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Role of Essential Oils in Controlling Fungi that Cause Decline Disease of Guava

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ABSTRACT

The effect of 11 essential oils was in-vitro and in-vivo evaluated against guava decline disease fungi namely, Botryodiplodia theobromae, Fusarium oxysporum and Rhizoctonia solani isolated from guava shoots, roots and rhizosphere. Six concentrations of each were studied in vitro on potato dextrose agar. Using of clove oil and judean wormwood oil separately at 250 ppm, pepper and tangerine oils at 500 ppm, anise oil at 750 ppm and onion oil at 1000 ppm led to full inhibition of radial growth of B. theobromae. In the case of F. oxysporum, full inhibition of radial growth was occurred by clove oil at 500 ppm or judean wormwood, onion and cinnamon oils at 750 ppm as well as tangerine, anise, black seed and thyme at 1000 ppm. The growth of R. solani has been full inhibited using clove, and judean wormwood oils at 250 ppm or onion oil at 500 ppm. While, other tested oils have inhibitory effects at concentrations higher than previously mentioned. In vivo study showed that, the effect of the treatment of 1.5 years old guava seedlings roots with clove and judean wormwood oils separately using three concentrations (500,1000 and 1500 ppm) for 20 minutes before planting in contaminated soil with decline fungi led to significant differences between treatments and the only contaminated ones (control). The best result were obtained using clove oil at 1500 ppm which inhibited completely the infection (severity =0) and gave significant increase in plant height and dry weight of both shoot and root systems as well as leave contents of chlorophyll a and chlorophyll b.

Key words: *Essential oils, Guava decline disease, Plant pigments.*

INTRODUCTION

Essential oils are among the promising strategies not only to manage plant diseases but also to have a safe environment and low toxicity to people due to their natural properties. They have a low risk for resistance development by pathogenic microorganisms. They are also biodegradable compounds and used efficiently in integrated pest management programs. Essential oils have been widely used against bacteria, viruses, fungi and nematodes[13,23]. Many studies showed that these oils have antifungal activity through their inhibitory effects on mycelial growth against many phytopathogenic fungi such as *B. theobromae*, *R. solani*, *Pythium irregulare*, *Ceratocystis pilifera*, *Phragmidium violaceum*, *Colletotricum capsici*, *Phytophthora capsici*, *F. solani* and *F. oxysporum*[3,8,15,21].

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Wilson *et al*[34]., reported that essential oil extracted from red thyme (*Thymus zygis* L.) has a great inhibitory effect on *Botrytis cinerea* spore germination compared with other tested essential oils. Rahhal[25] reported that clove oil completely inhibited the mycelial growth of *Alternaria tenuis, Sclerotinia sclerotiorum, F. solani and R. solani*. Moreover, clove oil decreased faba bean disease index with 57.08% and chickpea disease index with 51.92 %. The lesion number of faba bean decreased with 26.67 % whereas in chickpea the lesion number decreased by 31.25%. Seven essential oils, namely: citral, eugenol, geraniol, limonene, and linalool, inhibited the growth of 14 phytopathogenic fungi, including *F. oxysporum*, *F. Moniliforme* and *R. bataticola*[19]. During studies conducted by Abdelgaleil[6] and Soad & Abdelgaleil[29] where the antifungal activities of 8 essential oils against ten phytopathogenic fungi, a concentration of 500 ppm *Mentha microphylla* oil completely inhibited the growth of *F. culmorum, F. oxysporum*, *Penicillium digitatum*, *R. solani* and *Rhizopus stolonifer*. The effect of essential oils on postharvest (e.g. *P. digitatum, Aspergillus flavus, Colletotricum gloeosporioides*) and soilborne fungi (e.g. *Pythium ultimum, R. solani, Bipolaris sorokiniana*) have been also tested. Katooli *et al*[18]., showed that eucalyptus essential oil in all tested concentrations (give a range of the concentrations) had completely inhibition effect on mycelial growth of *Pythium ultimum* and *R. solani*.The *Eucalyptus tereticornis* Sm. essential oil caused damages and changes in hyphae and chlamydospores of *F. oxysporum* and also decreased the number of conidia[4]. Barrera-Necha *et al*[7].,studied the antifungal effects of 10 essential oils against *F. oxysporum* f. sp. *gladioli*. They found that essential oils of cinnamon, clove and thyme inhibited the mycelial growth of *Fusarium* sp. totally. Abdel-Kader *et al*[2].,found that clove, caraway (*Carum carvi* L.), thyme and peppermint essential oils inhibited the mycelial growth of *F. solani, R. solani, Sclerotium rolfsii* and *Macrophomina phaseolina* under *in vitro* conditions.

