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Biological effects of two *Trichoderma harzianum* isolates and mint oil on some postharvest fungal pathogens of sugarbeet

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Abstract

Sugarbeet (*Beta vulgaris* L.) stored in outdoor piles prior to processing, during this storage period the crop is subject to multiple postharvest rots. Some fungal pathogens (*A. niger, A. flavus, Fusarium* sp. and *Rhizopus* sp.) were isolated and identified from rotted sugarbeet. Pathogenicity test of the isolated fungi had different pathogenicity rates using different concentrations of spore suspension at different incubation periods. *A. flavus* showed little number of lesions and negative effect on sugarbeet when tested for pathogenicity. Two antagonists of *Trichoderma harzianum* were tested for their effectiveness on sugarbeet rot fungal pathogens and compared with mint oil. In dual culture, size of clear zone is not correlated with the occurrence of pathogen overgrowth by antagonists. The antagonistic action of the two tested isolates of *T. harzianum* against the isolated phytopathogens indicated different degrees of antagonism. The non-volatile and gaseous volatile organic compounds by the antagonists inhibited growth of phytopathogens on agar plates, and no inhibition was investigated for *Rhizopus* sp. and *Fusarium* spp. by the tested volatile and non-volatile compounds, respectively. Moreover, mint oil showed inhibition degrees as well as volatile compounds of antagonists for all of the tested pathogens except against *Rhizopus*. Sugarbeet treated with mint oil as well as culture filtrate of *T. harzianum* suppressed pathogen spore germination on the surface of sugarbeet root resulted in decreasing rot incidence. This work provides strong evidence that *T. harzianum* and mint oil are competitors to control sugarbeet rot.

1. Introduction

Around 400 vegetable crops include root and tuber are known to be commercially cultivated worldwide [1]. The term "root and tuber crops" is general called for wide subterranean storage organs [2].

Statistics show that, depending on the storage environment and the species type, losses of postharvest agricultural products are considerably high ranging from 30-60 % of the stored produce in 3-6 months of storage duration [3, 2]. Hence, the need for improved, adequate and effective postharvest storage and preservation facilities is emphasized.

The two most important sources of sugar (sucrose) are sugarbeet (*Beta vulgaris* L.) together with sugar cane. However, sugar cane can only be grown in tropical and subtropical regions, sugarbeet is mostly grown all over the world which generally temperate regions [4].

Sugarbeets with its high content of convertible carbohydrates make them suitable for the production of renewable energy [4]. For using sugarbeets in renewable energy (bioenergy), sugarbeets need to be stored with minimum losses of sucrose in a good way for usage through whole of year. Biological control treatments might be the solution for storage problems.

Sugar losses occur and the quality of the sugarbeet decreases during storage. The extent of the decrease depends on the condition of the harvested beet, the storage conditions and the length of the storage period. Guidelines are needed to minimize the sugar losses and the decrease in quality. Even more negative effects on beet processing can be caused by rotting beet and rotten beet parts. Part of the rotten material is removed during washing of the beet, thus contaminating the wash water. Control of rot diseases using fungicides are normally recommended which are not economical and causing environmental hazards. Sharma *et al.* [5] suggested that, biological control is the use of microorganisms to suppress postharvest pathogens, which might be the only one way to prolong sugarbeet storage.

Sucrose losses in addition to accumulation of invert sugar are always accompanied during storage of sugarbeet. It was reported that, the losses in sugar yield between 0.018 to 0.143% per day go together with a fourfold increase in invert sugar [6]. It would be believed that, these losses and enzymatic conversions can be attributed either respiration of plant respiration or the microbes colonization go together with rot development.

Moreover, the mechanical harvesting and topping constitute entry sites for wound pathogens might cause severe injuries (e.g., bruising, cracks, and root tip breakage) which are unable to readily penetrate intact cell wall barriers of the periderm [7-9] might help in postharvest losses. Thus, apparently healthy harvested sugarbeet can display rot symptoms after storage [10, 11]. The most commonly fungal species isolated from

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deteriorated beets were *Botrytis cinerea*, *Fusarium* spp., *Penicillium* spp., and *Phoma betae* [12-14]. Rots of storage beet have been also associated with the bacterial colonization of *Leuconostoc mesenteroides* subsp. *dextranicum* [14, 15].

The control of plant diseases by introduction of antagonistic bacteria, *Bacillus subtilis* and *Pseudomonas cepacia* as well as fungi, *Trichoderma* and *Rhodotorula* [5, 16, 17] was performed. Even after, more of other antagonists have been identified to be used in controlling various plant diseases.

Roots and tubers diseases were controlled by fungal antagonists. Suppress plant pathogens by the fungal and bacterial biological controls use some naturally occurring mechanisms. It was suggested that, the modes of action of biological control might be directly as competition for space, nutrients, siderophore-mediated suppression, antibiosis production, parasitism, cell-wall lytic enzymes, or indirectly by inducing the plant systemic resistance [5]. General, no single mechanism found to be responsible or suitable for biocontrol of pathogen, whereas, more than one mechanism is implicated.

The fungal *Trichoderma* is characterized by its presence everywhere in the environment, especially in the soil. *T. harzianum* is a soil saprophytic fungus which able to become hyperparasitic to several species of fungal pathogen. Since Weindling [18] recognized *Trichoderma* as an antagonist, the antagonistic effect of *Trichoderma* species against fungal plant pathogens have been extensively studied in the field of biological control [19-22].

Efforts of several research groups are performed to find cheap and low toxic alternatives to fungicides application, to control soil-borne pathogens [23, 24]. Plant extracts and essential oils, as safer natural chemicals [25], have long been known to have antimicrobial properties and used to control some plant diseases (26-29]. To increase the efficacy of essential oils in sugarbeet fungal pathogens management, the products need to be studied further. Plant essential oils are produced commercially, and many of these oils correspond to members of the mint family (Lamiaceae). Methanol was reported as the major compound in mint oil. Moreover, the produced essential oils from mint species, when tested on three different fungal species showed its effectiveness for inhibiting fungal mycelial growth *in vitro* at a concentration 1600 µl/l [30]. In addition, menthone and menthol were identified as two major chemical components that collectively comprise 55.8 % of the chemical constituents of mint oil.

Therefore, our goal of this investigation were planned to evaluate two selected *Trichoderma harzianum* isolates for their antagonistic potentials and identify their competitive effects that can be used for the treatment of sugarbeet roots against the isolated rotting fungi in addition to determination of the role of these selected antagonists in protection of sugarbeet from rotting by the isolated postharvest fungal pathogens compared with the essential mint oil.

2. Materials and Methods

2.1. Chemicals

Most of the chemicals used in this study were analytical grade. Sigma (USA), Merck (Germany) and SD Fine Chemicals (India) were companies of the used chemicals in this study where purchased. Mint oil was purchased from "El Baraka" which is located in *Egypt*.

2.2. Plant material

Sugarbeet roots used in these studies were grown in the experimental farms of Faculty of Agriculture, El-Minia University. One sugarbeet (*B. vulgaris* L.) certified hybrid *variety seed* quality *(*Pleno) (multigerm seeds) was grown in a greenhouse during November-April 2017/2018 (~18 weeks with 13 h day and 11 h nights). Beet roots for all experiments were harvested after 16 – 18 weeks of planting, all leaves, vegetative buds and petioles tissue were removed, and the roots were gently washed to remove adhering soil.

Extra cultivation was done to examine effect of mint oil on seed planting, whereas, seeds were treated first with mint oil before grown in the experimental farms of Faculty of Science, El-Minia University. Roots were harvested as well as mention previously.

