Application of *Trichoderma harzianum* and essential oils as seed dressing against charcoal rot disease incidence of sunflower under field conditions

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The efficacy of the bioagent *Trichoderma harzianum* and some essential oils individually or based on CMC and Arabic gum carriers as seed dressing was evaluated against sunflower charcoal rot incidence under field conditions. Essential oils, Caraway, Bitter orange and Lavender were used in this study. Also, the fungicide Topsin-M (70% WP) was used as the comparison treatment. Laboratory tests showed that the reduction in fungal mycelia growth increased significantly in parallel to the increase in concentrations of essential oils to reach its maximum at the highest concentration used. *T. harzianum* was found to be affecting negatively on the linear growth of *M. phaseolina* in dual culture test against *T. harzianum*. Under field conditions, the obtained results indicated that all applied treatments could reduce disease incidence. In this regards, *T. harzianum* recorded the best applied seed treatments that disease incidence at the first and second growing seasons, respectively. Seed dressing with essential oils proved to have high potential effect for reducing charcoal rot disease incidence under field conditions. Superior effect of lavender oil on disease incidence either alone or based on CMC and Arabic gum. These treatments followed by bitter orange and caraway oils compared with the fungicide and untreated control. The present findings demonstrate that application of bio control agents and essential oils on a commercial scale for controlling such diseases is promising. Considering their attribute and broad-spectrum activities as antifungal compounds they could have promise success as alternatives to conventional fungicides for the management of plant diseases.

**Keywords:** charcoal rot, disease control, essential oils, sunflower, *T. harzianum*.

**INTRODUCTION**

Sunflower (*Helianthus annuus* L.) belongs to the family Asteraceae, which is an important oil seed crop. A variety of fungal pathogens attacked sunflower causing losses in its yield and oil quality (Sangawan et al., 2005). *Macrophomina phaseolina* (Tassi) Goid, is considered the main fungal pathogen affecting sunflower in Egypt and worldwide and causing the charcoal-rot disease. The fungus is not only soil-borne, but also seed-borne and infects plants from seedling stage to maturity (Purkayastha et al., 2006). Humid tropical weather is suitable conditions for *Macrophomina phaseolina* (Tassi) Goid the causal pathogen of Charcoal rot disease. Several field crops, *i.e.* bean, maize, sorghum, soybean, sesame and cotton are attacked by *Macrophomina phaseolina* (Mayek-Perez et al., 2001; Mayek-Perez et al., 2002). The common name of the disease caused by *M. phaseolina* came from the appreaed symptoms which showed the growth of numerous microsclerotia and pycnidia on the stem of diseased plants. *Macrophomina phaseolina* (Tassi) Goid was reported to cause charcoal rot
on more than 500 plant species worldwide (Wyllie, 1988; Das et al., 2008). Moreover, black microsclerotia (dormant body) considered the way for fungal survive in soil that each cell is potential to germinate and cause disease (Dubey and Upadhyaya, 2001). Chemical control, biological control, resistant cultivars and control by cultural practices are common strategies for controlling sunflower charcoal rot. Developing tools and procedures that are simple, fast and accurate for suppressing the pathogen populations, particularly, Macrophomina phaseolina are needed. Scientists paid more attention towards the development of alternative methods which are safe, non-toxic to humans and animals and are rapidly biodegradable to avoid hazardous control strategies. One of such strategy is the use of biocontrol agents to control fungal plant diseases. The genus Trichoderma is reported to have promising and effective for controlling wide range of phytopathogens (Chet et al., 1977). Reduction of disease losses requires the application of several control measures for a long term program. In this concern, biocontrol agents, plant extracts, essential oils, antioxidant, growth regulators and natural compounds were used as an IPM program for plant disease control (Bindu and Kumar, 2009; Li et al., 2009; Sharma et al., 2012). Recently, the strategy of preventing bacterial and fungal growth has considerable concern for discovering plant-derived antimicrobial agents such as essential oils and volatile compounds as alternatives to chemical pesticides (Jenny, 2000; Michael, 2000; Sagdic et al., 2003; Lasciotti et al., 2004). Therefore, the objective of the present study was planned to evaluate the integrated treatment of T. harzianum and different essential oils as seed dressing against sunflower charcoal rot disease incidence under natural field conditions.