Moreover, essential oils used to coat seeds resulted in a significant reduction of root rot incidence of bean, at both pre- and post-emergence stages under greenhouse conditions. Under field conditions seeds coated with essential oils sown in soil treated with the bio-agent *T. harzianum*, gave pronounced protection to emerged bean seeds against the invasion of root rot pathogenic fungi. These results show that application of essential oils in integration with the bio-agent *T. harzianum* may be considered as an applicable, safe and cost-effective method for controlling such soilborne diseases. Study of the antifungal activity of 15 essential oils against six plant pathogenic fungi (*R. solani, M. phaseolina, F. oxysporum, Helminthosporium* sp., *A. alternata* and *Diplodia* sp.) indicated that the essential oils of caraway, clove, fennel and thyme were effective against the tested fungi. In addition, the antifungal activity was dramatically enhanced against all the tested fungi, particularly in the case of clove oil with triton X-100 mixture which proved to be the most effective one[14]. Huang *et al*[16].,showed that the fruit essential oil of star anise (*Illicium verum* Hook.) and *trans*-anethole from anise could be developed as the natural fungicides (*i.e.,* fumigants) for plant disease control in fruit and vegetable preservation. Seema & Devaki[27] tested the fungicidal properties for 12 essential oils against *R. solani*. The results confirmed that 5 essential oils namely, cinnamon, clove and fennel have shown promising results against *R. solani*. Recent work shows that in eukaryotic cells, essential oils can act as prooxidants affecting inner cell membranes and organelles such as mitochondria[12,17]. The objectives of this study were to 1) evaluate the effect of eleven different plant volatile oils on the mycelial growth of the fungi (*R. solani*, *B. theobromae* and *F. oxysporum*) causing the decline disease of guava, 2) assess the effectiveness of these oils in decreasing disease severity on guava plants infected by guava decline fungi, and 3) evaluate the effect of these oils on both leaf chlorophyll content and the growth of guava plants.

MATERIALS AND METHODS

Isolation and identification of decline disease fungi:

Plant samples of guava trees showing typical decline symptoms were collected from 39 fields from Alexandria (three counties), El-Behera (five counties) and Matruh (one county) governorates, Egypt. The plant samples included 300 from roots, 106 from main stems, 195 from branches, 218 from leaves and 146 from fruits. In addition, 980 samples were collected from the soil. The isolation of pathogenic agents from diseased roots, stems and leaves was carried out according to Ashour & Saber[5].

Biocontrol agents were recovered from guava roots and soil samples. Fungal cultures were then isolated and purified by either single spore isolation or hyphal tip techniques[30]. The purified fungal isolates were identified to the genus level by using the morphological characteristics according to Barnett & Hunter[6]. Sun *et al*[32].,Watts *et al*[33].,Sneh *et al*[28].,and Leslie & Summerell[20]. The pure cultures of the isolated fungi were kept on PDA slants at 4°C.

Extraction of essential oils

Essential oils were extracted from eleven flowering plants (Table 1) according to Pramila *et al*[24].,These oils were evaluated against guava decline fungi both *in vitro* and *in vivo*. Various parts of plants were collected for the extraction of essential oils. The plant materials were identified according to the guidance of the students' flora of Egypt[31]. One kilogram of each plant tissues was used for extracting the essential oils. The extraction method of essential oils were carried out according to Pramila *et al*[24].,with some modifications, e.g. each volatile oil was dried over anhydrous sodium sulphate and then kept in sealed clean glass vials at 4°C for further biological studies.

