), chloroform (6.6 g), ethyl acetate (5.4 g) and methanol (6.3 g) extracts. Solvents (analytical grade) for extraction were obtained from commercial sources (Sigma–Aldrich, St. Louis, MO, USA).

2.4. Fungal pathogens

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 *F. oxysporum* KACC 41083, *Phytophthora capsici* KACC 40157, *C. capsici* KACC 410978, *F. solani* KACC 41092, *Rhizoctonia solani* KACC 40111, *S. sclerotiorum* KACC 41065 and *B. cinerea* KACC 40573.

2.5. Preparation of spore suspension and test samples

The spore suspensions of *B. cinerea, C. capsici, F. oxysporum, F. solani*, *P. capsici* and *S. sclerotiorum* in sterile distilled water were obtained from 10 days old cultures. The spore suspension was collected and then centrifuged. A hemocytometer was used to obtain a homogenous spore suspension of 1×10^8 spores/mL. To prepare the stock solutions of essential oil and extracts, the essential oil was dissolved in dichloromethane separately, whereas the extracts were dissolved in their respective solvents (hexane, chloroform, ethyl acetate and methanol). Samples with known weights were further diluted with 5% of the respective solvents used to prepare test samples, where the final concentration of the solvent was 0.5% (v/v).

2.6. Antifungal activity of essential oil and organic extracts

Petri dishes (9 cm diameter) containing 20 mL of potato-dextrose agar (PDA), containing per liter 4 g potato infusion solids and 20 g dextrose (Acumedia Manufacturers, Inc., Lansing, MI, USA) were used for antifungal activity assay, performed on solid media by the disc diffusion method [12]. Sterile Whatman paper discs of 6 mm diameter were placed on the agar, equidistant and near the border, where the essential oil (1000 ppm) and the extracts of hexane, chloroform, ethyl acetate and methanol (1500 μg/disc) were added separately. An agar plug of fungal inoculums (6 mm diameter) was removed from a previous culture of all the fungal strains tested and placed in the center of the petri dishes. Petri dishes were sealed with parafilm to prevent the leak of test oil and extracts. The plates were incubated at 25 °C for 5–7 days, until the growth in the control plates reaches the edge of the plates. The plates without the essential oil and extracts were used as negative control. The plates were prepared in triplicate for each treatment. The relative growth inhibition of treatment compared to negative control was calculated by percentage, using the following formula:

$$\text{Inhibition } (\%) = [(C - T)/C] \times 100,$$

where C and T are the radial growth (mm) of fungus in the control and treated plates, respectively.

2.7. Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of essential oil and various organic extracts against fungal pathogens was determined by agar dilution method as described before [13]. Appropriate quantities of oil and various extracts were diluted in DMSO to produce the concentration ranging from 62.5 to 2000 μg/mL. The final concentrations of DMSO in the assay did not exceed 2%. A 10 μL spore suspension (1×10^8 spores/mL) of each test strain was inoculated in the test tubes in PDB medium and incubated for 3–7 days at 28 °C. The control tubes containing PDB medium were inoculated only with fungal spore suspension. The minimum concentration at which no visible growth was observed was defined as the MIC, which was expressed in μg/mL.

2.8. Spore germination assay

For spore germination assay of *B. cinerea, C. capsici, F. oxysporum, F. solani*, *P. capsici* and *S. sclerotiorum*, essential oil samples (2 μL) in 5% dichloromethane were dissolved with water to obtain 31.25, 62.5, 125, 250, 500 and 1000 μg/mL concentrations of the oil, where the final concentration of dichloromethane was 0.5% [14]. The samples were inoculated with spore suspension of each fungal pathogen containing 1.0×10^8 spores/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 generated germ tubes were enumerated and percentage of spore germination was calculated. The control (0.5% dichloromethane) was tested separately for spore germination of different fungi.

2.9. Growth kinetics assay

P. capsici which appeared to be more resistant compared to other tested fungi to the essential oil in the spore germination assay was chosen as test fungus for kinetic study and evaluation of antifungal activity of essential oil. A 10 μL spore suspension (about 1.0×10^8 spores/mL) of this fungal species was inoculated to different concentrations of essential oil (31.25, 62.5 and 125 μg/mL) in a test tube and a homogenous suspension was made by inverting the test tubes 3–4 times. After specific intervals of 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, a 100 μL of spore suspension was taken onto the glass slide and incubated at 24 °C for 24 h. The spores that generated germ tubes were enumerated and percentage of spore germination was calculated. All experiments were conducted in triplicate.