MATERIALS AND METHODS

Tested materials

Essential oils used in the present study were obtained from CID Company, Egypt. The essential oils tested in present study were Caraway (Carum carvi), Bitter orange (Citrus aurantium) and Lavender (Lavandula officinalis). These essential oils were stored in dark bottles at 4ºC for further studies. The used essential oils were used individually or based on 2% of CMC and Arabic gum carriers. Pathogenic and antagonistic fungal isolates kindly obtained from Culture Collection Unit, Plant Pathology Department, National Research Centre (NRC), Egypt were used in present study. The virulent isolate of the pathogenic fungus Macrophomina phaseolina and antagonistic fungus Trichoderma harzianum have been showed high plant pathogenic and antagonistic ability against several plant pathogens and used successfully in previously various works at the same Department.

Laboratory test

The efficacy of some essential oils, Caraway, Bitter orange, Lavender, antagonism of T. harzianum individually or based on CMC and Arabic gum carriers and fungicide Topsin-M on Macrophomina phaseolina the causal of sunflower charcoal rot was evaluated under in vitro conditions.

Three essential oils at concentrations of 0.5, 1, 2, 4 and 8% were evaluated for their inhibitory effect on M. phaseolina fungal radial growth. Emulsified stocks at high concentrations of tested essential oils were prepared by dissolving in sterilized distilled water. A few drops of the emulsifier Tween 20 (Sigma Co.) were added to the essential oil volumes to obtain an emulsion feature. The tested essential oils were prepared as three stocks, individually, or based on 2% of either CMC or Arabic gum. Different volumes of each feature of essential oil emulsion were added to conical flasks containing 100 mL sterilized PDA medium before its solidification, to obtain the proposed concentrations. The supplemented media were poured into Petri-dishes (9 cm) about 20 ml each. The control treatment was PDA medium which was free of essential oils. Disks (5 mm-diameter) of M. phaseolina growth taken from seven day-old culture were placed on the centre of Petri dishes.

The inhibitory effect of the fungicide Topsin-M (70%WP) against the growth of M. phaseolina was evaluated as comparison treatment for both essential oils and the bioagent. Three concentrations, 2.5, 5.0 and 10 ppm based on the active ingredient were prepared in PDA medium poured in Petri-dishes. Control treatment was fungicide-free medium. Petri-dishes were inoculated with 5mm disc of 10-day-old tested fungal cultures.

As for antagonistic bioagents test, T. harzianum efficacy was also evaluated in vitro performed on PDA medium using the dual culture technique (Ferreira et al., 1991). Treatment of the bioagent T. harzianum (growth suspension 1X10⁶) individually or based on either CMC or Arabic gum carriers were placed into a well (5mm diameter) of PDA plate 10 mm from the edge of the Petri dish. Another 5-mm disk of the pathogen M. phaseolina growth was placed on the opposite side of the
dish at the same distance. The control treatment was inoculated with a well of the bioagent treatment or mycelial disk of the pathogen alone. Ten replicates (dishes) were used for each pathogen-treatment concentration combination and for the untreated checks. All plates were incubated at 25±2°C until the fungal reached full growth in the control treatment. Linear growth was measured (mm) and mycelial growth reduction (%) was calculated as follows:

\[
\text{Growth reduction } \% = \frac{(C-T)}{C} \times 100
\]

Where:

\( C \) = linear fungal growth in control.
\( T \) = linear fungal growth in treatment.