Table 1. Aromatic plants used as a source of biocontrol oils for guava decline fungi

Scientific name	Family	Common name	Tissue type
1. *Zingiber officinale* Roscoe	Zingiberaceae	Ginger	Rhizome
2. *Piper nigrum* L.	Piperaceae	Pepper	Seed
3. *Cinnamomum cassia* (Nees& T. Nees)	Lauraceae	Cinnamon	Dried bark
4. *Eucalyptus globulus* Labill.	Myrtaceae	Eucalyptus	Leaves
5. *Thymus vulgaris* L.	Apiaceae	Thyme	Seed
6. *Allium cepa* L.	Amaryllidaceae	Onion	Leaves
7. *Eugenia caryophyllata* Thunb.	Myrtaceae	Clove	Flower buds
8. *Nigella sativa* L.	Ranunculaceae	Black seed	Seed
9. *Pimpinellaanisum* L.	Apiaceae	Anise	Dried fruit
10. *Artemisia judaica* L.	Asteraceae	Judean wormwood	Arial parts
11. *Citrus tangerine* Tanaka	Rutaceae	Tangerine	Dried peel

***In vitro* studies**

The fungitoxic activity of the essential oils was evaluated using the poisoned food technique according to Soad & Abdelgaleil[29]. Fungi were grown on potato dextrose agar (PDA) medium amended with different concentrations of essential oils (150, 250, 500, 750 and 1000 mg/l). Different concentrations of essential oils were prepared by dissolving the required amounts in 0.5 ml of 1% dimethyl sulfoxide or liquid soap (pril) as an emulsifier at a rate of 10 ml/l and then mixed with PDA medium immediately before pouring into the petri plates. A mycelial disc (0.6 cm in diameter) of the tested fungi from the periphery of a fungal colony grown on PDA for a week at 25°C. The inoculated PDA plates were incubated at 30°C. Five replicates were prepared for each treatment. PDA Petri dishes amended with dimethyl sulfoxide were used as a control. The mycelial linear growth was measured when the full growth of the tested fungus observed in the control treatment. Percentage of mycelial growth inhibition was calculated using the following formula:

Mycelial growth inhibition = [(DC – DT) /DC] * 100

DC= average diameters of the fungal colony of the control plate.

DT= average diameters of the fungal colony of each treatment.

***In vivo* studies**

Based on the *in vitro* studies, the most potent essential oils were selected and evaluated under greenhouse conditions against pathogenic fungi causing decline disease of guava. Plastic bags filled with sterilized aerated sandy clay soil were inoculated with 15gm/Kg of each fungal inoculum which had previously been growing for two weeks on barley medium at 30°C. The treatments were as follows:

1) Guava transplants cultivated in non-infested soil bags (control).
2) Guava transplants cultivated in infested soil bags with the hyphal mixture of decline fungi.
3) Roots of guava transplants treated with essential oil at concentration 500 mg/l, for 20 minutes then cultivated in infested soil bags with decline fungi.
4) Roots of guava transplants treated with essential oil at concentration 1000 mg/l, for 20 minutes then cultivated in infested soil bags with decline fungi.
5) Roots of guava transplants treated with essential oil at concentration 1500 mg/l, for 20 minutes then cultivated in infested soil bags with decline fungi.

Disease severity (affected area per tree) was evaluated after nine months from the transplanting date. Plants were uprooted and different measurements were determined, e.g. chlorophyll content, plant height, and dry weights of shoots and roots.

Statistical analysis:

All the experiments were conducted with five replicates for each treatment and arranged in a randomized complete block design. The quantitative data obtained were analyzed by the statistical analysis of variance with SAS software (SAS institute)[26] and the level of significance was determined by LSD comparisons at the 5% probability level.