2.10. In vivo antifungal activity assay

Based on the *in vitro* susceptibility, *P. capsici* (leaf spot/scorch) was selected as the test fungus for the *in vivo* study conducted on greenhouse-grown pepper plants. The *in vivo* antifungal activity of test samples was determined by a whole plant method as described previously [15].

In brief, for the *in vivo* study, the tested pepper plants, possessing an average of 8–12 leaves were kept under following greenhouse conditions: A day and night temperatures of 70–82 °F and 62–64 °F, optimum for pepper plants were maintained. Because, during cloudy weather, a temperature closer to the lower end of these ranges is preferred, while in sunny weather, temperatures closer to the higher end are better for pepper plants. Below 60 °F, nutrient deficiencies may occur because plants can not absorb some elements at cool temperatures, indicating lack of phosphorus uptake (even though there may be adequate phosphorus in the nutrient solution). Where day temperatures might exceed 85–90 °F, cooling equipment is needed to maintain the regular growth of the plants. Therefore, ideally, the thermostat was located at blossom height of the greenhouse for good temperature control. The optimum relative humidity for greenhouse-grown pepper plants was maintained at 65–75%. The higher light intensity required for pepper plants was also maintained. For that greenhouse was equipped with spectral filters that can alter red and far-red light balance of sunlight. Further, to prepare the test solutions at the concentration of 1000 μg/mL, 4 μL of essential oil was dissolved in 5% dimethylsulfoxide (DMSO) followed by diluting it with water containing a surfactant Tween 20 (200 μg/mL), where the final concentrations of dimethylsulfoxide and Tween 20 were 0.5% and 0.1%, respectively. The initial concentration of the test solution was 1000 μg/mL, in further; test dilutions of 500 and 250 μg/mL of essential oil were employed. For applying the test samples of the oil, 4 mL of each test sample solution was sprayed onto each pot at the same time. Further, 6 mL of fungal spore suspension (1.0×10^8 spores/mL) of *P. capsici* was sprayed onto each pot. Controls were sprayed with dimethylsulfoxide and Tween 20 solutions, where the final concentrations of DMSO and Tween 20 were 0.5% and 0.1%, respectively. The area of lesions on treated plants was measured in millimeter using a vernier caliper. All tests were conducted in three replicates. The effect of antifungal efficacy of the test samples on disease was evaluated after 12 days as a percentage of inhibition calculated by the formula:

$$\text{Percent inhibition } (\%) = [(A - B)/A] \times 100,$$

where *A* and *B* represent the disease area on the untreated and treated plants, respectively.

2.11. Statistical analysis

The essential oil and various organic extracts were assayed for antifungal activity. Each experiment was run in triplicate, and mean values were calculated. A Student's *t*-test was computed for the statistical significance of the results.

3. Results

3.1. Antifungal activity assay

The essential oil of *C. nocturnum* exhibited a moderate to high antifungal activity against all the tested fungi. At the concentration of 1000 ppm, the essential oil showed potent inhibitory effect on the radial growth of all phytopathogens such as *P. capsici* (80.6%), *R. solani* (80.5%), *B. cinerea* (70.6%), *F. solani* (69.7%), *S. sclerotiorum* (66.2%), *F. oxysporum* (63.6%) and *C. capsici* (59.2%), as shown in Table 1. Also, hexane, chloroform, ethyl acetate and methanol extracts showed radial growth inhibition against some of the phytopathogens but not for all. According to the results given in Table 2, methanol extract of *C. nocturnum* showed a remarkable antifungal activity against *F. solani* (78.9%), *B. cinerea* (76.3%), *S. sclerotiorum* (75.2%), *R. solani* (72.1%), *P. capsici* (68.4%), *C. capsici* (68.4%) and *F. oxysporum* (67.0%). Ethyl acetate extract showed inhibition (46.6–60.7%) against *R. solani, P. capsici, S. sclerotiorum, F. oxysporum* and *C. capsici*. Chloroform extract had good antifungal activity against *P. capsici*, *F. oxysporum, S. sclerotiorum* and *C. capsici* with radial growth inhibition ranged from 50.8% to 56.8%, while hexane extract inhibited 50.2% to 53.7% radial growth of *P. capsici*, *S. sclerotiorum* and *F. oxysporum*.

