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CONTROL OF GARDENIA LEAF SPOT AND BUD ROT DISEASES USING SOME NATURAL PLANT OILS.

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ABSTRACT

This study was conducted to through light on the most important fungi affected gardenia (*Gardenia jasmenoides* Ellis) plant with leaf spot and bud rots diseases and the effect of some plant essential oils as safe management against these fungi *in vitro* and *in vivo*. Isolation trials from infected gardenia plant tacked from Giza governorate during 2005-2006 growing season revealed eleven fungal species related to eleven genera. The isolates were differed in there frequency depending on the infected plant part and the isolation periods, *Botrytis cinerea*, *Alternaria alternata*, *Pestalotia langloissii* and *Cladosporium* sp. Were the most dominant fungi. These four isolates were differed in there pathogenic capabilities depending on the infected plant part, *B. cinerea* was exhibited the highest percentage of rotted buds while *A. alternata* and *P. langloissii* were only infected the leaves. *A. alternata* was exhibited the highest disease severity. Among twenty plant essential oils tested *in vitro*, Cumin (*Cuminum cyminum*) oil was the most effective one, completely inhibited the mycelial growth of the tested fungi at 500 ppm. concentration or more, while Anise (*Pimpinella anisum*), Peppermint *(Mentha piperita*), Thyme (*Thymus vulgaris*), Clove (*Syzigium aromaticum*) and French basil (*Ocimum basilicum*) oils at concentrations ranging between 750 and 1000 ppm depending on the fungal species occupied the second position in this respect. *In vivo* studies with cumin oil individually or in mixture with anise or clove oils were showed the best treatment under artificial inoculation than under natural infection in reducing the disease incidence. Generally spraying gardenia plant by cumin oil at 2500 ppm. mixed with clove oil at 5000 ppm. concentration was the best treatment that significantly decreased the disease incidence under greenhouse conditions.

INTRODUCTION

Production of ornamental plants is a considerable sector of the economical agricultural income. Nowadays, the indoor plants became necessary to overcome serious problems of air and environmental pollution, particularly in the closed place or small apartments.

The Gardenia genus is considered one of the most important cutting flower plants which includes over 200 species, with the most important ones *Gardenia jasminoides* Ellis (native to china) and *Gardenia thubergia* L.F (White gardenia, native to South Affrica). This genus is belonging to Rubiaceae (Coffee) family.

The richly scented *Gardenia jasminoides* Ellis is suffering from several diseases such as leaf spot (Barrett and Hardman, 1947; Shoemaker and Straby; 1965; Gupta and Prased, 1983; kamal *et al.*, 1983; Ciccaron, 1985; Cappelli, 1996; Dreistadt, 2001; Kobayashi *et al.*, 2003; Zheng-shiweii and Lao-Chong, 2004 and Hilal, 2004), Flower blights and bud rots (Dimock, 1940; Mullen and Jacobi, 2001; Pal *et al.*, 1983; Dreistadt, 2001 and Hilal, 2004) and stem canker (Calvino, 1939; Ghillini, 1940; Mc-Kenzie *et al.*, 1940; Barret and

Hardman, 1947, Verneau, 1949 Buddin and Wakefield, 1938 and Hansen and Barrett, 1938).

Gardenia leaf spot caused by *Alternaria alternata* and *Pestalotia langloissii* as well as bud rots caused by *Botrytis cinerea* become to be serious on *G. jasminoides* under Egyptian greenhouses, Particularly in moist conditions where the disease can lead to heavy defoliation despite the excessive and indiscriminate use of synthetic fungicides.

The excessive and indiscriminate use of synthetic fungicides are cause many hazards to humans and animals due to their possible carcinogenicity, teratogenicity, high and acute toxicity, long degradation periods and environmental pollution (Lingk, 1991), Also, spraying these materials on the foliages of gardenia plant results in a decrease in plant quality because of its deposits on the leaves. So, the exploitation of natural substances such as plant essential oils is urgently needed as alternatives to these synthetic fungicides (Daferera *et al.*, 2003), as they are easily decomposable, not environmental pollutants and posses no residual or phytotoxic properties (Tewari, 1990; Badei *et al.*, 1996; Bishop and Thornton, 1997 and Tripathi *et al.*, 2002).

Essential oils are volatile, natural and complex compounds characterized by a strong odour and are formed by aromatic plants as secondary metabolites. Production of these oils by plants is believed to be predominant a defense mechanism against pathogens (Oxenham, 2003) and indeed, they have been shown to possess antifungal properties both *in vitro* and *in vivo* (Wilson *et al.*, 1997 and Bhaskara Reddy *et al.*, 1998). The complexity plant essential oils relates to their highly contents from natural components that ranging between 20 and 60 components at quite different concentrations. Among these large numbers of components there are two to three components called the major components are find at fairly high concentrations 20-70 % compared to others components present in trace amounts (Bakkali *et al.*, 2008). Although the major components are reflect quite well the biophysical and biochemical features of the essential oils (Ipek *et al.*, 2005) it is difficult to correlate the fungitoxic activity to single compound or class of compounds (Begamboula *et al*., 2004), where the synergistic or antagonistic effect of one compound in minor percentage in the mixture must be considered, as each of the essential oil components has its own contribution on biological activity of the oil (Daferera *et al.*, 2003). Generally, inhibition of fungal growth by essential oils often involves induction of changes in cell wall composition (Ghfir *et al.*, 1997), plasma membrane disruption, mitochondrial structure disorganization (de Billerbeck *et al.*, 2001), and interference with enzymatic reactions of the mitochondrial membrane, such as respiratory electron transport, proton transport and coupled phosphoration steps (Knobloch *et al.*, 1989).

Plants have evolved physiological and biochemical mechanisms, including increases in the activities of oxidative and reductive enzymes associated with a biotic and biotic factors (Melo *et al.*, 2006). This response has been observed by several investigators related to plant defense (Farmer, 2001) and constitutes an evolutionary strategy of plants for defending themselves against pathogens.

The objective of this study is to through light on the most important fungi affecting gardenia (*Gardenia jasminoides* Ellis) plants causing leaf spot and bud rot diseases and the effect of some plant essential oils as safe management against these fungi *in vitro* and *in vivo*.

MATERIALS AND METHODS

1-Isolation, purification and identification of the associated fungi.

Gardenia plants (*Gardenia jasminoides* Ellis) grown under greenhouse conditions in commercial nurseries located at Giza governorate were suffered from leaf spot and bud rots diseases all over the season. During February, April, July and November of 2005-2006 growing season, diseased plant were selected and transferred in their pots to the Lab of Plant Pathology Dept. Fac. Agric. Cairo University.