RESULTS

Isolation of guava pathogenic fungi

Seven fungal pathogens namely, *F. oxysporum*, *F. solani*, *F. culmorum*, *Pythium* sp., *Macrophomina phaseolina*, *Sclerotinia sclerotiorum*, and *R. solani* were isolated from both guava roots and soil samples. Out of these seven pathogenic fungi, *F. oxysporum* and *R. solani* were the most abundant. However, the isolation from guava branches, twigs, leaves and fruits resulted in 11 pathogenic fungi namely, *F. oxysporum*, *B. theobromae*, *Alternaria alternate*, *A. solani*, *Aspergillus niger*, *Cercospora* sp., *Cladosporium* sp., *Curvularia* sp., *Colletotrichum gloeosporioides*, *Helminthosporium* sp. and *Phytophthora* sp. The most abundant fungi from guava aerial parts were *F. oxysporum* and *B. theobromae*. Based on the abundance of the isolated pathogenic fungi and Koch's postulates, the most virulent isolates of *F. oxysporum*, *B. theobromae* and *R. solani* which cause decline disease of guava were used for further studies.

***In vitro* studies**

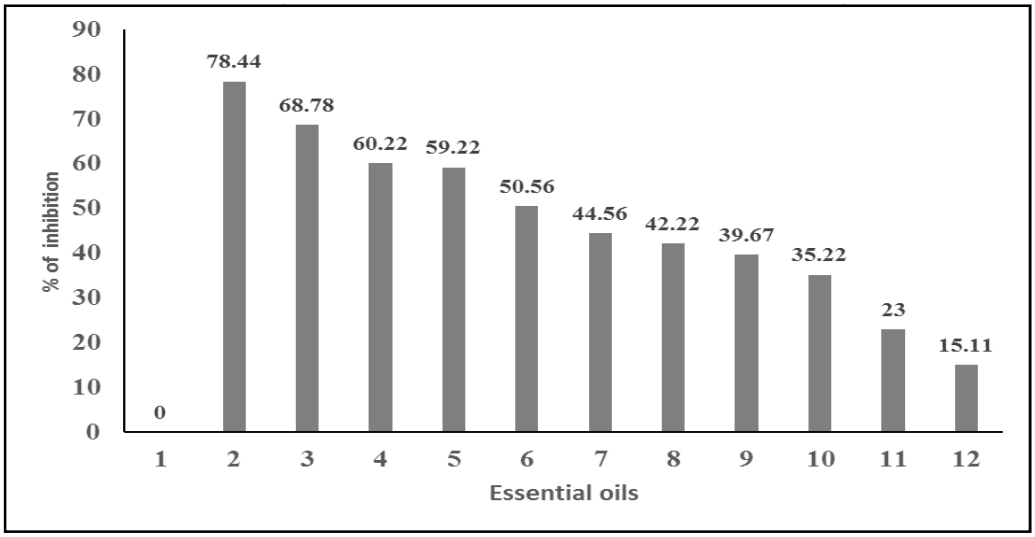
Fungitoxic activity of eleven different oils was tested by the poisoned food technique using potato dextrose agar medium at six different concentrations on radial growth of guava decline pathogenic fungi named *R. solani*; *B. theobromae* and *F. oxysporum*. All tested volatile oils showed toxicity to the three decline fungi (Table 2). The highest inhibition of mycelial radial growth was 78.44% in case of clove oil. Conversely, the lowest reduction was 15.11% in case of ginger oil (Figure 1). *R. solani* was more sensitive to volatile oils than *B. theobromae* which was more sensitive than *F. oxysporum* (Table 3). Fungal toxicity was increasing with concentration. Clove, judean wormwood, onion, and tangerine volatile oils were significantly more toxic to the tested fungi. The 11 studied essential oils differed in their inhibition effectiveness on the mycelial growth of guava decline disease fungi. In the case of *B. theobromae*, the linear growth was completely inhibited by the clove and judean wormwood oils at concentration of 250 ppm, while 500 ppm concentration of both pepper and tangerine oils inhibited completely the mycelial growth (Table 3). Higher concentrations of anise (750 ppm) and onion (1000 ppm) were needed to completely inhibit *B. theobromae* growth.