3.2. Minimum inhibitory concentration (MIC)

According to the results given in Table 1, MIC of essential oil was found more effective against *P. capsici*, *B. cinerea*, *F. solani* and *S. sclerotiorum* (62.5, 125, 250 and 250 μg/mL, respectively) as compared to those of *C. capsici* and *F. oxysporum* (500 μg/mL for each). On the other hand, the methanol and ethyl acetate extract were found more susceptible than hexane and chloroform extract against the tested fungi (Table 3). The MIC values of methanol extract against *P. capsici*, *F. oxysporum*, *C. capsici, F. solani*, *S. sclerotiorum* and *B. cinerea* were found in the range between 125 and 500 μg/mL. The ethyl acetate extract displayed antifungal activity against *F. oxysporum*, *P. capsici*, *C. capsici*, and *S. sclerotiorum* with MIC values of 250–1000 μg/mL, whereas chloroform extract showed activity against *F. oxysporum*, *P. capsici*, *C. capsici*, and *S. sclerotiorum* with MIC values of 500–1000 μg/mL. However, hexane extract did not show desirable results against all the phytopatho-

Table 1
Radial growth of phytopathogenic fungi by the essential oil (1000 ppm) of *C. nocturnum* L.

Fungal strains	Essential oil[a]		MIC[d] (μg/mL)
	RG[b] (mm)	*I*[c] (%)	
F. oxysporum (KACC 41083)	15.8 ± 0.6	63.6 ± 1.0	500
F. solani (KACC 41092)	12.8 ± 0.3	69.7 ± 0.4	250
C. capsici (KACC 410978)	16.6 ± 0.5	59.2 ± 0.7	500
P. capsici (KACC 40157)	8.1 ± 0.1	80.6 ± 0.1	62.5
R. solani (KACC 40111)	7.7 ± 0.5	80.5 ± 1.0	na
S. sclerotiorum (KACC 41065)	13.9 ± 0.5	66.2 ± 1.1	250
B. cinerea (KACC 40573)	11.9 ± 0.3	70.6 ± 0.4	125

[a] Values are represented as means ± SD of three experiments.
[b] Radial growth of fungus pathogens.
[c] Inhibition percentage.
[d] Minimum inhibitory concentration; na: not applicable.

Table 2
Radial growth of phytopathogenic fungi by the various organic extracts (1500 μg/disc) of *Cestrum nocturnum* L.

Fungal strains	Organic extracts[a]							
	HAE		CHE		EAE		ME	
	RG[b] (mm)	*I*[c] (%)	RG[b] (mm)	*I*[c] (%)	RG[b] (mm)	*I*[c] (%)	RG[b] (mm)	*I* (%)
F. oxysporum (KACC 41083)	21.7 ± 0.4	50.2 ± 0.6	20.7 ± 0.4	52.3 ± 0.5	20.7 ± 0.4	52.3 ± 0.5	13.9 ± 0.5	67.0 ± 2.4
P. capsici (KACC 40157)	19.6 ± 0.5	53.7 ± 1.4	18.3 ± 0.3	56.8 ± 0.3	17.9 ± 0.3	57.8 ± 0.9	12.7 ± 0.3	68.4 ± 0.9
C. capsici (KACC 410978)	nd	nd	20.1 ± 0.3	50.8 ± 0.2	21.8 ± 0.4	46.6 ± 0.5	12.9 ± 0.3	68.4 ± 0.3
F. solani (KACC 41092)	nd	nd	nd	nd	nd	nd	8.8 ± 0.3	78.9 ± 0.5
R. solani (KACC 40111)	nd	nd	nd	nd	19.5 ± 0.3	50.8 ± 0.3	11.0 ± 0.2	72.1 ± 0.3
S. sclerotiorum (KACC 41065)	20.0 ± 0.2	51.1 ± 0.4	18.9 ± 0.3	53.8 ± 0.6	16.1 ± 0.2	60.7 ± 0.4	10.2 ± 0.2	75.2 ± 0.5
B. cinerea (KACC 40573)	nd	nd	nd	nd	nd	nd	10.0 ± 0.2	76.3 ± 0.4