In clean big polythene bags fruits of beet roots were kept and transferred to the laboratory, and were stored at temperature ranged between -3 ℃ to -21 ℃ before use. Tap water and Detol soap were used to wash roots before doing any treatments, then the surface was disinfected by spraying with 70% ethanol and cleaned with sterile water, then by spraying with 10% hypochlorite and cleaned again with sterile water, and finally, dried prior to use.

2.3. Fungal Antagonists and their culture media

The two fungal antagonists T3 and T24 of *Trichoderma harzianum* were taken from Microbial Research of Botany and Microbiology MRBM Department, Faculty of Science, Minia University, Egypt, where they were stored at 4 ℃. The two antagonists were previously isolated from the soil samples collected from Minia Governorate and morphological identified by DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany [22]. El-Katatny [22] studied previously the antagonistic actions of these isolates, and their potentials as biocontrol agents against some phytopathogenic fungi were suggested [31, 32].

The culture filtrates of *Trichoderma* spp. were obtained by culturing fungi in flasks of potato dextrose broth (PDB). Flasks were inoculated with agar plugs of 7 day-old cultures, and incubated for 5 days at 28±2 °C. The biomass was removed by filtration and the culture filtrate was used for anti-fungal tests. Culture filtrate of PDB cultures were separately suspended in a full strength (undiluted, 100 %), 50 % and 25 % dilution of the filtrate. Culture filtrate of the fungus was sterilized and filtered through 0.22 µm Millipore filters.

2.4. Isolation of pathogenic fungi from healthy sugarbeet fruits

Three healthy sugarbeet roots were washed with 100 ml sterilized distilled water for 5 minutes. Before pouring potato dextrose agar (PDA) contains Rose Bengal to a sterilized Petri dish, the washing water (One ml) was transferred aseptically. In addition, six sugarbeet fruits were incubated in sterilized clean plastic bags for 7 days at 28 ± 2 °C. The rotten fragments that contain growing moulds were transferred to Petri dishes contain PDA with Rose Bengal. Six plates containing PDA were prepared for each. Plates were kept for 7 days, at 28 ± 2 °C. The resulted fungal organisms were examined microscopically to be identified.

2.5. Isolation of pathogenic fungi from diseased sugarbeet fruits

Six sugarbeet roots with fungal rot symptoms (diseased) were collected from different farm sites at Minia governorate, then samples were transferred to our Lab. in clean plastic bags. The rotten fragments from the fruit surface were picked up aseptically and transferred to PDA contain Rose Bengal. Plates were incubated at 28 ± 2 °C for 3-5 days. The fungal growing molds were sub-cultured on separate sterile PDA plates and the resulting fungi were microscopically examined to be identified.

2.6. Preparation of antagonist and pathogen inocula

Trichoderma harzianum (T3 and T24) spore suspension was prepared by mixing the harvested conidia from grown slants cultures on PDA (7-day-old) in sterile water and the density of population was determined by dilution plate method [33]. Concentration of spore suspension was adjusted as colony forming unit (CFU) per milliliter of distilled water as required for each experiment.

Inocula of fungal pathogens were prepared by using pure culture plates of 7 days old, except for *Fusarium* species; the incubation period of cultures was 14 days. After plates were flooded with sterilized distilled water, conidia were scraped and the unwanted mycelial debris was removed by filtration. As previously mentioned, conidial concentration was determined according to Chung and Hoitink, [33], and the concentration for each experiment was calculated to concentrations that required for experiments using sterilized distilled water and expressed as CFU ml^{-1}. The population of spore suspension always reached to concentrations of 1×10^5, 1×10^6 and 1×10^7 colony-forming units (CFU) ml^{-1} for either antagonists or sugarbeet pathogens.

2.7. Pathogenicity test on healthy sugarbeet fruits

In this study, suagrbeet fruits free of visible damage and disease symptoms were used. Before infection with pathogen the fruits were washed and their surface was disinfected, then the fruits were individually sprayed with pathogen spore suspension at different concentrations (10^5, 10^6 and 10^7 CFU ml^{-1}) for about 5 minutes with stirring. Sterile distilled water instead was used for control. The sprayed roots were placed in sterile polythene clean bags contain moistened wet filter papers to produce a micro-humidity chamber; the incubation was done at 28 ± 2 °C. Three roots were used as a replicate in each polythene bag. Roots were

monitored after incubation period (7 days) and the intensity of fruit rotting was evaluated by the equation of decay index DI [34]:

DI equation: $$\frac{1 \times N1 + 2 \times N2 + 3 \times N3 + 4 \times N4 + 5 \times N5}{5 \times NT}$$

Where, *N*1 to *N*5 is fruit's respective quantity of each scale and *NT* is the total number of fruit examined. Each treatment consisted of three replications and each replication contained 15 fruit roots. The experiment was repeated twice.

2.8. Growth of the antagonists and fungal pathogens on different agar media

Three different types of media, i.e. Czapek's agar (synthetic medium), potato dextrose agar PDA (semi-synthetic medium) and beet extract agar (natural medium) in Petri dishes were prepared. PDA was prepared by boiling 200 g unpeeled, sliced potato per distilled water for 30 min, filtration through cheesecloth was done, and then the effluent was kept. And then was mixed with dextrose (20 g), Agar (20 g), and followed by boiling to dissolve the mixture. Czapek's agar medium contains the following; glucose, 20 g, NaNO3 2.0 g, KH2PO4, 1g, KCl 0.5 g, MgSO4.7H2O, 0.5 g, FeSO$_4$.7H$_2$O, 0.01, agar, 20 g in 1000 ml distilled water. Beet extract agar medium contained 200 g of sugarbeet cooked in 1000 ml distilled water and 20 g agar. Media were autoclaved and poured into sterilized plates. The inoculum was a circular disc (8 mm diameter) for each of the selected fungal species (antagonists or pathogens) that were individually cut using sterile Cork borer from the edge of colony growing on PDA, then it was placed in the center of agar plate culture. Three replicates were prepared for each investigation, and plates were incubated at 28 ± 2 ° C, whereas, the growing colonies were daily observed. The radial growth was measured daily for 6 days until the colony reached to the edge of the plate. Growth percentage of each fungus on the three different media was compared with the least values in the schedule (daily growth rate of fungi on Czapek's medium).

2.9. In vitro antagonistic activity of Trichoderma isolates

Antagonistic potential of *Trichoderma* spp. against the isolated phytopathogens was tested on solid agar plates by the technique of dual culture; in another investigation production of volatile and non-volatile organic compounds (VOCs) was tested.

2.9.1. Dual-culture-plate method

As described previously phytopathogen were individually tested in dual-culture plated method with either *T. harzianum* T3 or T24 [35]. The area between the two colonies of dual plates at the interaction point was investigated for measuring of clear zone size (mm) after three days of incubation. Antagonist overgrowth on the test fungal pathogen was examined and photographed using digital camera. Assessment of interactions was made for further 4 days. An assessment scale from S1-S5 was done for the antagonistic potential on modified Bell's scale [36].

2.9.2. Test of volatile organic compounds (VOCs)

Method of inverted plates or the sandwich plate method was used to examine the inhibition effects of *T. harzianum* isolates (T3 and T24) against the four postharvest fungal pathogens of sugarbeet by production of volatile compounds *in vitro*. The method followed that of Dennis and Webster [37] (sealing agar plate method), whereas, individual disc of antagonist was inoculated on PDA and the lid of the plate was replaced by the bottom of fresh disc culture of the tested fungal phytopathogen. The two halves of the plate were taped (sealed) together by Parafilm. Control of phytopathogen was considered when Petri dish of pathogen inverted on fungi-free agar media. All of the incubation procedures were performed at 28$\pm$°C under light-limited conditions to control the sporulation of *Trichoderma*. The tests were conducted in triplicate. After different incubation periods (1-7 days), diameter of the tested phytopathogen was measured, and the growth inhibition percentage was calculated by the following formula [38]:

$$inhibition\ [\%] = \frac{A_{control} - A_{treated}}{A_{control}} \times 100$$

A $_{control}$ is daily growth rate of pathogen in control;

A $_{treated}$ daily growth rate of pathogen in treatment

An extra investigation was done using the method of sandwich plate or inverted plate method to examine the *in vitro* inhibition effects of mint oil against the four isolates of postharvest fungal pathogens by making wells contain oil in the lower PDA dish. After the same incubation period (1-7 days), diameter of the tested phytopathogen was measured, and the growth inhibition percentage was calculated by the same formula of Fahri and Dikilita, [38].