Table 2.Total inhibition of guava decline disease fungi using 11 essential oils with five concentrations

Essential oil	Fungal growth	% of inhibition	Essential oil	Fungal growth	% of inhibition
Control	9.00	00.00	Thyme	4.99	44.56
Clove	1.94	78.44	Pepper	5.20	42.22
Judean wormwood	2.81	68.78	Anise	5.43	39.67
Onion	3.58	60.22	Black seed	5.83	35.22
Tangerine	3.67	59.22	Eucalyptus	6.93	23.00
Cinnamon	4.45	50.56	Ginger	7.64	15.11

Each number is a mean of 3 fungi×5 concentration×5 replicates

Fig. 1: Percentage of inhibition in radial growth of guava decline fungi treated with 11 essential oils



1-Control, 2- Clove, 3- Judean wormwood, 4- Onion, 5-Tangerine, 6- Cinnamon, 7- Thyme, 8- Pepper, 9- Anise, 10- Black seed, 11- Eucalyptus and 12- ginger

However, complete inhibitory concentrations of oils extracted from eucalyptus, black seed, ginger, thyme and cinnamon, were more than 1000 ppm (Table 3). In the case of *F. oxysporum*, the linear growth was completely inhibited by clove oil at 500 ppm, judean wormwood, cinnamon and onion oils at 750 ppm, and tangerine, anise, black seed and thyme oils at 1000 ppm (Table 3). More than 1000 ppm concentrations of oils extracted from pepper, eucalyptus, and ginger needed to completely inhibit the mycelial growth of *F. oxysporum*. The 250 ppm concentrations of clove, tangerine, and judean wormwood oils were sufficient to completely inhibit the growth of *R. solani* (Table 2). Moreover, the growth of *R. solani* was completely inhibited by onion oil at 500 ppm and black seed, cinnamon and thyme oils at 750 ppm. To completely inhibit the mycelial growth of *R. solani* by oils extracted from pepper, anise, eucalyptus and ginger, the concentrations were 1000 ppm (Table 3).

Table 3. Effective concentrations of essential oils (ppm) that completely inhibited the mycelial growth of guava decline disease fungi

Essential oil (A)	Fungi (B)		
	B. theobromae	*F. oxysporum*	*R. solani*
Clove	250	500	250
Pepper	500	>1000	1000
Tangerine	500	1000	250
Judean wormwood	250	750	250
Anise	750	1000	1000
Eucalyptus	>1000	>1000	1000
Black seed	>1000	1000	750
Ginger	>1000	>1000	1000
Cinnamon	>1000	750	750
Onion	1000	750	500
Thyme	>1000	1000	750

***In vivo* studies**

The most two potent essential oils, extracted from clove and judean wormwood tissues, were selected for further *in vivo* studies on 1.5 years old guava seedlings under open field conditions to evaluate their efficacy against guava decline disease fungi. Clove oil at concentration 1500 ppm prevented completely the infection of guava plants with decline fungi, while, the same concentration of judean wormwood oil reduced the infection to 24.3%. Moreover, the 1500 ppm concentration of clove oil increased significantly guava infected-plant height and dry weights of shoots and roots compared with the healthy control.

However, the judean wormwood oil at 1500 ppm enhanced significantly all the characters of infected treated plants in comparison with the only infected ones. There was a slight increase in the content of chlorophyll a and b in infected plants treated with 1500 ppm clove oil in comparison with healthy control ones. With respect to judean wormwood oil, the 1500 ppm concentration caused significant increasing of guava infected-plant height, dry weights of shoots and roots and the content of chlorophyll a and b in comparison with the only infected ones but still less than the healthy control (Table 4).