[a] Values are represented as means ± SD of three experiments. HAE: hexane extract; CHE: chloroform extract; EAE: ethyl acetate extract; ME: methanol extract.
[b] Radial growth of fungus pathogens.
[c] Inhibition percentage; nd: no detection of antifungal activity.

Table 3
Minimum inhibitory concentrations of various organic extracts of *C. nocturnum* L. against phytopathogenic fungi.

Fungal strains	MIC (μg/mL)			
	HAE	CHE	EAE	ME
F. oxysporum (KACC 41083)	1000	1000	500	500
P. capsici (KACC 40157)	500	500	250	250
C. capsici (KACC 410978)	nd	1000	1000	250
F. solani (KACC 41092)	nd	nd	nd	125
R. solani (KACC 40111)	na	na	na	na
S. sclerotiorum (KACC 41065)	1000	500	250	500
B. cinerea (KACC 40573)	nd	nd	nd	125

HAE: hexane extract; CHE: chloroform extract; EAE: ethyl acetate extract; ME: methanol extract; nd: no detection of antifungal activity; na: not applicable.

gens tested except *S. sclerotiorum*, *P. capsici* and *F. oxysporum* (MIC: 500–1000 μg/mL).

3.3. 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 negative 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 *B. cinerea* at 62.5 and 125 μg/mL concentrations of essential oil, respectively. Essential oil also exhibited a potent inhibitory effect on the spore germination of *F. oxysporum*, *S. sclerotiorum*, *F. solani* and *C. capsici* in the range of 50–80% at concentrations ranging from 125 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.

3.4. Inhibition of fungal infection in vivo

The antifungal activity of essential oil of *C. nocturnum* against *P. capsici* was assessed by the presence or absence of disease area on the tested pepper plants (Fig. 3). According to the results given in Table 4, the oil exhibited wide range of antifungal activity. The blind controls such as DMSO (0.5%) and Tween 20 (0.1%) did not inhibit the growth of test strain.

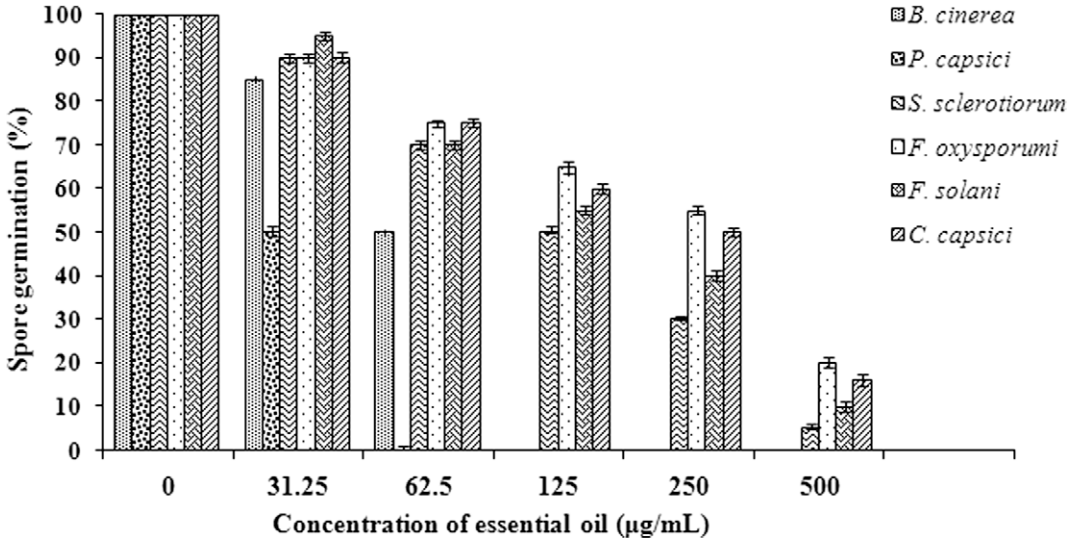

Fig. 1. Effect of different concentrations (μg/mL) of the essential oil of *C. nocturnum* on spore germination of tested fungi.