Isolation trials were carried out during the previously mentioned periods on potato dextrose agar medium (PDA). Spots with different sizes and colours were carefully cut using sterilized forceps. The fragments were surface sterilized using 1% sodium hypochlorite. Under aseptically conditions, fragments were transferred to place onto the surfaces of sterilized PDA medium in Petri-dishes (9 cm. in diam.). Petri-dishes were then incubated at 23° ±1°C for 3 days. The emerged fungi were picked up and subcultured onto fresh PDA medium. Fungi were purified using hyphal tip or single spore technique adopted by Dhingra and Sinclair (1985). Purified fungi were identified according to their morphological characters using the keys given by Ellis, (1971); Raper and Fennel, (1977) and O'Donnell, (1979).

Occurrence and frequency of fungi isolated at the end of the incubation period were determined according to the following formula:

$$\%X = N/T \times 100$$

Where:

% X = frequency of the fungus.
N = Number of colonies for the fungus.
T = Total number of fungal colonies for the isolated fungi.

2- Pathogenicity testes:

Pathogenicity testes were conducted for the most dominant fungi isolates namely, *Botrytis cinerea, Alternaria alternata, Pestalotia langloissii* and *Cladosporium* sp. Each isolate was separately grown on PDA medium at 23° ±1°C for 7 days. A spore suspension was prepared by adding 10 ml. of distilled water to each plate and tapering the spores using a camel hair brush. The spore concentration was adjusted to $5x10^3$ conidia/ml. using a haemicitomiter. Under greenhouse conditions, where the degree of temperature was 25º±2 º C and relative humidity (RH) was 65%, healthy gardenia plants (6-month old) grown in pots 20 cm in diameter containing autoclaved peatmos were used in this investigation. Before artificial inoculation, a drop of Tween 20 was added to the spore suspension as a wetting agent. Spore suspension of any of the four tested fungi was sprayed on both leaf surfaces and buds of the plant with an atomizer and each plant received 20 ml of spore suspension. Treated plants were covered with polyethylene bags to maintain high relative humidity for 24h then removed. Control plants, were similarly treated only by sterile

distilled water mixed with a drop of Tween 20. Three plants were used for each particular treatment. Plants were observed daily for three weeks following inoculation looking for leaf spot and bud rot symptoms.

Rotted buds on each treated plant were determined after 7 days following inoculation period as percentage of rotted buds to the healthy one. Whereas, for leaf spot determination, disease index was measured within three weeks following inoculation. Areas of visible symptoms were scored for disease index on a scale of 4 points as follows:

0 = no symptoms.
1 = few scattered lesions covering about 1-10% of the leaf.
2 = spots covering about 11-25% of the leaf.
3 = spots coalescing and covering about 26-50% of the leaf.

Disease index was converted according to the equation suggested by Baudion (1988) as follows:

$$\text{Disease index \%} = \Sigma\, n/N \times 100$$

Where:

(n) Is the number of leaves in each numerical grade

(r) and (N) is the total number of inoculated leaves multiplied by the maximum numerical grade (4).

3- Plant oils treatments:

A- Source of plant oils.

Several essential oils were tested for their antagonistic effects against the three tested fungi i.e., *B. cinerea, A. alternata, and P. langloissii* the causal agents of leaf spot and bud rots of gardenia plant either *in vitro* and *in vivo*. The tested oils were from clove (*Syzigium aromaticum* L.), anise (*Pimpinella anisum*), peppermint (*Mentha piperita*), cumin (*Cuminum cyminum*), coriander (*Coriandrum sativum*), French basil (*Ocimum basilicum*), local basil (*Ocimum kilimandscharium*), caraway (*Carum carvi*), thyme (*Thymus vulgaris*), fennel (*Foeniculum vulgare*), Egyptian geranium (*Pelargonium graveolens*), sage (*Salvia officinalis*), rosemary (*Rosmarinus officinalis*), chamomile (*Ormenis mixta*), parsley (*Petroselinum crispum*), marjoram (*Origanum vulgare*), dill (*Anethum graveolens*), celery (*Apium graveolens*), eucalyptus (*Eucalyptus citriodora*) and tagets (*Tagetes patula*). These plant oils were obtained from Medicinal and Aromatic Pl. Res. St. El-Quanter El-Khairiah, Qulubyiah governorate, Hort. Res. Inst., Agric. Res. Center (ARC), Giza, Egypt.

B- *In vitro* experiments:

1- *In vitro* effect of the individual plant oils on the linear growth of the tested fungi:

The minimum inhibitory concentration of the tested plant oils against the three tested fungi was conducted using a poisoned plate technique or the agar dilution method described by Gulluce *et al.* (2003). The oils were added in a separated mean to sterile melted PDA medium containing drop of Tween 20 to produce the concentrations 500, 750, 1000, 1500 and 3000 ppm. The resulting PDA solutions were immediately poured into sterilized Petri-dishes (9 cm. in diam.) at the rate of 20 ml/plate. Dishes were inoculated at the center with 4 mm mycelial disc cut from the periphery of 7- day-old culture of any of the three tested fungi. The inoculated plates were incubated at the optimum

temperature for each fungus, i.e. 20°C for *B. cineria* and 25°C for *A. alternata* and *P. langloissii*. Three replicate dishes were used for each treatment. Plant oil free PDA medium with drop of Tween 20 was used as control. The diameter of the developed colonies was measured when the mycelial growth of the fungus covered the plates of check treatment. The inhibition in mycelial growth rate was calculated according to formula suggested by Deans and Svoboda (1990) as follows:

$$I=C-T/C \times 100$$

Where:

(I) is the inhibition percentage of mycelial growth, (C) is the mean colony diameter (mm) of the control set and (T) is the mean colony diameter of treatment sets.

2-Effect of mixing cumin, clove, thyme, peppermint and anise oils on the growth of the tested fungi:

Five of the most effective plant oils were selected to study their toxicity on the tested fungi when mixed together*, i.e*. cumin (*Cuminum cyminum*), thyme (*Thymus vulgaris*), anise(*Pimpinella anisum*), peppermint (*Mentha piperita*), and clove (*Syzigium aromaticum* L.) oils, where the mixture was conducted between each two oils at the rate of 1:1 (500:500 ppm.), 1:2 (250:500 ppm.) and 2:1 (500:250 ppm) concentrations. The toxicity of each mixture was tested by the method mentioned above and described by Gulluce *et al.* (2003). Three replicates were used for each treatment. Plant oil free PDA medium with a drop of Tween 20 was used as control. Also, mycelial parts of fungi which could not grow during the assay were transferred into sterile essential oil-free PDA media and then observed for a week to determine the fungicidal or fungistatic effect for each mixture of plant oils. The days needed for mycelium reactivation for each fungus were also calculated.

3- Effect of cumin oil individually or in mixture with anise or clove oil on percentage of mycelial growth and germinated sclerotia of *Botrytis cinerea*.

Sclerotia of *Botrytis cinerea* the causal organism of gardenia bud rot disease were dipped in cumin oil at 500 ppm. as individual treatment and on the mixure of cumin and anise or clove oils at the rate of 1:2 (i.e. 250:500 ppm.) concentrations for 15 minutes. Sclerotia were removed with the help of sterilized fine forceps, placed on sterilized blotting paper to remove access of water and each sclerotium placed at the center of PDA medium. For control, sclerotia were only dipped in sterilized water then placed on PDA medium. Petri-dishes were incubated at the optimum temperature (20°C) for the fungus. Three replicates were used for each treatment. The averages of the linear growth in mm were calculated when the mycelium reached its maximum growth in the check treatment.