Table 4: Effect of clove and judean wormwood (JW) oils at three concentrations on disease severity and some plant characters of guava infected with decline fungi

Treatments (ppm) A	Severity and some plant characters(B)											
	Severity %		Plant height (cm)		Dry weight of shoots(gm)		Dry weight of roots(gm)		Chlorophyll a (mg/g)		Chlorophyll b (mg/g)	
	Clove	JW	Clove	JW	Clove	JW	Clove	JW	Clove	JW	Clove	JW
Healthy/ 0	00.0*	00.0	41.2	41.2	48.9	48.9	25.7	25.7	11.6	11.6	6.7	6.7
Infected/ 0	90.0	90.0	26.6	26.6	16.5	16.5	6.4	6.4	0.8	0.8	0.5	0.5
Infected/ 500	38.5	59.1	31.4	27.8	29.6	29.4	15.1	11.3	4.0	1.8	2.1	0.9
Infected/ 1000	23.9	41.7	36.6	30.4	40.2	35.6	18.0	17.4	8.5	4.5	4.2	2.4
Infected/ 1500	00.0	24.3	43.4	32.4	56.1	41.5	27.8	20.5	11.8	7.4	7.00	3.6
Mean	**30.48**	**43.02**	**35.84**	**31.68**	**38.26**	**34.38**	**18.60**	**16.26**	**7.34**	**5.22**	**4.10**	**2.82**

Severity: L. S. D.$_{0.05}$ (B) = 0.62, L. S. D. $_{0.05}$(A*B) = 1.38
Dry weight of shoots L. S. D. $_{0.05}$(B) = 1.60, L. S. D. $_{0.05}$(A*B) = 3.58
Dry weight of roots: L. S. D. $_{0.05}$(B) = 1.06, L. S. D. $_{0.05}$(A*B) = 2.37
Plant height: L. S. D. $_{0.05}$(B) = 0.87, L. S. D. $_{0.05}$(A*B) = 1.95
Chlorophyll a L. S. D. $_{0.05}$(B) = 0.20, L. S. D. $_{0.05}$(A*B) = 0.46
Chlorophyll b: L. S. D. $_{0.05}$(B) = 0.19, L. S. D. $_{0.05}$(A*B) = 0.43

DISCUSSION

Essential oils and their components are gaining increasing interest because of their relatively safe status, low toxicity for people and environment due to their natural properties, low risk for resistance development by pathogenic microorganisms, their biodegradability, their wide acceptance by consumers and their exploitation for potential multipurpose functional use[9,11,22]. In some plant species, one main constituent of the oil may predominate while in many species no single compound predominates and instead there is a balance of various components[10]. Essential oils have been widely used for its effect as bactericidal[13], virucidal, fungicidal and nematicidal[23]. Several workers in different countries focused on the oils displayed great potential of antifungal activity as a mycelial growth inhibitor against the tested phytopathogenic fungi such as *B. theobromae, R. solani*, *Pythium irregulare*, *Ceratocystis pilifera*, *Phragmidium violaceum, Colletotricum capsici, Phytophthora capsici, F. solani* and *F. oxysporum*[8,15,21]. Our results indicated that the effect of eleven different plant volatile oils at six different concentrations on radial growth of three pathogenic fungi isolated from guava showed that the highest reduction of mycelial radial growth was 78.44% in case of clove oil and the lowest reduction was 15.11% in case of ginger oil. *R. solani* was more sensitive to volatile oils than *B. theobromae* which was more sensitive than *F. oxysporum*. Fungal toxicity was increasing with the increasing of concentrations. Clove, judean wormwood, onion, and tangerine volatile oils were significantly more toxic to the tested fungi. Clove oil inhibited completely growth of *B. theobromae* and *R. solani* at 250 ppm while, the complete inhibition of *F. oxysporum* occurred at 500 ppm. The effect of judean wormwood oil was similar to that of clove oil on *B. theobromae* and *R. solani* but *F. oxysporum* tolerated concentration more than 500 ppm and completely suppressed at 750 ppm. Onion oil stopped the growth of *R. solani* at 250 ppm, *F. oxysporum* at 750 ppm and *B. theobromae* at 1000 ppm. Tangerine volatile oil prevented the growth of *R. solani* at 250 ppm, *B. theobromae* at 500 ppm and *F. oxysporum* at 1000 ppm. Kishore *et al*[19].,reported that clove oil, cinnamon oil and five essential oil components (citral, eugenol, geraniol, limonene, and linalool) inhibited growth of 14 phytopathogenic fungi, including *F. oxysporum, F. moniliforme* and *R. bataticola*. Seema & Devaki[27] tested the fungicidal properties for 12 essential oils against *R. solani*. The results confirmed that three essential oils namely, cinnamon, clove, and fennel have shown promising results against *R. solani.*