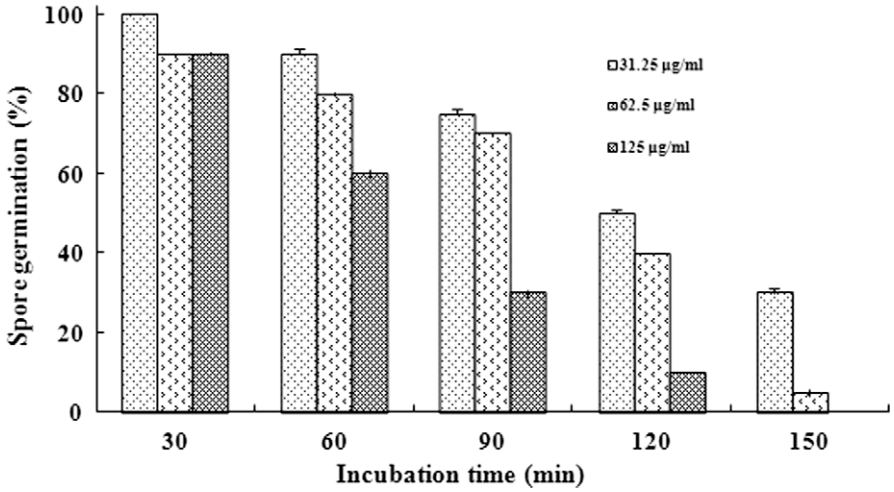

Fig. 2. Kinetics of inhibition of *P. capsici* spores by the essential oil of *C. nocturnum*.