2.9.3. Test of non-volatile organic compounds

Non-volatile compounds were checked following the agar plate diffusion method using PDA. The antagonists (T3 and T24) were grown in agitated (120 rpm) PDB liquid culture. The 7-day-old cultures filtrate was filter and sterilized for bioassay at 100 % on PDA against the postharvest fungal pathogens using well diffusion method; briefly, sterilized culture filtrate (0.2 ml) was transferred to wells (8 mm) on Petri dish agar plates containing conidial suspension of the tested pathogens. The ability of the sterilized culture filtrate for inhibition was done by measuring size of clear zone around well [39].

2.10. Effect of antagonist culture filtrates and mint oil on beet fruits rot

Culture filtrates of *T. harzianum* (T3 and T24) were obtained by growing these fungi in liquid culture potato dextrose broth PDB. Flasks (100 ml) were inoculated with 8 mm agar plugs from 7 day-old cultures of antagonists on (PDA) and incubated for 7 days at approximately 28$\pm$ °C. Biomass was removed by filtration and the culture filtrate was sterilized through a membrane of 0.22 μm pore size to be used in the antifungal test. Roots previously infected with individual pathogens (1x10^7) were sprayed with the full strength culture filtrate (100%) of *T. harzianum* (T3 and T24) and also with 50% and 25% dilutions for about 5 min. Moreover, samples of sugarbeet roots were sprayed with mint oil in addition to sterile water (as control). The beet roots then were placed in clean polythene bags, and incubated at 28±2 ℃. Each particular treatment consists of three replicates in polythene bags and the bag contained 3 beet roots as a replicate as well as untreated check (-ve control, healthy; +ve control, infected). In this experiment the harvested sugarbeet roots were performed using seeds of sugarbeet treated with and without mint oil. 18 bags were used for each postharvest isolated fungal pathogen (9 bags for beet roots of seeds treated with mint oil and the other 9 bags were beet roots of seeds without mint oil). The experimental design was performed as follows; seeds without mint oil: treatment 1, healthy (-ve control, not infected, untreated); treatment 2 +ve, infected only and untreated roots; treatment 3, roots sprayed with mint oil; treatment 4, roots sprayed with T3 culture filtrate 25 %; treatment 5, 50 %; treatment 6, 100 %; treatment 7, roots sprayed with T24 culture filtrate 25 %; treatment 8, 50 %; treatment 9, 100 %.

Sugarbeet roots were monitored after periods of 3, 5 and 7 days. Root rot intensity was measured for each fungal isolate and calculated using the decay index (DI) as previously mentioned [34].

2.11. Statistical analysis

In all experiments of this study, samples were used randomly. Data were expressed as mean$\pm$ SE and the statistical analysis were done using SAS Software (Version 10.0 for Windows). In addition, statistical difference between treatments was analyzed by one-way analysis of variance (ANOVA).

3. Results and Discussion

Economical losses for freshly harvested fruits and vegetables were caused greatly by postharvest pathogens. Each of fungi and bacteria are responsible for postharvest diseases [40]. Fungal biocontrol products with pesticidal activity are being explored in order to make available as bio-pesticides, which are easily biodegradable, selective and can be easily produced, especially after the wide range of harm effects of synthetic pesticides.

Commonly it is believed that vegetables with their less acidic nature than fruits are more prone to be attacked by bacteria; however, in many cases fungi also are equally considered responsible for rapid spoilage. Numerous minutes' spores or conidia are produced by fungi, and these structures distribute readily in the surrounding causing rapid spoilage of vegetables and fruits. In this study, postharvest rots of sugarbeet were caused by fungi. The isolated fungi were *Aspergillus*, *Fusarium*, and *Rhizopus*. These pathogens were previously reported and

described as serious postharvest pathogens causing rapid spoilage of vegetables and fruits [41].

Biological control is one of the most promising alternatives method to fungicides, whereas, microorganisms of biocontrol agents protect fruits and vegetables from phytopathogens infection [42-44]. *Trichoderma* as a biological control agent has been proved to suppress certain diseases in a great number of studies [35, 45-47]. In this investigation the potentials of *T. harzianum* (T3 and T24) as antagonists in suppressing fungal postharvest sugarbeet roots pathogens were examined, whereas, some of these fungal pathogens were previously reported as heavy losses in storage and transit of beet roots [11, 48]. Moreover, this study addresses the antagonistic actions of *T. harzianum* against some isolated fungal postharvest pathogens of sugarbeet. The tested *T. harzianum* had inhibitory effect against these fungal pathogens which could be suggested to produce extracellular metabolites that have potential to control sugarbeet fungal root rot.

Several *Trichoderma* spp. have been performed to protect commercially important fruits and vegetables such as banana, apple, strawberries, mango, potato, and tomato during their storage [49]. In addition, biofungicide of *Trichoderma* based, TRICHODEX (Makhteshim Chemical Works Ltd., Beer Sheva, Israel) is commercially known and be available for suppressing of *Botrytis cinerea* [50, 51].

3.1. Fungi associated with healthy and diseased Sugarbeet roots

Several molds are responsible for the postharvest beet root decay; however, *Botrytis cinerea*, *Fusarium* spp., *Penicillium* spp., and *Phoma betae* were frequently isolated species from deteriorated beets [12, 13, 52]. Many of these pathogens are known to cause postharvest losses in storage clamps of beets [53].

A total of six fungal pathogens were isolated from healthy or rotten sugarbeet roots (**Figure 1**). All of these isolates were identified morphologically on the basis of colony, hyphal morphology of fungi cultures and spores characteristics. The six isolated postharvest fungal pathogens of sugarbeet were belonged to three genera and five different species. These fungal pathogens were identified as *Rhizopus* sp., *A. flavus* and *Fusarium* sp. (1) from the diseased sugarbeet, whereas, *Rhizopus* sp., *A. niger* and *Fusarium* sp. (2) from healthy roots (**Table 1**). Each isolate were sub-cultured into a PDA agar plates and stored at 4°C for further studies. All fungal pathogens were deposited in the strain culture collection of MRBM Department, where they were stored at 4°C.

In this study, *Rhizopus, Aspergillus* and *Fusarium* species were most commonly involved in suagrbeet rot and are consistent with previous reports [11, 54-56]. Previously, Hanson [55] suggested that, *Rhizopus* and *Fusarium*, were the most pathogenic isolated (50- 60 %) from these beets, in addition to *Rhizoctonia solani* Kühn.

(**Figure 2**) shows the percentage of frequency isolated fungi from healthy (Groups I) and diseased (Groups II) groups of the tested samples of beet roots. Mycological analysis of rotten

sugarbeet samples (Grousp II) showed three fungal species belonged to three genera. *Rhizopus* sp. caused rotting in 60% of the examined diseased sugarbeet roots. *A. flavus* and *Fusarium* sp. (1) came next with 20 % for each of the total samples. According to healthy beets of Group (I), *Rhizopus* sp. was recorded again as a high frequency of occurrence (50 %) followed by *A. niger* (40 %). Finally, healthy beet roots in Group (I) reported low frequency of occurrence by 10 % samples as *Fusarium* sp. (2) (**Figure 2**).