**Field experiment**

Field experiment was carried out at Researches and Production Station of the National Research Centre at Nubaria region, Beheira Governorate, Egypt during two successive summer growing seasons 2015 and 2016. The efficacy of *Trichoderma harzianum* and some essential oils as seed dressing treatments for controlling charcoal rot disease incidence of sunflower was evaluated. The essential oils, *e.g.* Caraway (*Carum carvi*), Bitter orange (*Citrus aurantium*) and Lavender (*Lavandula officinalis*) used as seed dressing at the rate of 2% (v:w). The fungicide Topsin-M (70WP) 2g/Kg seed was used in this study as a comparison treatment. *Trichoderma harzianum* (growth suspension 1X10^8) and essential oils were applied individually or based on either CMC and Arabic gum carriers. At the first growing season 2015, the experimental field consisted of plots (7x6 m) each comprised of 12 rows with five plots as replicates for each particular treatment as well as untreated check in a completely randomized block design. Sunflower seeds (cv Sakha 53) were sown in all treatments. All plots received the traditional agricultural practices. Percent of charcoal rot disease incidence was recorded throughout the growing season. Percentage of disease incidence was recorded referring to the numbers of emerged seedlings. The same procedures were also repeated for the second growing season 2016. Average of disease incidence for the two seasons was calculated.

**Statistical analysis**

The obtained data were subjected to IBM SPSS software version 14.0. Analysis of variance was determined and the mean values were compared by Duncan’s multiple range test at \( P < 0.05 \).

**RESULTS AND DISCUSSION**

**Laboratory tests**

Presented data in Table (1) showed that the tested essential oils in this work, caraway, bitter orange and lavender essential oils have a great variety of phytochemicals (Table 1) that, could be considered as responsible for a larger or smaller antifungal activity, such as: flavonoids, triterpenes carvone, limonene, carveol, coumarin, dihydrocarveol, pinen, thujone, and other minor constituents [C.f. Herb Information Herb Information (www.holisticonline.com/Herbal-Med/_Herbs/h280.htm)].

The inhibitory effect of caraway, bitter orange and lavender essential oils; antagonistic bioagent as well as fungicide against the linear growth of *Macrophomina phaseolina* the causal of sunflower charcoal rot are presented in Table (2). Reduction in fungal mycelia growth increased significantly via the increase in concentrations of essential oils to reach its maximum at the highest concentration used. Complete growth inhibition was only observed at 8% of bitter orange oil individually or based on CMC and Arabic gum carries. At the same concentration the minimum fungal growth was recorded at lavender oil individually or based on CMC and Arabic gum treatments which recorded as 4,3 and 3 mm, in respective order. Caraway treatments showed the lowest effect on fungal growth. That the fungal linear growth was measured as 7,6 and 5 mm at the highest concentration (8%) of caraway oil individually or based on CMC and Arabic gum, respectively.

The efficacy of essential oils as antifungal inhibitors was reported to be used in plant disease control as antimicrobial compounds (Kaur and Arora, 1999). Many of essential oils have the power to inhibit fungal growth showing fungicidal and fungistatic activities.

In general, they are composed of complex mixtures of volatile, lipophilic, liquid and odiferous substances. They may also be described as volatile oils, ethereal oils and essences (Simões et al. 2003). Volatile oils, flavonoids, cumarins and miscellaneous are the most important components of caraway, bitter orange and lavender oils polyphenols because they possess many biological activities, such as antioxidant, cardioprotective, and anticancer, anti-inflammation, antiaging and antimicrobial properties.
**Table (1): Botanical plant classification and main active principles of their essential oil**

<table>
<thead>
<tr>
<th>Common name</th>
<th>Scientific name</th>
<th>Family</th>
<th>major active components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caraway</td>
<td>Carum carvi</td>
<td>Umbelliferae</td>
<td><strong>Volatile oil</strong>, consisting of carvone (40-60%) and limonene, with dihydrocarvone, carveol, dihydrocarvone, pinen, thujone, and other minor constituents. <strong>Flavonoids;</strong> mainly quercetin derivatives. <strong>Miscellaneous;</strong> polysaccharide, protein, fixed oil calcium oxalate.</td>
</tr>
<tr>
<td>Bitter orange</td>
<td>Citrus aurantium</td>
<td>Rutaceae</td>
<td><strong>Flavonoids,</strong> including limonene, hesperidin, neohesperidin, naringin, and tangeretin</td>
</tr>
<tr>
<td>Lavender</td>
<td>Lavandula officinalis</td>
<td>Labiatae</td>
<td><strong>Volatile oil,</strong> containing linalyl acetate, with linalool, lavandulyl acetate, borneol, camphor, limonene, cadinene, caryophyllene, 4-butanolide, 5-pentyl-5-pentanolide. <strong>Coumarins;</strong> Umbelliferone, herniarin, courmarin, dihydrocoumarin. <strong>Miscellaneous:</strong>* triterpenes e.g. ursolic acid, flavonoids e.g. luteolin</td>
</tr>
</tbody>
</table>