On the other hand, the sclerotia were centrifuged with the three oils at the same concentrations mentioned above as described by Zewani *et.al.*, (2004) at 1000 rpm for 15 min. and decanted to remove mycelial fragments. Sclerotia were removed with the help of sterilized fine forceps, placed on sterilized blotting paper to remove access of water and then placed on PDA medium. For control, sclerotia were centrifuged at 1000 rpm for 15 min. with sterilized distilled water only. Five sclerotia were kept for each treatment and

replicated three times. Percentages of germinated sclerotia were calculated 24h following incubation at the optimum temperature *i.e.* 20°C.

C-*In vivo* experiments:

1-Effect of cumin oil individually or in mixture with anise or clove oils on controlling bud rot caused by *B. cinerea* and incidence of leaf spot disease caused by *A. alternata* and *P. langloissii*.

Healthy Gardenia plants (6-month old) grown in sterilized pots (25cm in diam) containing peatmos were used to study the effect of cumin oil individually and mixurally with anise or clove oil on controlling the infection caused by the three tested fungi under greenhouse conditions. The tested oils were diluted to 5000 ppm for cumin oil treatment and 2500:5000 ppm for the mixtures, where the concentration of cumin oil in the mixture was 2500 ppm, meanwhile both clove and anise oils were mixed by 5000 ppm for each. The tested oils sprayed onto the upper leaf surfaces to run-off using an atomizer 24h before inoculating the plants with a spore suspension of each fungus ($5.0x10^3$ conidia/ml).Control treatment consisted of sterilized distilled water containing 0.5 % Tween 20. The percentage of rotted buds was assessed 8 days following the inoculation. Meanwhile, the disease index of leaf spot symptoms was assessed 3 weeks following inoculation. Three pots were used for each treatment.

2-Effect of cumin oil individually and in a mixture with anise or clove oil on controlling bud rot and leaf spot diseases under natural infection under greenhouse conditions.

To study the effect of cumin oil individually or in mixture with anise or clove oil under greenhouse conditions, healthy Gardenia plants (6-month old) grown in sterilized pots (25cm in diam.) containing peatmos were divided into three groups. At the beginning of March ,2007 the first group sprayed by cumin oil at 5000 ppm concentration, the second group sprayed by a mixture of cumin oil at 2500 ppm and anise oil at 5000 ppm concentration and the third group sprayed by a mixture of cumin oil at 2500 ppm and clove oil at 5000 ppm concentration. The plants were sprayed randomly by atomizer each week through 2007 season. Three replicate pots were used for each treatment. Control treatment consisted of healthy plants sprayed with sterilized distilled water containing 0.5 % Tween 20. The rotted buds were observed at May 2007 and the percentage of rotted buds was calculated as mentioned under pathogenicity test. The resulting leaf spots symptoms were observed at September 2007 and the disease index was calculated as mentioned before.

RESULT AND DISCUSSION

1-Isolation, purification and identification of the associated fungi.

In the present study (Table 1) Isolation trails, from naturally infected gardenia plant by leaf spot and bud rot symptoms collected from greenhouses located at Giza governorate resulted in the presence of eleven fungal species belonged to eleven fungal genera. These fungi were identified as *Botrytis cinerea, Alternaria alternata, Pestalotia langloissii, Cladosporium* sp., *Stemphylium* sp*., Phomopsis* sp*., Myrothecium roridum, Coniotherium* sp*., Fusarium oxysporum., Botryodiplodia* sp. and *Trichoderma viride*. These

isolates were differed in their occurrence and frequencies according to the infected plant part and the seasonal isolation periods. Generally, *B. cinerea, A. alternata, P.langloissii and C*. sp. were the most prevailing fungi. *B. cinerea* was isolated only from the rotted buds formed in spring season at the rate of 77%, where it was recorded the highest frequency of occurrence during this period. *A. alternata*, *P.langloissii* and *C*. sp. were isolated from both infected leaves and buds through all the isolation periods except for *A. alternata* which not isolated in spring season. The heights frequency of *C*. sp. was recorded in winter season at the rate of 50% and 75% from infected leaves and buds, respectively followed by spring season by 57.1% and 15.4% from leaves and buds, respectively. Meanwhile, the highest frequency of *A. alternata* and *P. langloissii* were recorded in summer season. The corresponding percentages were 40% and 15.4% for *A. alternata* and 30% and 30.8% for *P. langloissii* from infected leaves and buds, respectively.

Table 1: Occurrence and frequency of fungi isolated from gardenia plants suffering from leaf spot and bud rot diseases through 2005-2006 growing season.

Isolated fungi	% frequency / season							
	Spring		Summer		Autumn		Winter	
	Leaves	Buds	Leaves	Buds	Leaves	Buds	Leaves	Buds
Botrytis cinerea	00.0	77.0	00.0	00.0	00.0	00.0	00.0	00.0
Alternaria alternata	00.0	00.0	40.0	15.4	20.0	00.0	16.7	00.0
Pestalotia langloissii	14.6	00.0	30.0	30.8	20.0	00.0	16.7	25.0
***Cladosporium* sp**	57.1	15.4	00.0	23.2	35.0	100.0	50.1	75.0
Myrothecium roridum	14.3	00.0	00.0	00.0	00.0	00.0	16.7	00.0
***Coniotherium* sp**	00.0	00.0	10.0	00.0	20.0	00.0	00.0	00.0
***Stemphelium* sp**	14.0	00.0	00.0	00.0	00.0	00.0	00.0	00.0
Fusarium oxysporum	00.0	00.0	15.0	00.0	05.0	00.0	00.0	00.0
***Botryodiplodia* sp**	00.0	7.6	00.0	00.0	00.0	00.0	00.0	00.0
***Phomopsis* sp**	00.0	00.0	05.0	00.0	00.0	00.0	00.0	00.0
Trichoderma viridae	00.0	00.0	00.0	30.8	00.0	00.0	00.0	00.0

According to the available literature, the recorded causal organisms of leaf spot and/or bud rot diseases of *Gardenia jasminoides* Ellis in Egypt were *Fusarium solani, Nigrospora* sp*., Rhizoctonia solani* on the flowers and *Myrothecium roridum* on the leaves (Hilal, 2004), but in other countries Numerous investigators have shown that leaf spot and bud rots diseases of *Gardenia jasmenoides* Ellis are caused by different fungi such as *Botrytis primer* (Mullen and Jacobi, 2001); *Botrytis cinerea* (Dimock, 1940 and Dreistadt, 2001); *Alternaria alternata* (Gupta and Prasad, 1983); *Pestalotia langloissii* (Shoemaker and Straby, 1965); *Pestalotia* spp. (Gonsalves and Ferreira., 2002); *Myrothecium roridum* (Barrett and Hardman, 1947; Cappelli, 1996 and Dreistadt, 2001); *Rhizoctonia* spp. (Dreistadt, 2001); *Septoria gardeniae* (Kobayashi *et al.*, 2003); *Cercosporidium okinawaense* (Kobayash

et al., 2002); *Phyllosticta gardiniicola* (Zheng and Lao, 2004 and Kamal *et al.*, 1938); *Phyllosticta* sp. (Dreistadt, 2001); *Mycosphaerella luzonensis* (Kobayashi *et al.*, 1988 and *Colletotrichum gloeosporides* (Ciccaron, 1985; and Zheng-Shiweii and Lao-Chong, 2004). This higher numbers of the recorded fungi that associating with gardenia plant may be attributed to the physiobiochemical processes in the plant.