Previously, *Rhizopus* was isolated from diseased vegetables like potato, beet and pumpkin, and this disease was commonly called as *Rhizopus* rot, which was reported as the second most leading postharvest rots of vegetables. The diseased fruit and vegetable with *Rhizopus* is known to be quickly decomposed, whereas, the skin readily slips of infected fruit. The flesh becomes brown, very soft, and soon collapses which is consistence with results of this study [56].

3.2. Pathogenicity test on healthy sugarbeet roots

Pathogenicity test had different pathogenic rates by measuring the decay index (DI) of each of the isolated fungi (5 different isolates) from both infected and healthy sugarbeet roots (**Table 2**). The concentration of pathogen spore suspension had significant effect on pathogenicity effectiveness, whereas, the higher concentration of 10^7 CFU ml^{-1} for most pathogens showed the greater decay indexes (DI, 270, *Rhizopus* sp.) at 7-days of incubation period. Moreover, the more prolongation of the incubation period of roots with the pathogen, the greater Decay Index was recorded. In addition, root rots intensity after 7 days was higher than incubation period of 3 or 5 days.
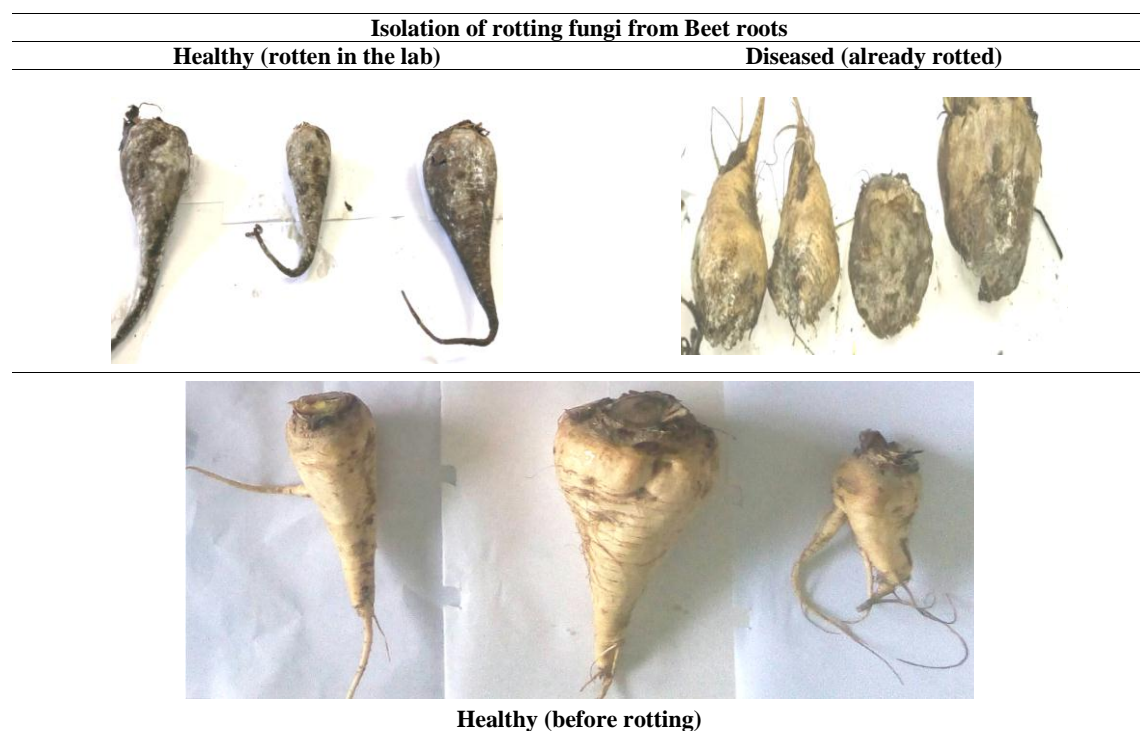
In this study all the tested fungi were virulent; however, *Rhizopus* sp. was the most virulent pathogen showing the highest decay index for all examined concentrations (10^5, 10^6, 10^7 CFU ml^{-1}) as well as at the three different incubation periods (3, 5 and 7 days). Soft rots of fruits, vegetables, and root crops, were well reported for causing by *Rhizopus* especially in postharvest storage conditions [57, 58]. Moreover, *Rhizopus* grows intensively even on refrigerated fruits [59].

In contrast, *A. flavus* showed a little number of lesions (-ve effect) after the three tested incubation periods using either low or high concentration of spore suspension (**Table 2**). Enzymes production is responsible and correlated with pathogen virulence (against plants) as well as living tissues maceration. *A. flavus* is known as a common saprophyte and is also having an opportunistic as a pathogen where it has been isolated from insects, birds, mammals, and plants. However, it is not typically highly virulent in healthy living tissues [60].

The enzymes could be responsible in nutrient capture carbon source accessibility, rather than as pathogenicity or virulence factors [60]. In another study, low or high virulence isolates of *A. flavus* investigated the hydrolytic capacity of enzymes; it found increasing in the levels and numbers of amylase isozymes for the high virulence isolate [61]. The Functional analysis of *A. flavus* library expressed sequence tag (EST) revealed a number of genes that could be correlated with fungal virulence or pathogenicity, that responsible for enzymatic activities [62].

Table 1: The frequency of fungi occurred on healthy which left for one week in the lab. and diseased postharvest sugar beet root.

Frequency of fungi occurred on healthy and diseased beet roots			
Healthy		Diseased	
Species	%	Species	%
- *Rhizopus* sp.	50	- *Rhizopus* sp.	60
- *A. niger*	40	- *A. flavus*	20
- *Fusarium* sp. (2)	10	- *Fusarium* sp. (1)	20

Isolation of rotting fungi from Beet roots	
Healthy (rotten in the lab)	Diseased (already rotted)

Healthy (before rotting)

Figure 1: Diseased (already rotted), healthy (then rotted in lab) and healthy beet roots

Table 2: Pathogenicity of the isolated fungi (5 different organisms) using three different concentrations (10^5, 10^6, 10^7 CFU ml^{-1}) after 3, 5 and 7 days of inoculation period.

Incubation period (day)	Decay Index (DI) of the isolated fungal pathogens				
	Rhizopus sp.	*A. niger*	*Fusarium* sp. (1)	*Fusarium* sp. (2)	*A. flavus*
3 d.					
- 10^5	32.2	30.6	25	27.3	
- 10^6	81.6	76	32	40	
- 10^7	101.6	97.3	48	90	
5 d.					
- 10^5	53.3	48.2	42	45.5	Little number
- 10^6	151.6	140	54.7	80.3	of leisons
- 10^7	200	166.4	90.4	118.5	(-ve effect)
7 d.					
- 10^5	150	66.7	59.1	64.4	
- 10^6	218.4	153.1	145.2	167.3	
- 10^7	270	250	173.3	180	

DI equation: $\dfrac{1 \times N1 + 2 \times N2 + 3 \times N3 + 4 \times N4 + 5 \times N5}{5 \times N_T}$

N1: Healthy fruit
N2: one lesion smaller than 1cm in diameter
N3: one lesion bigger than 1cm but the decay area smaller than 25% of fruit surface
N4: Decay area between 25% to 40% of beet fruit surface
N5: Decay area bigger than 40% of fruit surface
N_T: The total number of fruits examined

Regardless the prominence of enzymes maceration tissues as important factor of fungal virulence, there are probably some factors other than enzymes might play significant roles in successful invasion of plants by *A. flavus*.