**Table (2): Effect of some essential oils and T. harzianum on Macrophomina phaseolina the causal of sunflower charcoal rot**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Linear growth of Macrophomina phaseolina (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Essential oil (%)</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>Caraway oil (Carum carvi)</td>
<td>67 ± 4.35 bc</td>
</tr>
<tr>
<td>CMC + Caraway oil</td>
<td>61 ± 2.0 bc</td>
</tr>
<tr>
<td>Arabic gum + Caraway oil</td>
<td>60 ± 2.0 bc</td>
</tr>
<tr>
<td>Bitter orange oil (Citrus aurantium)</td>
<td>54 ± 1.0 d</td>
</tr>
<tr>
<td>CMC + Bitter orange oil</td>
<td>56 ± 1.0 d</td>
</tr>
<tr>
<td>Arabic gum + Bitter orange oil</td>
<td>55 ± 2.64 d</td>
</tr>
<tr>
<td>Lavender oil (Lavandula officinalis)</td>
<td>66 ± 2.64 bc</td>
</tr>
<tr>
<td>CMC + Lavender oil</td>
<td>62 ± 2.0 bc</td>
</tr>
<tr>
<td>Arabic gum + Lavender oil</td>
<td>61 ± 2.0 bc</td>
</tr>
</tbody>
</table>

**Antagonism**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Linear growth of Macrophomina phaseolina (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Essential oil (%)</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
</tr>
<tr>
<td>T. harzianum</td>
<td>16.0 ± 1.73 b</td>
</tr>
<tr>
<td>CMC + T. harzianum</td>
<td>16.0 ± 1.73 b</td>
</tr>
<tr>
<td>Arabic gum + T. harzianum</td>
<td>16.0 ± 1.73 b</td>
</tr>
</tbody>
</table>

**Fungicide Concentration (ppm)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Linear growth of Macrophomina phaseolina (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Essential oil (%)</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
</tr>
<tr>
<td>Topsin-M</td>
<td>18 ± 1.73 b</td>
</tr>
<tr>
<td>Control</td>
<td>90 ± 0.0 a</td>
</tr>
</tbody>
</table>

Means ± standard deviations within each column followed by the same letter are not significantly different by Duncan multiple range test at $P < 0.05$. 

Black seed extracts have been reported to possess anti-inflammatory, antioxidant activities and also suppress medical therapy purposes (Ahmad et al., 2013; Al-Khalaf and Ramadan, 2013; Al-Khalaf and Ramadan, 2013; Al-Khalaf and Ramadan, 2013).
2013). Moreover, caraway products are used as sprouting inhibitors, and they also considerably reduce losses caused by storage fungi (Bang, 1999). Caraway essential oil delayed the symptoms appearance of late blight for about 10–14 days throughout on prevention of fungal sporangia production and growth at a low oil concentration under field conditions.

Presented data in Table (2) showed that the tested bioagent *T. harzianum* affected negatively on the *M. phaseolina*. The linear growth of pathogenic fungus measured as 16, 18 and 20 mm in dual culture test against *T. harzianum* individually or based on CMC and Arabic gum, respectively. Similarly, Khaledi and Taheri (2016) found that the biological control capability among 11 *Trichoderma* spp. isolates against *M. phaseolina*, varied from 20.22 to 58.67% in dual culture tests.