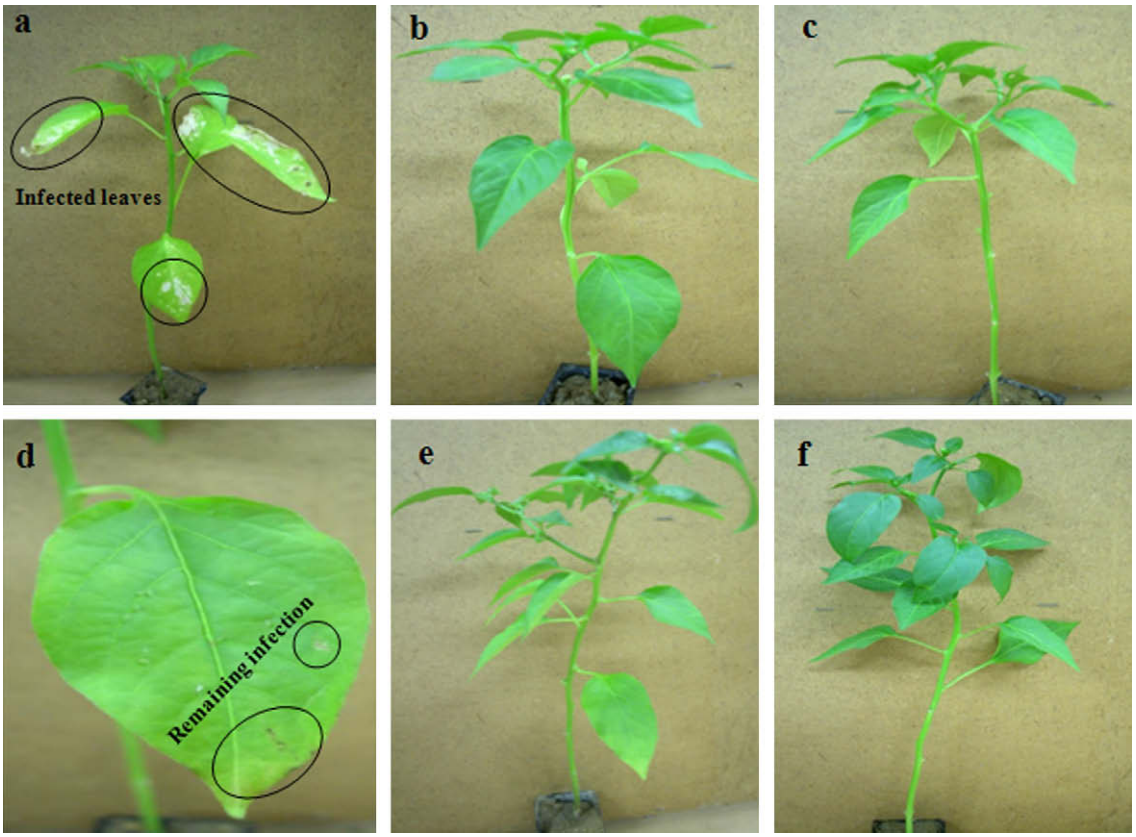

Fig. 3. *In vivo* antifungal activity of the essential oil of (*Cestrum nocturnum* L.) against plant pathogenic fungus of *Phytophthora capsici* on pepper plants. (a) Treated with pathogen (*P. capsici*) in vehicle; (b) no treatment (normal control); (c) treated with vehicle (0.5% DMSO + 0.1% Tween 20 in water); and (d–f) treated with pathogen and different concentrations of essential oil (250, 500 and 1000 µg/mL, respectively) in vehicle.

At the initial concentration of 1000 µg/mL the oil exhibited 100% antifungal effect against leaf spot/scorch of pepper caused by *P. capsici*. Further dilutions of the oil applied onto the plants were 500 and 250 µg/mL. Also at the concentration of 500 µg/mL, potential antifungal effect of the oil was observed with 100% antifungal effect against *P. capsici*. However, the oil at the concentration of 250 µg/mL had a moderate antifungal effect (82.4%) against *P. capsici* (Table 4).

4. Discussion

Plant essential oils are potentially useful source of antimicrobial compounds. It is often quite difficult to compare the results obtained from different studies, because the compositions of the essential oils can vary greatly depending upon the geographical region, the variety, age of the plant, the method of drying and the method of extraction of the oil. Many essential oils and their con-

Table 4
In vivo antifungal activity of the essential oil of *Cestrum nocturnum* L. against plant pathogenic fungus of *P. capsici* on greenhouse-grown pepper plants.

Groups	Treatment	Essential oil concentration (μg/mL)	Disease suppression efficacy (%)
Control (normal)	–	0	100 ± 0.0
Control (vehicle only)	VH	0	100 ± 0.0
Control + pathogen	VH	0	0.0 ± 0.0
	VH + EO	250	82.4 ± 0.6ab
Treatment	VH + EO	500	100 ± 0.0a
	VH + EO	1000	100 ± 0.0a

–: control without treatment; EO: essential oil.
VH: Vehicle solution (0.5% DMSO + 0.1% Tween 20 in water).
Values in the same column with different significance ($P < 0.05$).

stituents 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, currently, there is much research performed for the development of safer antifungal agents such as plant-based essential oils and extracts to control phytopathogens in agriculture [6]. Thus, essential oils and plant extracts are promising natural antifungal agents with potential applications in agro industries to control phytopathogenic fungi causing severe destruction to crops.

In brief, the hydrodistillation of the flowers of *C. nocturnum* gave dark yellowish oil with the major components of the oil having phenolic compounds, oxygenated mono- and sesquiterpenes, and their respective hydrocarbons [16]. 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 strongly inhibit microbial pathogens [17]. In general, the active antimicrobial compounds of essential oils are phenolic terpenes. It would seem reasonable that their antimicrobial or antifungal mode of action might be related to that of other compounds. Most of the studies on the mechanism of phenolic compounds have focused on their effects on cellular membranes. Actually, phenolic compounds not only attack cell walls and cell membranes, thereby affecting the permeability and release of intracellular constituents but they also interfere with membrane function. Thus, active phenolic terpenes might have several invasive targets which could lead to the inhibition of plant pathogenic fungi.