3.3. Growth of the selected fungal species on different media

The effects of the natural medium of sugarbeet juice agar and the synthetic one (Czapek's agar) as well as semisynthetic (PDA) on the radial growth of the tested fungal isolates were presented in **(Table 3)**. The growth of the all tested fungal organisms was promoted in various degrees on PDA or sugarbeet juice agar compared with growth on Czapek's agar, except for the two tested antagonists (T3 and T24) in which the mycelial growth was lowered on beet extracts compared with Czapek's agar medium **(Table 3)**. The two tested antagonists may have low metabolic capability to assimilate beet pulp nutrients. Low growth of antagonsists on beet extracts could be the advantage for using formulations of these microorganisms against the causal agents for sugarbeet rots.

Previously some different fungal genera such as *Mycochaetophora, Periconia, Alternaria*, *Chaetomium*, *Lyconectria, Drechslera, Penicillium, Dactylonectria, Absidia* and *Embellisia* were reported to have well growth in the beet pulp medium, as deduced from visual inspection. However, other fungal genera belonging to other taxa such as *Epichloë, Tolypocladium, Slopeiomyces, Darksidea* and *Microdochium,* did not grow well on the beet pulp medium although these strains grew well on PDA [63].

Although mycelial growth of *Rhizopus* sp. wasn't highly promoted on beet extract agar, it showed the same range of growth on Czapek's agar medium **(Table 3)**. The most promoted isolate on beet extract agar medium was *Fusarium* sp. 1 which showed 68.84 % increasing in growth diameter, however, *A. niger* and *Fusarium* sp. 2 came next and the growth diameter was promoted more than 50 % which recorded 55.39 and 50.15 %, respectively **(Table 3)**. Growth of the tested fungi was found to occur on all tested culture media, but on the semisynthetic solid medium was more favorable for fungal growth. The present finding is in conformity with the previously suggestion where PDA has the simple formulation and more nutrient contents, supporting the best mycelial growth of fungi [64]. Behavior of fungal growth on liquid media for all the tested fungi (antagonists and phytopathogens) showed the same pattern of growth on agar solid media (data not shown).

3.4. In vitro antagonistic tests of Trichoderma isolates

3.4.1. Antagonism in dual culture

On agar dual plates, first contact between the antagonists and any of the four tested fungal pathogens was observed within three days of incubation, except for *Rhizopus* sp., where the contact came earlier (two days). There are different degrees of antagonism by *Trichoderma harzianum* (T3 and T24) against the tested postharvest fungal pathogens **(Table 4).** Differences were found previously in *Attamyces* sp. mycelial growth inhibition caused by the antagonistic strains of *Gliocladium* sp.

and *Trichoderma* sp., which suggested to be among the physiological differences between these strains, and these variations could be due to the mechanism involved in the antagonistic activity by differential secretion of antifungal substances or killing pathogen by direct exploitation [51, 65]. It was reported that species of *Trichoderma* were effectively selective against pathogenic fungi [66, 67].

In dual plates, clear zone was observed after the 3$\underline{rd}$ day of incubation, in addition size of clear zone varied between treatments according to the phytopathogen and the antagonist **(Table 4)**. Mostly, *T. harzianum* T24 represented narrow clear zone against the tested sugarbeet pathogens compared with T3. The range of clear zone measured as 3-6 and 1-4 mm when T3 and T24, respectively were tested in dual culture plates against pathogens. Although, no inhibition zone was shown against *Rhizopus* sp., it was overgrown by S2 (more than 60 %) and S1 (more than 80 %) of Bell scale by T3 and T24, respectively **(Table 4)**. T3 as antagonist against *Fusarium* sp. (2) gave the widest clear zone of 6mm **(Table 4).** Generally, T3 showed relatively wide clear zones in dual plate against *Fusarium* spp., although the antagonist failed to overgrow these pathogens **(Table 4)**. There was no correlation between zone of inhibition and the occurrence of overgrowth by antagonists [22]. T3 showed wide clear zone against *A. niger* and *Fusarium* spp. **(Table 4)** and couldn't able to overgrow it. Furthermore, T3 and T24 produced no clear zone against *Rhizopus* sp. **(Table 4)**, at the same time both of them overgrew it by 60 % and more than 80 %, respectively (S1, S2 of Bell scale).

Mukherjee and Raghu [68] reported that, there was no correlation for the ability of *Trichoderma* to produce antifungal antibiosis and their activity to control the disease. This is consistent with our result. Moreover, it was reported before the metabolites production as fungitoxic compounds might not be the primary mechanism of biocontrol agents. Whereas, it might be according to other mechanisms, such as, competition, inhibition or direct killing of the pathogen hyphae [68]. In addition, the increase in fungal inhibition corresponded with incubation period, whereas, pathogen aggressive inhibition was noticed at 12 days of incubation period

3.4.2. Inhibition effect of volatile compounds

The gaseous metabolites released by the antagonists inhibited the growth of the tested postharvest pathogens that inoculated in the inverted Petri dish agar plates. The pathogens grew covering whole plates in control cultures, while in contact with volatile compounds from antagonistic strains (*Trichoderma harzianum* T3 and T24) the microbial growth of pathogen was significantly restricted **(Table 5)**. The maximum inhibition was 55.84 % and produced by *T. harzianum* T3 against *A. niger* after 5 days of incubation period. The weakest recorded effect of VOCs by the tested antagonists was shown against *Rhizopus* sp. **(Table 5)**, which started as low inhibition effect after the first day of incubation and decreased in the second day to be zero inhibition at the third day of incubation period. The other three postharvest pathogens were greatly inhibited by VOCs produced by *T. harzianum* T3 and T24 as well as mint oil. Interestingly, mint

Table 3: The colony radial growth and daily growth rate (Cm) for all tested fungal species on three types of agar media (Czapek's, potato dextrose agar PDA and beet extract media) during incubation period 1-6 days .

	Fungal Growth (Cm)																				
	Cz							PDA							Beet extract						
	1d	2d	3d	4d	5d	6d	Daily growth rate	1d	2d	3d	4d	5d	6d	Daily growth rate[a]	1d	2d	3d	4d	5d	6d	Daily growth rate[a]
Antagonists																					
T. harzianum T3	2.57	4.07	6.37	9.00	N.D	N.D	**5.50**	3.07	5.03	8.10	9.00	N.D	N.D	**6.30 (14.55)**	1.13	2.90	5.23	7.83	9.00	N.D	**5.22 (-5.09)**
T. harzianum T24	2.39	5.03	7.67	8.60	9.00	N.D	**6.54**	3.10	6.13	9.00	N.D	N.D	N.D	**6.08 (-7.03)**	1.21	3.50	6.73	9.00	N.D	N.D	**5.11 (-21.87)**
Pathogens																					
Rhizopus sp.	6.92	9.00	N.D	N.D	N.D	N.D	**7.96**	6.98	9.00	N.D	N.D	N.D	N.D	**7.99 (0.38)**	7.05	9.00	N.D	N.D	N.D	N.D	**8.03 (0.88)**
A. niger	0.87	2.77	5.30	6.23	6.37	6.83	**4.73**	2.50	3.40	5.62	6.25	6.43	7.92	**5.35 (13.11)**	3.92	6.03	7.87	8.37	8.93	9.00	**7.35 (55.39)**
Fusarium sp. 1	1.01	1.77	2.53	3.07	3.91	4.27	**2.76**	1.71	2.67	3.63	4.67	5.87	6.60	**4.19 (51.81)**	1.83	2.73	3.93	5.97	6.58	6.90	**4.66 (68.84)**
Fusarium sp.2	1.14	1.67	2.90	4.20	4.87	5.53	**3.39**	1.80	3.03	4.17	5.75	6.63	7.35	**4.79 (41.30)**	2.33	3.43	4.37	5.58	6.86	7.97	**5.09 (50.15)**

a: The number between parentheses indicates the percentage of promotion of growth on the two media (beet extract agar and potato dextrose agar (PDA media) comparable with that on Czapek's medium.