In this regards, *Trichoderma* spp. are considered as potential biocontrol agents and growth promoting fungi for many crop plants (Verma et al., 2007; Bai et al., 2008). The genus *Trichoderma* comprises a great number of fungal species. The antagonistic properties of these species are based on the activation of multiple mechanisms.

On the other hand, data also showed that the fungicide Topsin-M (70WP) could drastically inhibit the linear growth of *M. phaseolina*. Complete growth inhibition was recorded at concentration of 5ppm. Similarly, Topsin-M was reported to have high inhibitory effect on *M. phaseolina* growth in vitro. Complete fungal growth inhibition was observed at concentration of 4ppm Abdel-Kader et al., (2010). Also, azadirachtin, mancozeb and bavistin had announced effect on growth and sclerotial survival of *M. phaseolina* in vitro (Dubey and Kumar, 2003). Under greenhouse conditions the fungicides Benlate and Rizolex-T used successfully for controlling *M. phaseolina* and *Fusarium oxysporum* infections and increasing healthy sesame plants (Khalifa, 1997). Under field conditions, Benlate and Rizolex-T as soil treatments reduction in incidence of wilt and root rot diseases of sesame plants were recorded (Gabr et al., 1998). He added that Benlate was very effective in decreasing infection by *M. phaseolina* and *F. oxysporum* when applied at 0.5 g/hill.

**Field experiment**

It was reported that charcoal rot may be a difficult disease to control due to the nature of causal pathogen. At an early stage of plant growth, 1–2 weeks after planting, *Macrophomina phaseolina* invades the roots, whereas the disease symptoms appear later at mature plants (Pearson et al., 1984). Dates of sowing, crop rotation, planting densities, cultivars resistant and irrigation have all been suggested as important ways of disease management in soybean (Bowen and Schapaugh 1989; Todd, 1993). Since other management options are needed, chemical fungicides are being replaced with biocontrol agents because of the emergence of fungicide-resistant fungal isolates and public concerns regarding the health and environmental impacts of these chemicals. Therefore, several potential biocontrol organisms have been isolated, characterized, and commercialized subsequently biocontrol of plant diseases has received more consideration in disease management strategies (Shali et al., 2010).

In the present study, fungicide alternatives, some essential oils and *T. harzianum* individually or based on CMC and Arabic gum as sunflower seed dressing were evaluated throughout two successive growing seasons against charcoal rot disease incidence under natural field conditions. Presented data in Table (3) revealed that all applied seed treatments affect positively on disease incidence compared with untreated control. Data also showed that the recorded percentage of charcoal rot incidence was higher in the second growing season than that in the first one.

*Trichoderma harzianum* found to be the best applied seed treatments that disease incidence was recorded in range (10.0, 11.3, 8.6%) and (13.6, 14.5, 11.8%) at the first and second growing seasons, respectively. It was reported that *Trichoderma* spp. has different mechanisms of biocontrol activity against fungal phytopathogens which could be indirectly through competing for nutrients and space or directly by mycoparasitism and antibiosis (Benitez et al., 2004). These biocontrol agents are effective against soil and/or seed borne fungal diseases of various plants (Kubicek et al., 2001; Zeidan et al. 2005). It is relatively easy for *Trichoderma* populations to be established in different types of soil and the populations can survive for several months (Verma et al., 2007). Etebarian (2006) evaluate the potential biological agents *Trichoderma harzianum* (T39), *T. virens* (DAR74290), *T. viride* (MO), *T. harzianum* (M) and Trichdermin B a commercial formulation of *T. harzianum* (Bi) for the control of charcoal stem rot in melon caused by *Macrophomina phaseolina*. He found that the percentage of healthy plants
with the Trichdermin B, antagonist alone or in combination with the pathogen was significantly higher than in plants inoculated with the pathogen alone. Singh et al. (2004) suggested that cell surface hydrophobicity and electrostatic charge of antagonist Trichoderma spp. may contribute to non-specific adhesion onto the sclerotial surfaces of M. phaseolina that may be influenced by growth and environmental conditions.