2- Pathogenicity testes:

Pathogenicity tests by The most prevailing fungi isolated from gardenia plants namely, *B. cinerea, A. alternate, P. langloissii* and *Cladosporium* sp., indicated that these isolates were differed in there pathogenic capabilities to leaves and buds of the plant (Table 2.) The pathogenic fungi were *Botrytis cinerea, Alternaria alternata and Pestalotia langloissii* but *Cladosporium* sp was non-pathogenic. *B. cinerea, A. alternata* and *P.langloissii* were specialized in their pathogenic capabilities depending on the infected plant part. *B. cinerea* was only pathogenic to the plant buds, where it exhibited the highest percentage of rotted buds, being 41, 7% . Meanwhile, *A. alternata* and *P. langloissii* were pathogenic to the leaves. *A. alternata* exhibited the highest disease index, being 47.8% while, *P. langloissii* was the lowest one in this respect, being 21.1%.

Table (2): Pathogenicity tests using *Botrytis cinerea*., *Alternaria alternata*, *Pestalotia langloissii* and *Cladosporium.* sp.

Fungi tested	% Disease index of Leaf spots	% infection by Bud rots
Botrytis cinerea	00.0	41.7
Alternaria alternata	47.8	00.0
Pestalotia langloissii	21.1	00.0
Cladosporium sp	00.0	00.0

3- Plant oils treatments:

***A- In vitro* experiments:**

1-*In vitro* effect of the individual plant oils on the linear growth of the tested fungi:

Data obtained (Tables 3,4 and 5) clear that as the concentration of each oil increase, linear growth of the tested fungi decreased, and this effect differed according to the oil type. Cumin oil was the most effective one, completely inhibited the mycelial growth of the tested fungi at 500 ppm concentration or more, while clove, anise, thyme, coriander, French basil and peppermint oils at concentrations ranged from 750 to 1500 ppm according to the fungus type occupied the second position in this respect. Where, clove, anise, thyme and peppermint oils at 750, 1000, 1500 and 1500 ppm concentrations, respectively completely inhibited the mycelial growth of *A. alternata*. anise, peppermint and thyme oils at 750 ppm concentration as well as clove, French basil and geranium oils both at 1000 ppm also, rosemary oil at 1500 ppm concentration completely inhibited the mycelial growth of *P. langloissii*. Meanwhile, anise, thyme, peppermint, clove, coriander and French basil oils at 1000 ppm concentration completely inhibited the mycelial growth of *B. cinerea*.

Table 3: Linear growth (mm) and percentage of reduction in mycelial growth of *B. cinerea* on PDA medium mixed with different concentrations of 20 plant essential oils and incubated at 20° C for 6 days.

Oils tested (O)	linear growth (mm) and (%) reduction in mycelium growth at (ppm) concentration of essential oils										
	500	Red%	750	% Red	1000	% Red	1500	% Red	3000	% Red	Mean
Cuminum cyminum	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	٠٠,٠
Pimpinella anisum	٢٤,٦	٧٢,٧	١٨,٠	٨٠,٠	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	٠٠,٠
Mentha piperita	١٣,٠	٨٥,٦	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	٠٢,٦
Thymus vulgaris	29.8	66.9	16.1	82.1	00.0	١٠٠,٠	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	09.2
Syzigium aromaticum	39.1	57.0	19.8	78.0	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	11.8
Coriander sativum	52.0	42.2	37.7	58.1	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	18.0
Ocimum basilicum	68.0	24.4	43.2	52.0	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	22.2
Carum carvi	45.8	49.1	27.3	69.7	19.0	79.1	14.3	84.1	٠٠,٠	١٠٠,٠	21.3
O.kilimandscharium	62.2	30.9	56.7	37.0	23.5	74.0	15.0	73.3	٠٠,٠	١٠٠,٠	31.5
Foeniculum vulgare	61.0	32.2	51.8	42.4	33.0	63.3	21.0	76.7	٠٠,٠	١٠٠,٠	33.4
Pelargonium graveolens	90.0	00.0	45.0	50.0	29.3	64.4	17.0	81.1	٠٠,٠	١٠٠,٠	36.3
Salvia officinalis	٩٠,٠	٠٠,٠	90.0	00.0	37.0	58.9	18.7	79.2	٠٠,٠	١٠٠,٠	47.1
Rosmarinus officinalis	٩٠,٠	٠٠,٠	٩٠,٠	٠٠,٠	74.0	17.8	61.0	32.2	٠٠,٠	١٠٠,٠	27.0
Origanum vulgare	٩٠,٠	٠٠,٠	٩٠,٠	٠٠,٠	43.7	51.4	33.0	63.3	٠٠,٠	١٠٠,٠	51.3
Petroselinum crispum	٩٠,٠	٠٠,٠	٩٠,٠	٠٠,٠	68.3	24.1	41.7	58.3	18.2	79.8	61.6
Anethum graveolens	٩٠,٠	٠٠,٠	٩٠,٠	٠٠,٠	40.3	55.2	26.8	70.2	13.0	85.0	52.0
Ormenis mixta	43.3	51.9	37.5	58.3	29.0	67.8	23.7	73.7	20.7	77.0	30.8
Apium graveolens	90.0	00.0	90.0	00.0	72.0	20.0	64.8	28.0	46.5	48.5	72.7
Eucalyptus citriodora	٩٠,٠	٠٠,٠	٩٠,٠	٠٠,٠	90.0	00.0	71.0	21.1	25.2	72.0	73.2
Tagets patula	٩٠,٠	٠٠,٠	٩٠,٠	٠٠,٠	٩٠,٠	٠٠,٠	53.3	٢١,١	40.8	28.3	68.6
(Check)	٩٠,٠										
Mean	٦٢,٦		٥٤,٥		٣٥,٢		٢٣,٩		١٢,٠		----

LSD at 0.05 for:

Oils tested (O): 0.3
Concentration (C): 0.2
O×C: 0.8

On the other hand, caraway, local basil, fennel, marjoram, rosemary and geranium oils at 3000 ppm completely inhibited the mycelial growth of the tested fungi. Meanwhile, celery, parsley, chamomile, eucalyptus, tagets oils

were the lowest activity. Where each of them only resulted in reduced of mycelial growth of the tested fungi till the highest tested concentration 3000 ppm. These results are in harmony with several workers. Maruzzella (1962) arranged the active parts of volatile oils according to the antimicrobial activities in the decreasing orders as follows: Aldehyde, Phenols, Alcohols, Ketons and Hydrocarbons. Jaspal and Tripathi (1999) mentioned that the pure essential oils completely inhibited the mycelial growth of many pathogenic fungi, and the fungal sensitivity to the previous essential oils differed in their effect from one fungus to another, this might be due to the capability of essential oils to penetrate into the fungal cells. According to Aligianise *et al.* (2001), the activity of the plant essential oils was divided depending on the minimum inhibitory concentration (MIC) to three divisions i.e. strong (MIC more than 500 ppm), Moderate (MIC from 600 ppm to 1600 ppm) and weak (MIC more than 1600 ppm). So, the results obtained from the present study of cumin oil on the fungi tested lead to group the oil into a strong effect category, where it is completely inhibited the mycelial growth of the tested fungi at 500 ppm concentration The inhibitory effect of cumin oil might be attributed to the presence of the cuminaldehyde. Anise, peppermint, clove, coriander, thyme and French basil oils were showed a moderate affect. Where, they completely inhibited the mycelial growth of the fungi tested at concentrations ranging from 750 to 1500 ppm. ,except for coriander oil which showed a week effect only on *A. alternata* where it is completely inhibited the mycelial growth of the fungus at 3000 ppm concentration., This might be due to the presence of the great amount of phenolic and alcohols substances like thymol in thyme oil, eugenol in clove oil, menthol and carvacrol besides menthyl acetate ester in peppermint oil, chavicol beside linalool alchohol, methyl chavicol and camphen compound in French basil oil (Mohamed *et. al*., 2003; El-Baroty, 1988 and Moussa, 1998). The other tested oils i.e. caraway, local basil, fennel, marjoram, rosemary, geranium, celery, parsley, chamomile, eucalyptus, tagets oils were the lowest activity. Where each of them only resulted in a reduction of the mycelial growth of the tested fungi till the highest tested concentration 3000 ppm., except for rosemary and geranium oils which showed a moderate affect only on *P. langloissii* where they completely inhibited the mycelial growth of the fungus at 1500 and 1000 ppm concentration respectively. This week activity of the previously mentioned oils might be due to their poorness in phenolic compounds for example local basil contains the same compounds in French basil but in small quantities also, marjoram oil is very poor in its phenolic compounds except cineol compound which act as antifungal agent in a great amounts reaching to 30.1% but it was poor in other phenolic compounds (El-Baroty, 1988; Moussa, 1998; Bhaskara Reddy *et al.*, 1998 and Arras and Usai, 2001).The MIC and toxicity concentrations of the essential oils varied from study to study and this is probably due to the different methods of extraction of the essential oils and different sensitivity of the tested fungi (Saikaia *et al.*, 2001).

Table 4: Linear growth (mm) and percentage of reduction in mycelialgrowth of *A. alternata* on PDA medium mixed with different concentrations of 20 plant essential oils and incubated at 25° C for 10 days.

Oils tested (O)	linear growth (mm) and (%) reduction in mycelium growth at (ppm) concentration of essential oils										
	500	Red%	750	% Red	1000	% Red	1500	% Red	3000	% Red	Mean
Cuminum cyminum	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	٠٠,٠
Pimpinella anisum	٤٥,٠	٥٠,٠	٢٠,٥	٧٧,٢	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	١٣,١
Mentha piperita	32.5	63.9	27.8	69.1	24.5	72.8	00.0	100.0	00.0	100.0	١٧,٠
Thymus vulgaris	36.2	59.8	60.5	32.8	73.3	18.6	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	٠٧,٠
Syzigium aromaticum	34.7	61.4	00.0	100.0	00.0	100.0	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	٣٤,٠
Coriander sativum	68.0	24.4	66.3	26.3	33.2	63.1	12.6	86.0	٠٠,٠	١٠٠,٠	36.0
Ocimum basilicum	37.0	18.9	65.0	27.8	56.7	37.0	00.0	100.0	٠٠,٠	١٠٠,٠	٤٠,٠
Carum carvi	72.7	19.2	67.8	24.7	59.2	34.2	37.4	58.4	٠٠,٠	١٠٠,٠	٣٧,٠
O.kilimandscharium	60.7	32.6	55.8	38.0	43.7	51.4	23.0	74.4	٠٠,٠	١٠٠,٠	٤٠,٠
Foeniculum vulgare	79.5	11.7	62.8	30.2	56.2	37.6	٢٣,٠	72.1	٠٠,٠	١٠٠,٠	٥٠,٠
Pelargonium graveolens	90.0	00.0	61.7	31.4	52.8	41.3	44.0	51.1	٠٠,٠	١٠٠,٠	٧٥,٠
Salvia officinalis	٩٠,٠	٠٠,٠	90.0	00.0	73.8	18.0	66.0	26.7	54.7	39.2	74.0
Rosmarinus officinalis	٩٠,٠	٠٠,٠	84.2	06.4	74.5	17.2	61.0	32.2	53.0	42.2	58.3
Origanum vulgare	٩٠,٠	٠٠,٠	90.0	00.0	60.7	32.6	50.8	43.6	00.0	100.0	43.0
Petroselinum crispum	67.0	25.6	54.0	40.0	43.5	51.7	28.0	68.9	20.8	76.9	39.4
Anethum graveolens	65.2	27.6	63.2	29.8	59.3	34.1	42.0	53.3	22.8	74.7	51.0
Ormenis mixta	56.2	37.6	42.3	53.0	37.0	58.9	33.0	63.3	28.3	73.6	59.0
Apium graveolens	77.3	14.1	68.8	23.6	67.0	25.6	53.2	40.9	27.8	69.1	80.0
Eucalyptus citriodora	90.0	00.0	90.0	00.0	90.0	00.0	71.2	20.9	57.7	35.9	50.0
Tagets patula	57.7	36.0	57.2	36.4	55.8	38.0	43.8	51.3	34.5	61.7	34.0
(Check)	٩٠,٠										
Mean	61.0		٦٠,٠		٥٠,١		34.1		١٩,٠		-----

LSD at 0.05 for:

Oils tested (O):	**0.2**
Concentration (C):	**0.1**
O×C:	**0.8**

Table 5: Linear growth (mm) and percentage of reduction in mycelial growth of *P.langloissii* on PDA medium mixed with different concentrations of 20 plant essential oils and incubated at 20° C for 6 days.