This research work describes the complex effect of essential oil on fungal spore germination. During the kinetic study of *P. capsici*, the essential oil had a little effect on the fungicidal activity at lower concentration but at the concentration of 125 μg/mL, the fungicidal action was very rapid and showed 100% spore germination inhibition of *P. capsici* after a period of 120 min. In the present study, the essential oil of *C. nocturnum* showed potential *in vitro* and *in vivo* antifungal effects against the tested plant pathogens. Earlier *in vivo* studies on the analysis of antifungal effect of various oil/extracts showed that they had varying degree of antifungal effect against different plant pathogenic fungi [18–19].

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 [20,21]. The *in vitro* and *in vivo* antifungal activities observed in this study could be attributed to the presence of phenylethyl alcohol, benzyl alcohol, eicosane, eugenol, *n*-tetracosane, caryophyllene oxide, 1-hexadecanol, methoxyeugenol and benzaldehyde have been claimed to contain the antifungal properties [22–25]. Those claims are further supported by our findings; indicating high contents of phenylethyl alcohol, benzyl alcohol, eicosane, eugenol, *n*-tetracosane, caryophyllene oxide, 1-hexadecanol, methoxyeugenol and benzaldehyde; comprising 65.96% of the oil [16]. Besides, minor components present in our essential oil such as hexadecanoic acid, 1-nonadecanol, heneicosane, methyl anthranilate, nonadecene, nerolidol, tetradecanal and citronellal also contribute to antifungal activity of the oil involving some type of synergism with the other active components [26].

In this study, *C. nocturnum* mediated essential oil and flower extracts showed varying antifungal activities against plant pathogenic fungi. It would also be interesting to study the effect of essential oil and organic extracts of *C. nocturnum* against other important fungi for developing new antifungal agents to control serious fungal diseases in plant, animal and human beings.

Thus, it can be concluded that the use of essential oil and extracts from flower of *C. nocturnum* could be 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 microbial phytopathogens causing severe destruction to crop, vegetable and ornamental plants.