N.D. : Not determined

Table 4: Screening the potential of *Trichoderma* spp. (T3 and T24) against the pathogenic fungi (*Rhizopus* sp., *A. niger*, *Fusarium* sp. 1 and *Fusarium* sp. 2) after 7 days.

	Antagonists			
Phytopathogens	Type of interaction		Inhibition zone (cm) formed (after 3 days)	
	T_3	T_{24}	T_3	T_{24}
Rhizopus sp.	S2	S1	0.0 ± 0.00	0.0 ± 0.00
A. niger	S5	S5	0.3 ± 0.15	0.1 ± 0.00
Fusarium sp. (1)	S5	S4	0.3 ± 0.05	0.4 ± 0.05
Fusarium sp. (2)	S5	S4	0.6 ± 0.00	0.1 ± 0.00

The results of viable counts are expressed as mean ± SE after log transformation. Mean is the value among treatments and SE is the standard error value for each treatment

S1 – S5 : Antagonistic potential on modified Bell's scale (after observation 7 days)

S1 = Antagonist completely overgrew the pathogen (more than 80% overgrowth)

S2 = Antagonist overgrew at least 2/3 growth of the pathogen (more than 60 % overgrowth)

S3 = Antagonist colonized on half of the growth of the pathogen (more than 40 % overgrowth)

S4 = Antagonist colonized on quarter of the growth of the pathogen (more than 20 overgrowth)

S5 = Formation of clear zone between colonies and no overgrowth was observed
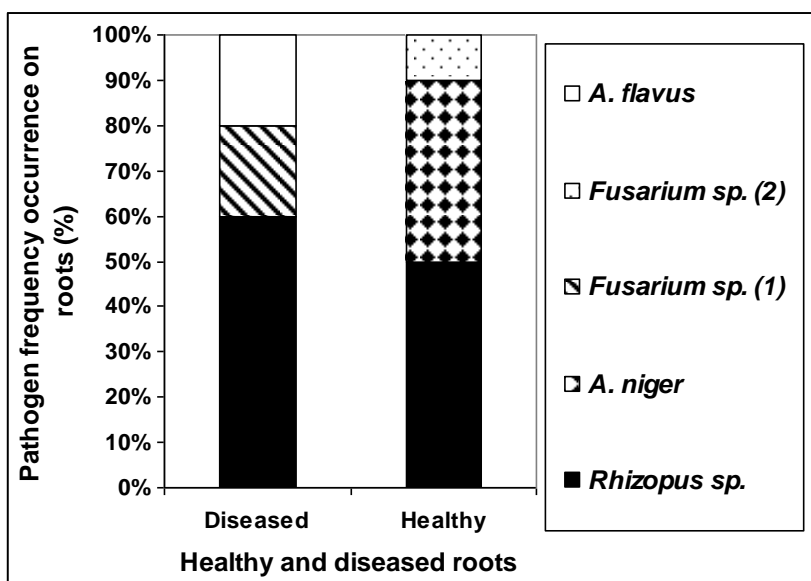
Figure 2: Frequency of fungi occurrence on both healthy and diseased sugarbeet roots.

Table 5: Test of volatile compounds produced by *Trichoderma* (T3 and T24) and mint oil against phytopathogenic fungi (*Rhizopus* sp., *A. niger*, *Fusarium* sp. 1 and *Fusarium* sp. 2) on PDA expressed as inhibition percentage of pathogen mycelia daily growth rate after different incubation periods (1-7 days) compared with control.

Antagonists	Pathogen of inhibition by volatile compounds of antagonists and mint oil (%)			
	Rhizopus sp	*A. niger*	*Fusarium* sp. 1	*Fusarium* sp. 2
After 1 day				
- *T. harzianum* **(T3)**	11.57 ± 0.96	20.36 ± 1.34	ND	ND
- *T. harzianum* **(T24)**	12.56 ± 0.96	20.36 ± 1.64	ND	ND
- Mint oil	8.88 ± 0.00	5.17 ± 1.23	ND	ND
After 2 days				
- *T. harzianum* **(T3)**	7.41 ± 2.14	36.0 ± 1.60	20.81 ± 2.69	2.89 ± 0.96
- *T. harzianum* **(T24)**	9.63 ± 1.032	38.58 ± 3.75	23.91 ± 0.00	0.00 ± 0.00
- Mint oil	0.00 ± 0.00	28.75 ± 3.12	21.58 ± 2.33	5.77 ± 1.92
After 3 day				
- *T. harzianum* **(T3)**	0.00 ± 0.00	44.66 ± 1.51	20.12 ± 2.08	11.6 ± 3.08
- *T. harzianum* **(T24)**	0.00 ± 0.00	23.91 ± 2.63	21.68 ± 0.52	15.89 ± 3.76
- Mint oil	0.00 ± 0.00	23.91 ± 1.05	23.67 ± 4.58	17.06 ± 1.17
After 4 day				
- *T. harzianum* **(T3)**	0.00 ± 0.00	55.07 ± 1.38	25.25 ± 3.34	22.99 ± 3.16
- *T. harzianum* **(T24)**	0.00 ± 0.00	27.64 ± 2.42	14.04 ± 2.91	29.16 ± 1.62
- Mint oil	0.00 ± 0.00	37.31 ± 3.41	31.48 ± 4.55	21.96 ± 3.97
After 5 day				
- *T. harzianum* **(T3)**	0.00 ± 0.00	55.84 ± 0.82	27.57 ± 1.35	24.56 ± 2.01
- *T. harzianum* **(T24)**	0.00 ± 0.00	27.64 ± 2.14	18.97 ± 2.55	30.98 ± 1.47
- Mint oil	0.00 ± 0.00	29.72 ± 3.65	39.68 ± 3.63	18.41 ± 4.41
After 6 day				
- *T. harzianum* **(T3)**	0.00 ± 0.00	51.04 ± 3.76	31.86 ± 3.02	34.83 ± 3.91
- *T. harzianum* **(T24)**	0.00 ± 0.00	28.33 ± 3.79	20.25 ± 4.88	34.83 ± 2.56
- Mint oil	0.00 ± 0.00	26.04 ± 3.61	40.43 ± 4.23	31.09 ± 3.95
After 7 day				
- *T. harzianum* **(T3)**	0.00 ± 0.00	49.26 ± 1.96	36.85 ± 3.78	42.28 ± 3.39
- *T. harzianum* **(T24)**	0.00 ± 0.00	30.55 ± 3.9	26.57 ± 3.92	43.37 ± 1.78
- Mint oil	0.00 ± 0.00	21.67 ± 1.7	45.91 ± 2.52	37.88 ± 2.36

The results of inhibition are expressed as mean ± SE. Mean is the value among treatments and SE is the standard error value for each treatment

oil for sometime gave lower inhibition against some fungal pathogens than *Trichoderm* species did, and it represented the same levels or more of inhibition against others. The antagonistic efficacy of volatile compounds by *Trichoderma* spp. against *F. oxysporum* was reported by Padmodaya and Reddy [69]. The major advantage of antibiosis *via* volatile compounds is the ability of these produced substances by the antagonists to be passed and diffused through air-filled space pores and help to check the target root rot pathogens without creating any physical contact with them.

Among VOCs of the two isolates of *T. harzianum* (T3 and T24), their effects on the tested fungal pathogens did not differ significantly. Morphological changes in the colonies by VOCs were not shown, which otherwise displayed normal growth characteristics (Data not shown). Prolongation of incubation period mostly improved VOCs inhibition effect (**Table 5**).