Oils tested (O)	linear growth (mm) and (%) reduction in mycelium growth at (ppm) concentration of essential oils										
	500	Red%	750	% Red	1000	% Red	1500	% Red	3000	% Red	Mean
Cuminum cyminum	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	٠٠,٠
Pimpinella anisum	٤٣,٨	٥١,٣	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	٠٩,٠
Mentha piperita	٤٥,٧	٤٩,٢	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	٠٩,١
Thymus vulgaris	١٦,٣	٨٢,٠	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	٠٣,٣
Syzigium aromaticum	٣٦,٣	٦٠,٠	٣١,٥	٦٥,٠	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	١٣,٦
Coriander sativum	١٩,٨	٧٨,٠	١١,٣	٨٧,٤	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	٠٠,٠
Ocimum basilicum	٦٥,٥	٢٧,٢	٥٥,٣	٣٨,٦	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	٢٤,٢
Carum carvi	٦٣,٠	٣٠,٠	٥٢,٥	٤١,٧	٢٨,٠	٦٨,٩	١٦,٤	٨١,٨	٠٠,٠	١٠٠,٠	29.0
O.kilimandscharium	٦٣,٠	٣٠,٠	٤٨,٧	٤٥,٩	٣٩,٦	٥٦,٠	٢٣,٠	٧٤,٤	٠٠,٠	١٠٠,٠	٢٢,٣
Foeniculum vulgare	٦٥,٨	٢٦,٩	٥٣,٥	٤٠,٦	٤٣,٧	٥١,٤	٢٧,٠	٧٠,٠	٠٠,٠	١٠٠,٠	33.0
Pelargonium graveolens	٥٦,٣	٣٧,٤	٥١,٢	٤٣,١	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	٢٢,٠
Salvia officinalis	٩٠,٠	٠٠,٠	٦٩,٧	٢٢,٣	٥٣,٥	٤٠,٦	١٧,٠	٨١,١	٠٠,٠	١٠٠,٠	٣١,٠
Rosmarinus officinalis	٧١,٠	٢١,١	٦٧,٣	٢٥,٢	٦٦,٠	٢٦,٧	٠٠,٠	١٠٠,٠	٠٠,٠	١٠٠,٠	١٧,٠
Origanum vulgare	٥٩,٥	٣٣,٩	٤٨,٧	٤٥,٩	٤٦,٧	٤٨,١	٣٦,٤	٥٩,٦	٠٠,٠	١٠٠,٠	٢٦,٠
Petroselinum crispum	٢١,٣	٧٦,٣	١٩,٢	٧٩,٠	١٧,٠	٨١,١	١٤,٧	٨٣,٧	١١,٠	٨٧,٨	٢٤,٠
Anethum graveolens	٥١,٠	٤٣,٣	٣١,٣	٦٥,٢	٢٧,٧	٦٩,٢	١٦,٢	٨٢,٠	٠٧,٨	٩١,٣	٣٨,٤
Ormenis mixta	٢٩,٣	٦٧,٤	٢٥,٠	٧٢,٢	٢٣,٣	٧٤,١	٢١,٣	٧٦,٣	١٩,٠	٧٨,٩	٧٢,٠
Apium graveolens	٦٥,٢	٢٧,٦	٤٩,٠	٤٦,٠	٥٥,٢	٦١,٠	٢٣,٥	٧٣,٩	١٩,٢	٧٨,٧	٧٠,٠
Eucalyptus citriodora	٩٠,٠	٠٠,٠	٩٠,٠	٠٠,٠	٧٤,٠	١٧,٨	٦٣,٨	٢٩,١	٤٢,٠	٥٣,٣	٤١,٠
Tagets patula	٩٠,٠	٠٠,٠	٩٠,٠	٠٠,٠	٦١,٧	٣١,٤	٥٦,٠	٣٧,٨	٤٦,٨	٤٨,٠	٧٠,٣
(Check)	٩٠,٠										
Mean	٥٣,٠		٤٢,٠		٢٧,٠		١٤,٤		١١,٢		----

LSD at 0.05 for:

Oils tested (O):	**0.2**
Concentration (C):	**0.1**
O×C:	**0.5**

2 -Effect of mixing cumin, clove, thyme, peppermint and anise oils on the growth of the tested fungi:

The mixture between the tested oils completely inhibited the mycelium growth of the tested fungi except for the mixture of (anise & thyme), (clove & thyme), (clove & peppermint) and (thyme & peppermint) where they only resulted in the reduction in mycelial growth of *A. alternata* from 90 mm in the absence of oil (check) to 85.6; 77.8; 71.1 and 79.4 mm, respectively. (Table 6). Boyraz and Ozcan (2006), mentioned that combinations of hydrosol, oleoresin, ground material and essential oils may provide an efficacious mixture for the inactivation of pathogenic and spoilage microorganisms in plant and foods.

Table (6): Effect of the tested plant oils mixtures on the linear growth of the tested fungi.

Oils tested (O)	Fungi						
	B. cinerea		*A. alternata*		*P. langloissii*		Mean
	Linear growth (mm)	% Red.	Linear growth (mm)	% Red.	Linear growth (mm)	% Red.	
Cuminum cyminum & Pimpinella anisum	00.0	100.0	00.0	100.0	00.0	100.0	00.0
Cuminum cyminum & Syzigium aromaticum	00.0	100.0	00.0	100.0	00.0	100.0	00.0
Cuminum cyminum & Thymus vulgaris	00.0	100.0	00.0	100.0	00.0	100.0	00.0
Cuminum cyminum & Mentha piperita	00.0	100.0	00.0	100.0	00.0	100.0	00.0
Pimpinella anisum & Syzigium aromaticum	00.0	100.0	00.0	100.0	00.0	100.0	00.0
Pimpinella anisum & Thymus vulgaris	00.0	100.0	13.0	85.6	00.0	100.0	04.3
Pimpinella anisum & Mentha piperita	00.0	100.0	00.0	100.0	00.0	100.0	00.0
Syzigium aromaticum & Thymus vulgaris	00.0	100.0	20.0	77.8	00.0	100.0	06.7
Syzigium aromaticum & Mentha piperita	00.0	100.0	26.0	71.1	00.0	100.0	08.7
Thymus vulgaris & Mentha piperita	00.0	100.0	18.5	79.4	00.0	100.0	06.2
Cuminum cyminum & Syzigium aromaticum	00.0	100.0	00.0	100.0	00.0	100.0	00.0
Cuminum cyminum & Thymus vulgaris	00.0	100.0	00.0	100.0	00.0	100.0	00.0
Cuminum cyminum & Pimpinella anisum	00.0	100.0	00.0	100.0	00.0	100.0	00.0
Mean	00.0	----	07.8	----	00.0	----	----

3- Fungicidal and fungistatic effects of the tested oils individually and in mixture on the tested fungi:

Cumin oil as individual treatment had a fungicidal effect on the tested fungi. Meanwhile, the other individual oils tested had a fungistatic effect and the days needed for mycelium reactivation ranged from 1 to 4 days according to the fungus type. (Table 7). On the other hand the mixture of cumin oil at 500 ppm with any of the tested oils at 500 ppm gave a fungicidal effect on the

tested fungi except for *A. alternata* where in case of the treatment with the mixture of (cumin & thyme) and (cumin &peppermint) a fungistatic effect was obtained and the mycelium reactivated through four days. this might be related to the differences in the ultrasruction of the fungus conidia .Jaspal and Tripathi (1999), mentioned that the pure essential oils completely inhibited the mycelial growth of many pathogenic fungi and the fungal sensitivity to the previous essential oils from one fungus to another, this also might be due to the capability of essential oils to penetrate into the fungal cell.