In vivo experiments, the most two potent essential oils, extracted from clove and judean wormwood tissues, were selected for further *in vivo* studies under green house conditions to evaluate their efficacy against guava decline disease fungi. Clove oil at concentration 1500 ppm prevented completely the infection of guava plants with decline fungi, while, the same concentration of judean wormwood oil reduced the infection to 24.3%. Moreover, the 1500 ppm concentration of clove oil increased significantly guava infected-plant height and dry weights of shoots and roots compared with the healthy control. However, the judean wormwood oil at 1500 ppm enhanced significantly all the characters of infected treated plants in comparison with the only infected ones. The content of chlorophyll a and b in infected plants treated with 1500 ppm clove oil was slightly increased in comparison with healthy control ones. In case of judean wormwood oil at concentration 1500 ppm, there is a significant increasing of guava infected-plant height, dry weights of shoots and roots and the content of chlorophyll a and b in comparison with the only infected ones but still less than the healthy control. Our results are in harmony with those reported by Rahhal[25] who tested the antifungal activity of 7 essential oils, while he found that the clove oil completely inhibited mycelia growth of *Alternaria tenuis, S. sclerotiorum, F. solani* and *R. solani*. Using infested soil with *R. solani*, pot experiment showed the clove oil decreased faba bean disease index with 57.08% and chickpea disease index with 51.92 %. Lesion number of faba bean decreased with 26.67 % and of chickpea with 31.25 and 18.75 %. Also, Barrera-Necha *et al*[7].,studied the antifungal effects of 10 essential oils viz., peppermint, thyme, cinnamon, clove, garlic, mexican lime peppers and eucalyptus against *F. oxysporum* f. sp. *gladioli*. They found that the essential oils of cinnamon, clove and thyme inhibited the mycelial growth of *Fusarium* totally. Abdel-Kader *et al*[2]., found that clove, caraway (*Carum carvi* L.), thyme and peppermint essential oils have inhibitory effects against the mycelial growth of *F. solani, R. solani, S. rolfsii* and *M. phaseolina* under *in vitro* conditions. El-Zemity & Soad[14] studied the antifungal activity of 15 essential oils against six plant pathogenic fungi (*R. solani, M. phaseolina, F. oxysporum, Helminthosporium* sp., *A. alternate* and *Diplodia* sp.). The results indicated that the essential oils of caraway, clove, fennel and thyme were effective against the tested fungi. In addition, the antifungal activity was dramatically enhanced against all the tested fungi, particularly in case of clove oil with triton X-100 mixture which proved to be the most effective one. Among the monoterpenoidal constituents, thymol, chlorothymol and carvacrol showed the highest fungicidal activity compared with other essential oil components used. Abdel-Kader *et al*[2]., indicated that the clove, caraway, thyme and peppermint essential oils used to coat seeds resulted in a significant reduction of root rot incidence of bean, at both pre- and post-emergence stages under greenhouse conditions. Under field conditions seeds coated with essential oils at a concentration of 4% sown in soil treated with the bio-agent *T. harzianum*, gave pronounced protection to emerged bean seeds against the invasion of root rot pathogenic fungi. These results show that application of essential oils in integration with the bio-agent *T. harzianum* may be considered as an applicable, safe and cost-effective method for controlling such soil borne diseases.

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