The lower growth inhibition of *Rhizopus* sp. by either volatile compounds of *Trichoderma* spp. or mint oil decreased with prolongation of incubation periods which became zero at the third day. Accordingly, the growth of the target fungus (pathogen) was stimulated instead of inhibition with prolongation of incubation period. It is likely that the weak antifungal effect of these metabolites from antagonists or mint oil stimulated the defense mechanisms of the target fungi, which were able to overcome of these stresses by growing more rapidly. Stimulated defense mechanism by the pathogens was previously reported [70], moreover, growth stimulation of wood decayers also occurs by chemical wood preservatives in low concentrations [71].

3.4.3. Inhibition effect of non-volatile compounds

Production of non-volatile organic compounds(non-VOCs) by the two tested *Trichoderma* spp. (T3 and T24) against the isolated postharvest fungal pathogens of sugarbeet are presented in (**Table 6**), whereas, clear zones were formed in well diffusion test on agar plates, and the inhibition zones reached to 0.69 cm. The low sensitive phytopathogen is *Fusarium* spp. of the all tested pathogens, which showed no clear zone of inhibition by *Trichoderma* non-volatile metabolites. On the other hand, the highest effect of antagonist metabolites was recorded against *Rhizopus* sp. which presented 0.69 cm and 0.58 cm of clear zone by metabolites of T3 and T24, respectively (**Table 6**). Some compounds as non-volatile substances belonging to terpene group presenting clear antifungal activity have been isolated from several species of *Trichoderma* like 3-4,dihydroxycarotane, trichodermin, harzianum A, mycotoxin T2, ergokinins A and B or viridin [72]. The inhibition effect of non-volatile compounds in this study might be according to the antimicrobial action of similar compounds produced as antibiotics. Generally, variation between and within *Trichoderma* isolates T3 and T24 in their antagonistic potential of the produced volatile and non-volatile metabolites were noticed.

3.5. Biocontrol potential of antagonists compared with mint oil

3.5.1. Effect of antagonist's culture filtrates and mint oil on beet fruits rot

The effects of *T. harzianum* (T3 and T24) culture filtrates (different concentrations) as well as mint oil on spore germination of postharvest fungal pathogens were evaluated. The treatment results of infected sugarbeet with postharvest fungal pathogens (four phytopathogens) by mint oil or culture filtrates of the two tested antagonists (T3 and T24) were presented in (**Tables 7 & 8**). In the case of *Fusarium* sp. (1) infection, all treatments were the higher as positive effect either by mint oil or culture filtrates of *T. harzianum* (T3 and T24), especially samples where sugarbeet roots of the seeds planted with mint oil, in which Decay Index (DI) gave zero infection (100% protection) by all treatments (**Table 8, Figure 3**). *Fusarium* sp. (2) came to next of positive effect treatments and in some investigation the protection reached 100% with zero infection (**Table 8**). On the other hand, the results of the treatments with mint oil or 100% culture filtrate concentration of T3 and T24 represented high efficiency of sugarbeet protection from rotting by the postharvest pathogens *Rhizopus* sp. and *A. niger* in which the protection for some cases became nearly 100 % at 3 and 5 days of incubation period compared with the other culture filtrate concentrations (25 and 50%) in which protections were lower (**Table 7**).

The high degree of DI was represented by *Rhizopus* sp. which was the most aggressive pathogen for untreated (+ve) samples (seed planted without mint oil), and the infected only presented 356 DI after 7 days incubation. These infected samples represented 32.8 DI when it was treated with 100 % concentration of T24 after the same incubation period (**Table 7**). Samples of sugarbeet roots in which seeds were treated with mint oil before planting showed high protection response after treatment with mint oil or culture filtrates of T3 and T24 compared with samples of sugarbeet root of seeds were untreated with mint oil (**Table 7 & 8**).

These results indicate that an extracellular diffusible metabolite(s) might be released from *T. harzianum* when it was grown on PDB and these metabolites prevented pathogen spores to germinate. Results of Odebode [73] suggested that, strains of *T. harzianum* Rifai and *T. pseudokoningii* Rifai produced metabolites in culture filtrates; these metabolites inhibited the growth of postharvest pathogens of some fruits. Moreover, random mutagenesis was employed for improving the production of antifungal metabolites of two important fungal antagonists (*Trichoderma* spp. and *Gliocladium* spp.) that were used to control a broad spectrum of phytopathogens [74].

Mint oil as a treatment on sugarbeet fruits decreased the rot intensity. On the other hand, treatments of *T. harzianum* (T3 and T24) culture filtrates on beet fruits suppressed pathogen spores from germination, and in some cases a complete inhibition occurred. This result is in agreement with the treatments of yams rots when sprayed with *T. viride* culture filtrate [75].

The naturally occurring materials would be the use of microbial inoculants or some of natural products like plant extracts and different types of essential oils. The former were suggested to

Table 6: Effect of non-volatile compounds produced after 5-days of incubation by culture filtrate of *T. harzianum* (T3 and T24) against postharvested fungal pathogens of beet roots on PDA measuring clear zone and expressed by (cm).

Postharvest Phytopathogens	Clear zone of antagonists by (cm)	
	T$_3$	T$_{24}$
Rhizopus sp.	0.69 ± 0.083	0.58 ± 0.01
A. niger	0.35 ± 0.058	0.24 ± 0.015
Fusarium sp. (1)	No clear zone	No clear zone
Fusarium sp. (2)	No clear zone	No clear zone

The results of clear zone size are expressed as mean ± SE. Mean is the value among treatments and SE is the standard error value for each treatment

Table 7: Decay index (DI) of beet roots (seeds planted with/without mint oil) infected with 1×10^7 postharvest fungal pathogens (*Rhizopus* sp. and *A. niger*) and treated with *T. harzianum* (T3 and T24) culture filtrate of different concentrations (25, 50 and 100%) or mint oil after

	Treatment of *Rhizopus* sp.							
Incubation period (days)	DI of beet roots (seeds planted without mint oil)							
	Infected	Mint oil	T$_3$ 25%	T$_3$ 50%	T$_3$ 100%	T$_{24}$ 25%	T$_{24}$ 50%	T$_{24}$ 100%
3 days	78.0	1.2	3.7	3.2	2.0	18.2	7.8	7.2
5 days	86.0	20.3	57.6	33.6	19.2	35	28.6	14.8
7 days	356	176	63.8	61.6	43.6	53.8	44.8	32.8
	DI of beet roots (seeds planted with mint oil)							
3 days	13.1	0.0	3.6	2.4	0.0	5.8	5.6	4.8
5 days	45.3	4.7	18.4	14.2	11.0	17.0	8.1	6.4
7 days	93.1	45.2	36	32.3	30.4	30.6	34.8	34.6
	Treatment of *A. niger*							
Incubation period (days)	DI of beet roots (seeds planted without mint oil)							
	Infected	Mint oil	T$_3$ 25%	T$_3$ 50%	T$_3$ 100%	T$_{24}$ 25%	T$_{24}$ 50%	T$_{24}$ 100%
3 days	27.5	7.2	24.9	22.8	6.3	23.0	15.8	6.7
5 days	33.1	13.3	30.3	24.9	6.7	29.5	20.9	10.9
7 days	180.9	21.1	49.9	32.5	25.4	51.7	41.3	26.1
	DI of beet roots (seeds planted with mint oil)							
3 days	10.5	1.7	3.7	0.9	2.5	4.5	2.6	1.9
5 days	39.3	7.3	9.2	4.5	4.1	13.3	10.3	5.2
7 days	60.3	8.5	12.7	9.1	7	18.2	13	6.5

DI equation:$\dfrac{1 \times N1 + 2 \times N2 + 3 \times N3 + 4 \times N4 + 5 \times N5}{5 \times N_T}$