Table 7. Fungicidal and fungistatic effects of the tested oils as individual treatment at 500ppm on the growth of the fungi tested.

Plant oils treatments	fungicidal and fungistatic effect of the oils / days on mycelium reactivation					
	B. cinerea		*A. alternata*		*P. langloissii*	
Cuminum cyminum	+		+		+	
Pimpinella anisum	±	٣*	±	٢	±	٢
Mentha piperita	±	١	±	٣	±	٣
Thymus vulgare	±	٣	±	٢	±	٢
Syzigium aromaticum	±	٣	±	٣	±	٣
Coriandrum sativum	±	٤	±	٢	±	١
Ocimum basilicum	±	٤	±	٢	±	٢
Carum carvi	±	٢	±	٣	±	٣
O. kilimandscharium	±	٢	±	٢	±	٢
Foeniculum vulgare	±	٤	±	٣	±	٢
Pelargonium graveolens	±	٢	±	١	±	١
Salvia officinalis	±	١	—	١	±	١
Rosemarinus officinalis	±	٢	—	١	±	١
Origanum vulgare	±	١	—	١	—	١
Petroselinium crispum	—	١	—	١	—	١
Anethum graveolens	—	١	—	١	—	١
Ormenis mixta	—	١	—	١	—	١
Apium graveolens	—	١	—	١	—	١
Eucalyptus citriodora	—	١	—	١	—	١
Tagetes patula	—	١	—	١	—	١

(+) Fungicidal effect (±) Fungistatic effect (—) No effect
*** mycelium reactivation after 1-4 days**

On the other hand, reduced the concentration of cumin oil to 250 ppm and fixed the concentration of any of the tested oils in the mixture at 500 ppm had a fungicidal effect only in case of the mixture of (cumin & anise) and (cumin & clove) oils on both *B. cinerea* and *P. langloissii*. Meanwhile, cumin oil at 250 ppm mixed with clove oil at 500 ppm had a fungicidal effect on and *A. alternata*. The other mixtures had a fungistatic effect on the tested fungi. While, fixed the concentration of cumin oil at 500 ppm and reduced the concentration of any of the tested oils in the mixture to 250 ppm had a fungicidal effect on the tested fungi (Table 8). Anderson *et al.* (1994), mentioned that the fungicidal activity of some essential oils constitutes such as trans-2-hexanal and citral aldehydes may be ascribed to the high electrophilic properties of the carponyl group adjacent to the double bond that make these compounds particularly reactive with neucleophiles, such as protein sulfhydryl and amino groups of the pathogen. Feng and Zheng (2007) mentioned that essential oils inhibited the

growth of fungi either temporarily (fungistatic) or permanently (fungicidal). Kishnora *et al.* (2007) reported that most essential oils are fungistatic effect than fungicidal. In this study, the fungicidal effect was only recorded for the cumin oil which contains cuminaldehyde as the major component. Also, the mixtures containing this oil with any of anise or clove oil which both rich in their content from phenolic compounds. So, this might be explained the fungicidal effect of the tested oils on the fungi tested.

Table (8): Fungicidal and fungistatic effect of the most effective oils used by 250:500ppm and 500:250ppm concentrations on the tested fungi.

Plant oil treatment	*Cuminum cyminum* C2	*Pimpinella anisum* C2	*Mentha piperita* C2	*Thymus vulgaris* C2	*Syzigium aromaticum C2*
C1					
	Botrytis cinerea				
Cuminum cyminum	-------	+	+	±	+
Pimpinella anisum	+	-------	±	±	±
Mentha piperita	±	±	------	±	±
Thymus vulgaris	±	±	±	------	±
Syzigium aromaticum	±	±	±	±	-------
	Alternaria alternata				
Cuminum cyminum	-------	+	+	±	+
Pimpinella anisum	+	-------	±	±	±
Mentha piperita	±	±	------	±	±
Thymus vulgaris	±	±	±	------	±
Syzigium aromaticum	+	±	±	±	------
	Pestalotia langloissii				
Cuminum cyminum	+	+	±	±	+
Pimpinella anisum	+	-------	±	±	±
Mentha piperita	±	±	--------	±	±
Thymus vulgaris	±	±	±	-------	±
Syzigium aromaticum	+	±	±	±	---------

C1=500ppm C2=250ppm

(+) Fungicidal effect (±) Fungistatic effect (—) No effect

4- Effect of cumin oil individually or in mixture with anise or clove oil on percentage of mycelial growth and germinated sclerotia of *B. cinerea*.

Cumin oil at 250 ppm mixed with clove oil at 500 ppm concentration recorded the heighst percent in reduced the mycelial growth raised from sclerotia and its germination followed by cumin oil at 500 ppm concentration. Meanwhile, cumin oil at 250 ppm mixed with anise oil at 500 ppm concentration had the lowest one in this respect (Table 9and 10).

Table (9): Effect of individual treating PDA medium with cumin oil alone or in a mixture with anise or clove oil on the germination percentage of sclerotia of *B. cinerea*, the causal pathogen of gardenia bud rot disease.

Plant oil treatment	Conc. (ppm)	Germinated sclerotia (%)
Cuminum cyminum	500	66.7
Cuminum cyminum & ***Pimpinella anisum***	250 + 500	53.3
Cuminum cyminum & ***Syzigium aromaticum***	250 + 500	40.0
(Check)	--------	100.0
Mean		65.0
LSD at 0.05		2.7

Table (10): Effect of cumin oil individually or in a mixture with anise or clove oil on percentage of reduction in mycelial growth of sclerotia of *B. cinerea* the causal pathogen of gardenia bud rot disease.

Plant oil treatment	Con. (ppm)	Linear growth (mm)	*% Red.*
Cuminum cyminum	500	53.4	40.7
Cuminum cyminum & ***Pimpinella anisum***	250 + 500	68.3	24.1
Cuminum cyminum & ***Syzigium aromaticum***	250 + 500	34.0	62.2
(Check)	--------	٩٠	٠٠,٠
Mean	--------	31.8	---------
LSD at 0.05		1.6	

B-*In vivo* experiments:

1- Effect of cumin oil individually or in mixture with anise or clove oils on controlling bud rot caused by *B. cinerea* and incidence of leaf spot disease caused by *A. alternata* and *P. langloissii*.