Also, the antifungal activity of T. harzianum and T. viride isolates against M. phaseolina, the causal agent of groundnut root rot was revealed by studies of Sreedevi et al. (2011). Abdel-Kader et al. (2010) reported that under greenhouse conditions application of T. harzianum and Topsin-M as integrated treatment had superior effect for suppressing 100% of the ashy stem blight disease incidence of cactus plants Aeonium canariense L. caused by M. phaseolina compared with each individual treatment of either fungicide (86.67%) or the bioagent (73.33%).

In the present study, seed dressing with essential oils proved to have high potential effect for reducing charcoal rot disease incidence under field conditions. It was observed in Table (3) that essential oils based on Arabic gum resulted in lower than that based on CMC or used individually, in ascending respective order. Illustrated data in Fig. (1) showed superior effect of lavender oil resulted in reduction of disease incidence either alone (57.8%) or based on CMC (60.1%) and Arabic gum (63.0%). These treatments followed by bitter orange and caraway oils, respectively. They recorded charcoal rot disease incidence as (45.3, 47.5, 46.9%) and (32.1, 40.8, 43.0%) when applied as seed dressing individually or based on CMC and Arabic gum, in relevant order, compared with the fungicide (22.5%) over untreated control treatment.

### Table (3): Effect of sunflower seed dressing with some essential oils and T. harzianum on charcoal rot incidence throughout two successive growing seasons (2015 – 2016)

<table>
<thead>
<tr>
<th>Seed dressing treatment</th>
<th>Charcoal rot incidence (%)</th>
<th>Charcoal rot incidence (%)</th>
<th>Charcoal rot incidence (%)</th>
<th>Charcoal rot incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caraway oil (Carum carvi)</td>
<td>Growing season, 2015</td>
<td>17.7 ±0.52 c</td>
<td>24.5 ±1.10 c</td>
<td>21.1</td>
</tr>
<tr>
<td>CMC + Caraway oil</td>
<td>16.8 ±0.60 c</td>
<td>20.0 ±1.21 d</td>
<td>18.4</td>
<td></td>
</tr>
<tr>
<td>Arabic gum + Caraway oil</td>
<td>14.5 ±0.96 d</td>
<td>20.9 ±1.25 d</td>
<td>17.7</td>
<td></td>
</tr>
<tr>
<td>Bitter orange oil (Citrus aurantium)</td>
<td>16.3 ±0.45 c</td>
<td>17.7 ±1.22 d</td>
<td>17.0</td>
<td></td>
</tr>
<tr>
<td>CMC + Bitter orange oil</td>
<td>15.9 ±0.20 c</td>
<td>16.8 ±1.32 d</td>
<td>16.3</td>
<td></td>
</tr>
<tr>
<td>Arabic gum + Bitter orange oil</td>
<td>15.4 ±0.72 c</td>
<td>17.7 ±1.83 d</td>
<td>16.5</td>
<td></td>
</tr>
<tr>
<td>Lavender oil (Lavandula officinalis)</td>
<td>11.8 ±0.50 e</td>
<td>14.5 ±1.17 e</td>
<td>13.1</td>
<td></td>
</tr>
<tr>
<td>CMC + Lavender oil</td>
<td>11.3 ±0.43 e</td>
<td>13.6 ±1.47 e</td>
<td>12.4</td>
<td></td>
</tr>
<tr>
<td>Arabic gum + Lavender oil</td>
<td>10.0 ±0.26 e</td>
<td>13.6 ±0.69 e</td>
<td>11.5</td>
<td></td>
</tr>
<tr>
<td>T. harzianum</td>
<td>8.6 ±0.52 f</td>
<td>11.8 ±1.10f</td>
<td>10.2</td>
<td></td>
</tr>
<tr>
<td>CMC + T. harzianum</td>
<td>8.1 ±0.26 f</td>
<td>10.0 ±0.34 f</td>
<td>9.1</td>
<td></td>
</tr>
<tr>
<td>Arabic gum + T. harzianum</td>
<td>7.2 ±0.95 f</td>
<td>9.0 ±1.05 f</td>
<td>8.1</td>
<td></td>
</tr>
<tr>
<td>Fungicide (Topsin-M 2g/kg)</td>
<td>20.4 ±1.51 b</td>
<td>27.7 ±0.90 b</td>
<td>24.1</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>28.6 ±0.72 a</td>
<td>33.6 ±1.05 a</td>
<td>31.1</td>
<td></td>
</tr>
</tbody>
</table>