References

[1] G.N. Agrios, Significance of plant diseases, in: Plant Pathology, fourth ed., Academic Press, San Diego, 1997, pp. 25–37.
[2] H.J. Williams, T.D. Phillips, E.P. Jolly, K.J. Stiles, M.C. Jolly, D. Aggrawal, Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions, Am. J. Clin. Nutr. 80 (2004) 1106–1122.
[3] N. Paster, L.B. Bullerman, Mould spoilage and mycotoxin formation in grains as controlled by physical means, Int. J. Food Microbiol. 7 (1988) 257–265.
[4] K.J. Brent, D.W. Hollomon, Fungicide Resistance: The Assessment of Risk, FRAC, Global Crop Protection Federation, Brussels, 1998, pp. 1–48.
[5] M. Barnard, M. Padgitt, N.D. Uri, Pesticides use and its measurement, Int. J. Pest Control 39 (1997) 161–164.
[6] T.R. Costa, F.L.F. Orionalda, S.C. Santos, M.A.O. Cecília, M.L. Luciano, H.F. Pedro, J.R. Paula, H.D. Ferreira, H.N.S. Beatriz, R.R.S. Maria do, Antifungal activity of volatile constituents of *Eugenia dysenterica* leaf oil, J. Ethnopharmacol. 72 (2000) 111–117.
[7] J.T. Roig, Galán de noche, in: Plantas medicinales, aromáticas o venenosas de Cuba, Editorial Científico-Técnica, Havana, 1988, pp. 443–444.
[8] V.U. Ahmad, F.T. Baqai, I. Fatima, R. Ahmad, A spirotanol glycoside from *Cestrum nocturnum* L., Phytochemistry 30 (1991) 3057–3061.
[9] H. Pérez-Saad, M.T. Buznego, Behavioral and antiepileptic effects of acute administration of the extract of the plant *Cestrum nocturnum* Lin (lady of the night), Epilepsy Behav. 12 (2008) 366–372.
[10] N.C. Backhouse, R. Delporte, P. Salinas, A. Pinto, S. Aravena, B.K. Cassels, Antiinflammatory and antipyretic activities of *Cuscuta chilensis*, *Cestrum parqui*, and *Psorela glandulosa*, Int. J. Pharm. 34 (1996) 53–57.
[11] N.N. Ntonifor, C.A. Ngufor, H.K. Kimbi, B.O. Oben, Traditional use of mosquito repellent to protect human against mosquito and other insect bites in rural community of Cameroon, East Afr. Med. J. 83 (10) (2006) 553–558.
[12] M.E. Duru, A. Cakir, S. Kordali, H. Zengin, M. Harmandar, S. Izumi, T. Hirata, Chemical composition and antifungal properties of essential oils of three *Pistacia* species, Fitoterapia 74 (2003) 170–176.
[13] L.A. Mitscher, R.P. Leu, M.S. Bathala, W.N. Wu, J.L. Beal, R. White, Antimicrobial agents from higher plants, introduction, rationale and methodology, Lloydia 35 (1972) 157–166.
[14] W. Leelasuphakul, P. Hemmanee, S. Chuenchitt, Growth inhibitory properties of *Bacillus subtilis* strains and their metabolites against the green mold pathogen (*Penicillium digitatum* Sacc.) of citrus fruit, Postharvest Biol. Technol. 48 (2008) 113–121.
[15] S.E. Lee, B.S. Park, M.K. Kim, W.S. Choi, H.T. Kim, K.Y. Cho, S.G. Lee, H.S. Lee, Antifungal activity of pipernonaline, a piperidine alkaloid derived from long pepper, *Piper longum* L., against phytopathogenic fungi, Crop Prot. 20 (2001) 523–528.
[16] S.M. Al-Reza, A. Rahman, S.C. Kang, Chemical composition and inhibitory effect of essential oil and organic extracts of *Cestrum nocturnum* L. on food-borne pathogens, Int. J. Food Sci. Technol. 44 (2009) 1176–1182.
[17] A. Cakir, S. Kordali, H. Zengin, S. Izumi, T. Hirata, Composition and antifungal activity of essential oils isolated from *Hypericum hyssopifolium* and *Hypericum heterophyllum*, Flav. Frag. J. 19 (2004) 62–68.
[18] J.K. Yoo, K.H. Ryu, J.H. Kwon, Y.J. Ahn, Antifungal activities of oriental medicinal plant extracts against phytopathogenic fungi, Kr. J. Agric. Chem. Biotechnol. 41 (1998) 600–604.
[19] V.K. Bajpai, H.R. Kim, C.T. Hou, S.C. Kang, Microbial conversion and *in vitro* and *in vivo* antifungal assessment of bioconverted docosahexaenoic acid (bDHA) used against agricultural plant pathogenic fungi, J. Ind. Microbiol. Biotechnol. 36 (2009) 695–704.
[20] P.P. Alvarez-Castellanos, C.D. Bishop, M.J. Pascual-Villalobos, Antifungal activity of the essential oil of flowerheads of garland chrysanthemum

(*Chrysanthemum coronarium*) against agricultural pathogens, Phytochemistry 57 (2001) 99–102.
[21] G. Singh, O.P. Singh, S. Maurya, Chemical and biocidal investigations on essential oils of some Indian *Curcuma* species, Prog. Crystal Growth Charact. 45 (2002) 75–81.
[22] F.S. El-Sakhawy, M.E. El-Tantawy, S.A. Ross, M.A. El-Sohly, Composition and antimicrobial activity of the essential oil of *Murraya exotica* L., Flav. Frag. J. 13 (1998) 59–62.
[23] G. Singh, P. Marimuthu, C.S. DE Heluani, C. Catalan, Antimicrobial and antioxidant potentials of essential oil and acetone extract of *Myristica fragrans* Houtt. (aril part), J. Food Sci. 70 (2) (2005) 141–148.
[24] N.U. Karabay-Yavasoglu, A. Sukatar, G. Ozdemir, Z. Horzum, Antimicrobial activity of volatile components and various extracts of the red alga *Jania rubens*, Phytol. Res. 21 (2007) 153–156.
[25] M. Omidbeygi, M. Barzegar, Z. Hamidi, N.H. Hassanali, Antifungal activity of thyme, summer savory and clove essential oils against *Aspergillus flavus* in liquid medium and tomato paste, Food Control 18 (2007) 1518–1523.
[26] M. Marino, C. Bersani, G. Comi, Impedance measurements to study the antimicrobial activity of essential oils from *Lamiaceae* and *Compositae*, Int. J. Food Microbiol. 67 (2001) 187–195.