The experiment of spraying gardenia plant by cumin oil as individual treatment or in a mixture with clove or anise oil on the percentage of reduction in the rotted buds caused by *B.cinerea* and the disease index of leaf spot disease caused by *A.alternata* and *P.langloissii* under artificial inoculation conditions showed that cumin oil at 2500 ppm mixed with clove oil at 5000 ppm concentration exhibited the heighst reduction percentage in the disease incidence by the three tested fungi under artificial inoculation. Where the treatment resulted in decreasing the incidence of gardenia bud rot disease caused by *B.cinerea* from 58.3% in (check) treatment to 16.7% and also, decreasing the incidence of gardenia leaf spot disease caused by *A.alternata* and *P.langloissii* to 26.7% and 11.1%, respectively compared with the (check) treatment 56.7 and 36.0%, respectively. Cumin oil at 2500 ppm mixed with

anise oil at 5000 ppm concentration treatment followed the previous mentioned treatment. Meanwhile, cumin oil at 5000 ppm concentration was the lowest one in this respect (Table 11).

Table (11): Effect of cumin oil individually or in mixture with anise or clove oils on controlling bud rot caused by *B. cinerea* and incidence of leaf spot disease caused by *A. alternata* and *P. langloissii*.:

Plant oils treatments	% infection of fungi			
	Conc. (ppm)	*A. alternata*	*B. cinerea*	*P.langloissii*
C. cyminum	5000	28.9	50.0	23.3
C. cyminum* & *P. anisum	2500 + 5000	32.2	41.7	21.0
Cu. cyminum* & *S.aromaticum	2500 + 5000	26.7	16.7	11.1
(Check)	--------	58.3	56.7	36.0
Mean	--------	36.5	41.3	22.9

LSD at 0.05 for:
Fungi (F) : 4.1
Concentration (C) : 4.2 F×C: 7.2

2- Effect of cumin oil individually and in a mixture with anise or clove oil on controlling bud rot and leaf spot diseases under natural infection in greenhouse

The *in vivo* treatments by the same oils and by the same concentrations were showed lower efficacy than those conducted under artificial inoculation conditions in the greenhouse but in general, cumin oil at 2500 ppm mixed with clove oil at 5000 ppm concentration exhibited the heighst percent of reduction in the disease incidence of gardenia bud rot and leaf spot disease Where, the treatment resulted in decreasing the incidence of gardenia bud rot disease from 43.3% in (check) treatment to 23.4% by 46% efficacy and also, decreasing the incidence of gardenia leaf spot disease from 41.7% in (check) treatment to 25.0% by 40.0% efficacy .followed by cumin oil at 5000 ppm. where, it reduced the incidence of gardenia leaf spot disease from 41.7% in (check) treatment to 33.3% by 20.1% efficacy (Table 12). Kishnora *et al* (2007), reported that very few studies have analyzed enough essential oils and biological endpoints to determine whether there is a specificity for different effects according to deferent oils or not . Clearly, it has been shown by Bakkali *et al.* (2005 and 2006) that the essential oils presented a specificity in the amplitude, but not in the mode of action, of the biological effects, i.e. cytotoxicity, cytoplasmic mutant induction, gene induction and antigenotoxicity effects. However, they did exhibit a specificity of the mode of action concerning production of ROS, probably due to differences in their actual composition corresponding to differences in compartmentation of the oxidative stress. Hansen *et al.* (2006) concerning antigenotoxicity, the essential oils showed the same protective activity. However the mode of action of protection differed, not

according to the type of oil, but according to the mutagens, i.e. to the type of lesions induced and thus, to the type of their enzymatic recognition and processing lead to translational synthesis or late apoptosis/necrosis (Bakkali *et al.,* 2005 and 2006).

Table (12): Effect of cumin oil individually and in a mixture with anise or clove oil on the percentage of reduction in rotted buds and the disease index of leaf spot disease under natural infection conditions in greenhouse.

Plant oils treatments	Leaf spot		Bud rot	
	% infection	% efficacy	% infection	% efficacy
C. cyminum	26.7	38.3	33.3	20.1
C. cyminum & P. anisum	29.4	32.3	41.7	00.0
C. cyminum & S.aromaticum	23.4	46.0	25.0	40.0
(Check)	43.3		41.7	
Mean	30.7		35.4	
LSD at 0.05	2.5		3.1	

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مقاومة امراض تبقعات الأوراق واعفان البراعم على نبات الجاردينيا باستخدام بعض الزيوت النباتية الطبيعية.

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أظهرت تجارب العزل من نباتات الجاردينيا المصابة بتبقعات الأوراق واعفان البراعم والمجموعة من صوب محافظة الجيزة خلال موسم ٢٠٠٥-٢٠٠٦ وجود أحد عشر نوعا فطريا تابعا لاحد عشر جنسا فطريا مختلفا. اختلفت نسب وجود كل فطر في العزل باختلاف كل من الجزء النباتي المعزول منه وفترات العزل. وكانت الفطريات بوترايتس سينريا، الترناريا الترناتا،نوع من الكلادوسبوريم وبيستالوشيا لانجلوسياي هي الأكثر تكرارا في العزل، كما أظهرت هذه العزلات الفطرية الأربعة المختلفة اختلافا في قدرتها المرضية على اصابة الأجزاء المختلفة لنباتات الجاردينيا حيث اقتصر الفطر بوترايتس سينريا في إصابته للبراعم قبل تفتح الأزهار وسجل أعلى نسبة مئوية لأعفان البراعم في حين تخصص كل من الفطرين الترناريا الترناتا وبيستالوشيا لانجلوسياي في إصابتهم للأوراق بالتبقع وكان الفطر الترناريا الترناتا هو الأعلى في شدة الإصابة. أما الفطر كلادوسبوريم ففشل في إصابته لاي من الأوراق أو البراعم.

أظهرت تجارب المقاومة للفطريات الثلاث المختبرة داخل المعمل بواسطة عشرين زيتا عطريا أن الزيت المستخلص من الكمون عند التركيز ٥٠٠ جزء في المليون أنه كان كافيا لتثبيط النمو الطولي للفطريات المختبرة، بينما احتلت الزيوت المستخلصة من الينسون، النعناع، الزعتر، القرنفل والريحان الفرنساوي عند تركيزات تتراوح بين ٧٥٠-١٠٠٠ جزء في المليون المرتبة الثانية في هذا الشأن واختلف التركيز المثبط لكل زيت باختلاف نوع الفطر.

كما أظهرت تجارب المقاومة تحت ظروف الصوبة بواسطة المعاملة بالزيت المستخلص من الكمون بمفردة أو بعد خلطه بالزيوت المستخلصة من الينسون أو القرنفل على التوالي أن المعاملة بخليط من زيت الكمون بتركيز ٢٥٠٠ جزء في المليون وزيت القرنفل بتركيز ٥٠٠٠ جزء في المليون هو الأفضل في خفض حدوث المرض، كما لوحظ أن كفاءة هذه الزيوت في خفض حدوث المرض كانت أعلى تحت ظروف العدوى الصناعية مقارنة بكفاءتها تحت ظروف العدوى الطبيعية داخل الصوبة.