Means ± standard deviations within each column followed by the same letter are not significantly different by Duncan multiple range test at $P < 0.05.$
During different stages of plant growth soilborne pathogens attacking host plant roots cause severe damage to most agricultural crops resulting in heavy losses of both yield and quality. The rhizosphere, which is defined as the region surrounding a plant roots, that is affected by microflora which occur in the dynamic environment at the interface of root and soil causing root diseases. Since the rhizosphere provides the front-line defense for roots against attack by pathogens, therefore, natural compounds that exist in the rhizosphere could consider ideal and adequate for use as safe fungicide alternative agents for agriculture. Essential oils considered one of potential economic tool for reducing population of plant pathogens in soil. Developed plant disease is difficulty curing, therefore, almost control methods are aimed to protect plants from becoming diseased rather than curing them after they have become diseased. Essential or volatile oils are aromatic oily liquids derived from plant materials, e.g. flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots which their constituents are basically complex mixture of terpenic hydrocarbons and oxygenated derivatives such as aldehydes, alcohols and esters (Table 1). In our study, results indicated that charcoal rot incidence was significantly decreased by using some essential oils and bioagent as seed dressing. These treatments surpassed the used fungicide Tospyn-M for the same purpose. These findings could be explained according to previous authors who stated that essential oils have important ecological functions. One of these functions is to protect the plant against infection by pathogens (Taiz and Zeiger, 1991). Helal et al., (2007) found that essential oil treatment caused plasma membrane disruption and disorganization mitochondrial structure of the pathogen. Some plants contain compounds able to inhibit the microbial growth well established (Naqui et al., 1994). Structures and mode of action of these plant compounds can be different when compared with antimicrobials conventionally used against microbial growth and survival (Nascimento et al., 2000). Plants could synthesize, by the secondary metabolism chemical compounds including flavonoids, isoflavonoids, tannins, cumarins, alkaloids, alkaloids, hencylpropanenes, alkaloids and organic acids which have antimicrobial activity (Nychas, 1996). Campo et al., (2003) Stated that the mode of action on/in the fungal cell as Fungistatic or fungicide effect of essential oils was reported. Cytoplasm granulation, cytoplasmic membrane rupture and inactivation and/or inhibition of intercellular and extracellular enzymes of mould fungi were known as inhibitory action of natural products. These biological events separately or concomitantly could take place with mycelium germination inhibition. Also, it is reported that plant lytic enzymes b-1,3 glycan, b-1,6 glycan and chitin polymers act in the fungal cell wall causing breakage (Brull and Coote, 1999). Inhibiting microorganisms could be attributed to different mechanisms of essential oils and their chemical compounds. Hypothetically the microbial inhibition
attributed to phenolic compounds, which increasing in the permeability and unavailability of vital intracellular constituents through sensitize the phospholipid bilayer of the microbial cytoplasmic membrane (Juven et al., 1994). Reports of Kim et al., (1995) indicated that essential oils containing phenolic compounds, i.e. carvacrol, eugenol and thymol had the highest antibacterial performances.

CONCLUSION
The present results may lead to the conclusion that application of biocontrol agent or essential oils could be considered as applicable, eco-friendly method for controlling such soilborne diseases. Also, the use of essential oils in agriculture could be a suitable alternative for inclusion in disease control systems and could act sometimes as main or adjuvant antimicrobial compounds and do not leave a toxic residue in the product.

CONFLICT OF INTEREST
The authors confirm that there is no conflict of interest for the information presented in this manuscript.

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AUTHOR CONTRIBUTIONS
NSE and MMNA suggested the point of research and designed the experimental work plan and participated in field application. NSE participated in field work and data collection. MMA participated in the field work and statistical analysis of the data. All authors read and approved the final version.

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