N1: Healthy fruit
N2: one lesion smaller than 1cm in diameter
N3: one lesion bigger than 1cm but the decay area smaller than 25% of fruit surface
N4: Decay area between 25% to 40% of beet fruit surface
N5: Decay area bigger than 40% of fruit surface
N$_T$: The total number of fruits examined

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Table 8: Decay index (DI) of beet roots (seeds planted with/without mint oil) infected with 1×10^7 postharvest fungal pathogens (*Fusarium* sp. 1 and *Fusarium* sp. 2) and treated with *T. harzianum* (T3 and T24) culture filtrate of different concentrations (25, 50 and 100%) or mint oil after

	Treatment of *Fusarium* sp. (1)							
Incubation period (days)	**DI of beet roots (seeds planted without mint oil)**							
	Infected	**Mint oil**	**T₃ 25%**	**T₃ 50%**	**T₃ 100%**	**T₂₄ 25%**	**T₂₄ 50%**	**T₂₄ 100%**
3 days	11.1	1.7	7.5	3.6	0.0	10.8	5.2	0.0
5 days	39.9	6.4	9.9	5.7	4.6	11.27	8.9	4.7
7 days	123.8	14.3	14.6	11.3	6.2	14.3	9.3	7.5
	DI of beet roots (seeds planted with mint oil)							
3 days	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5 days	5.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7 days	7.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Treatment of *Fusarium* sp. (2)							
Incubation period (days)	**DI of beet roots (seeds planted without mint oil)**							
	Infected	**Mint oil**	**T₃ 25%**	**T₃ 50%**	**T₃ 100%**	**T₂₄ 25%**	**T₂₄ 50%**	**T₂₄ 100%**
3 days	15.7	0.7	1.0	0.3	0.0	2.6	0.3	0.0
5 days	31.2	1.4	8.7	7.3	2.2	13.9	8.3	6.7
7 days	150.7	2.5	19.1	12.9	11.0	22.3	16.5	13.1
	DI of beet roots (seeds planted with mint oil)							
3 days	0.7	0.0	0.3	0.0	0.0	0.0	0.0	0.0
5 days	6.4	0.0	3.3	1.0	0.0	4.1	2.4	0.3
7 days	27.7	0.0	8.2	5.6	4.4	10.1	6.5	3.9

DI equation: $\frac{1 \times N1 + 2 \times N2 + 3 \times N3 + 4 \times N4 + 5 \times N5}{5 \times N_T}$
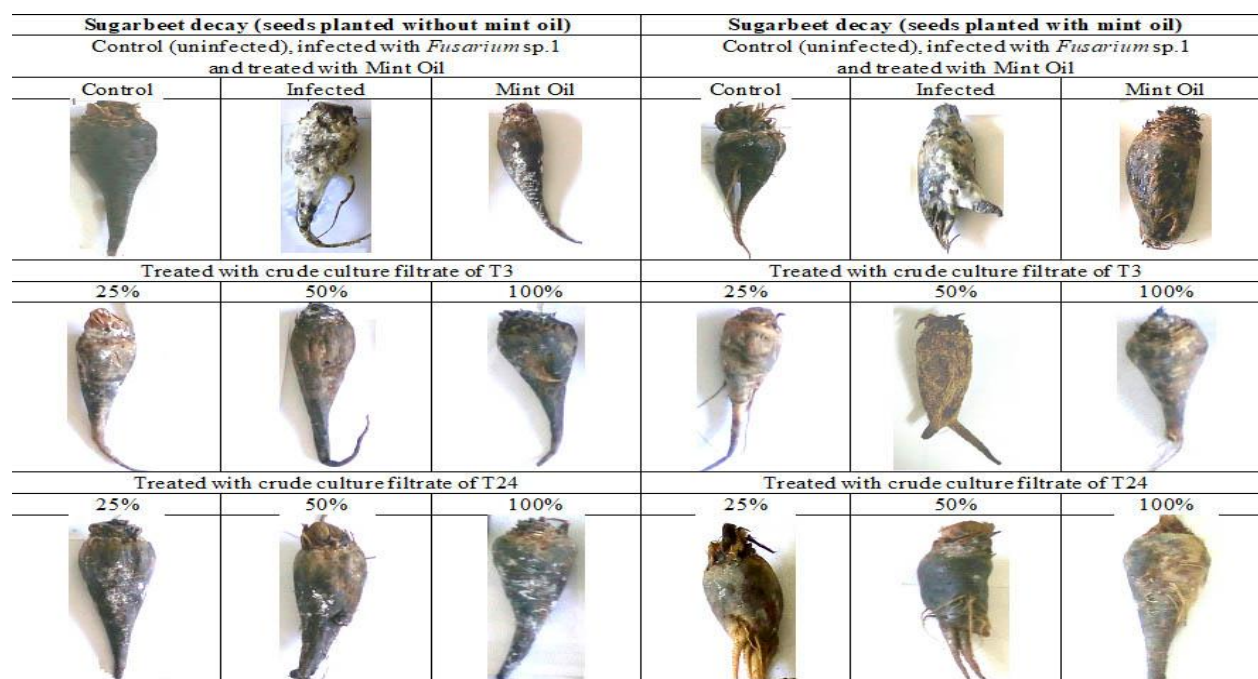
N1: Healthy fruit
N2: one lesion smaller than 1cm in diameter
N3: one lesion bigger than 1cm but the decay area smaller than 25% of fruit surface
N4: Decay area between 25% to 40% of beet fruit surface
N5: Decay area bigger than 40% of fruit surface
N_T: The total number of fruits examined

Figure 3: Efficacy of crude culture filtrate of antagonistic fungi (T3 & T24) on Decay index (DI) of sugarbeet (seeds planted with and without mint oil) which caused by the isolated postharvest fungal pathogens; *Fusarium* sp. 1 (1×10^7), after incubation period of 7 days.

be used in controlling postharvest diseases but there is little information on the latter in the technology of postharvest treatments. Recently, the naturally derived compounds are used extensively for managing food borne and postharvest pathogens because of their harmless and non-toxic nature. Naturally active antimicrobial biologically compounds that originated from plant are believed to be more acceptable with less hazardous compared with synthetic compounds; also it is represented as a rich source of disease-control agents. Essential oils of plant-derived compounds are used as antimicrobial agents. These oils are active with a wide spectrum of antimicrobial activity against plant pathogens. In this study, mint oil activity against sugarbeet rot agents is consistent with essential oils activities that were tested previously *in vivo* against *Geotrichum* on tomato fruits, whereas, treatment of tomato by oils prior to pathogen infection reduced the rot incidence to a considerable extent [76].

In the present study mint oil as a naturally occurring plant-derived compound could be potentially used for postharvest technology and to control postharvest pathogens. For effective control and management of postharvest pathogens by mint oils, different methods should be worked out. Antimicrobial effects would be as a result of many compounds synergistically together work that means the individual components by themselves may not be effective as their mixtures [76]. This might be the answer for the question of why a great affect of inhibition against the rotting agents by antagonists metabolites when seeds of sugarbeet were treated before planting with mint oil.

In the managing of postharvest rots, there are different parameters must to be considered like cold storage, chilling, sanitation and other physiological conditions. In spite of all these precaution, the rotting occurs due to the infection with microorganisms. Therefore, chemicals are mostly used to protect the product and become free from infection. Several fungicides are presently used in postharvest treatments to control a wide spectrum of microorganisms causing product decay. However, the extensive use of these chemicals and its inputs have causes several negative effects not only to the living organisms but also for the surround environments [56]. The significance of developing biodegradable and eco-friendly products to be as natural fungicides in the management and controlling various postharvest plant pathogens become the subject of increasing interest.

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