Pharmacophore

ISSN-2229-5402

Journal home page: http://www.pharmacophorejournal.com

Pharmacophore
an International Journal
ISSN 2229-5402

ANTIFUNGAL EFFECTS COMPARISON OF MEDICINAL PLANTS ESSENTIAL OIL AND TRICHODERMA FUNGI ISOLATES IN CONTROL OF THE CITRUS FRUIT GREEN MOLD

Ahmadreza Shahrokhi[1*], Behzad Hajieghrari[2]

1. ***Department of Plant Protection, Agricultural college, Higher Educational Complex of Saravan. Saravan. Iran***
2. ***Department of Biotechnology, Agriculture college, University of Jahrom. Jahrom. Iran***

ARTICLE INFO

Received:
03th Jun 2017
Accepted:
29th Nov 2017
Available online:
14th Dec 2017

Keywords: *Plant Essential Oil, Antifungal Effect, Fennel, Lavender, Trichoderma, Citrus.*

ABSTRACT

In this research, the effect of essential oils of Fennel (*Foeniculum vulgare*), peppermint (*Mentha piperita*) and Lavender (*Lavandula angustifolia*) and *Trichoderma harzianum* antagonist fungus isolates on *Penicillium digitatum*, causal agent of citrus green mold in in vitro and in vivo (on fruit) Was reviewed. In doula culture test, between essential oil and pathogen, the Fennel essential oil had the most effect (49.42% inhibition) and peppermint essential oil had the least effect (8.39% inhibitory) on preventing the growth of *Penicillium mycelium*. The essential oils of fennel and lavender with a 1500 µL / L concentration were used for in vivo tests. The 6 Trichoderma isolates were used in doula culture test. The isolate 2 had the highest effect (51.2% inhibition) and isolate 6 had the least effect (33.14% inhibitory) on inhibiting the growth of the disease agent. The isolates 2, 3 and 5 which had the highest inhibitory in laboratory were selected for in vivo tests. Orange fruits were inoculated with suspension pathogen at a concentration of 105 × 1 spore in 1 ml sterile distilled water and then treated with essential oil. Fennel essential oil was more effective than lavender in controlling Penicillium fungus on fruit. The antagonistic isolates effects on citrus green mold at 4 and 20 ° C was studied using two methods of immersion and spraying of fruits with suspension of antagonists. The highest prevention percentage of disease, against the infected control was observed of *Trichoderma harzianum* isolate 2 with 57.6% inhibitory and the least was isolate 5 with 13.41% inhibitory All essential oil and antagonist treatments compared to infected control, had significant difference in both in vitro and in vivo conditions, but among the all essential oils and antagonists, isolate 2 had the highest percentage of disease control in both conditions.

To Cite This Article: Ahmadreza Shahrokhi, Behzad Hajieghrari, (2017), "Antifungal Effects Comparison of Medicinal Plants Essential Oil and Trichoderma Fungi Isolates in control of the Citrus Fruit Green Mold", ***Pharmacophore, 8(6S),*** *e-1173885.*

Introduction

Fruit contamination to plant pathogens in post-harvest plants corrupts a significant amount of these products. A large number of chemical pesticides have been introduced to control these diseases, but in most cases due to the environmental problems of pesticide residues, toxicity to humans, the formation of resistant races and, in some cases, high costs, the use of such compounds has been limited [1-5]. Today, the use of natural and biological compounds, including essential oils and plant extracts, and antagonistic fungi and bacteria, is in the process of pathogens controlling and organic production. These compounds have no side effects and, due to their antioxidant properties, also increase the quality and length of fruit storage [5-9].

The identification of natural biological compounds that can be commercially used to control post-mortem disease is very valuable. The aromatic plants belong to the labiate and composite families, and Trichoderma isolates are rich in anti-microbial and antioxidant compounds. Trichoderma isolates control pathogens by several mechanisms, including mycoparasitism, antibiosis, competition for food and place, stress resistance, enzyme production and induction resistance [6,10]. Trichoderma produce a large number of antibiotic compounds, such as Alkyl Pyrones, Isonitriles, Polycations, and Steroids, which have direct link to biocontrol properties [3,11]. Inducing resistance and direct penetration to the pathogenic hyphae and lysozyme producing are Trichoderma mechanisms too [12]. Hajieghrari et al. that volatile metabolites of T. harzianum isolates had a

Corresponding Author: Ahmadreza Shahrokhi, Department of Plant Protection, Agricultural college, Higher Educational Complex of Saravan. Email: hafezreza@yahoo.com

significant inhibitory effect on the mycelial growth of *Rhizoctonia solani*, in which ThBI isolate with 89.63% showed the most inhibitory effect [12]. So far, a large number of Trichoderma species have been formulated to control diseases of the most important crop in the world.

Materials and Methods

In vitro tests

To pathogen prepare and isolation, some of the fungal spot margins on infected fruits, were selected and immersed in 70% ethanol in minute and then in 1% sodium hypochlorite solution for 1 minute. The samples rinsed 3 times with sterilized distilled water and cultured on PDA containing 0.1% streptomycin sulfate in petri dishes. Then they were placed in incubator at 25 ° C. The single-spore method was used to purify the fungi.

Preparation of medicinal plants and extraction of essential oils

The plant samples included Fennel seed, Peppermint vegetative part and Lavender flowers, were prepared from Fars Agricultural Research Center. Each of the samples was crushed by grinding, then the essential oil was extracted by distilled water with the help of a Clevenger Apparatus for 3 hours. Extraction of essential oil was performed in three replicates for each sample and were used 50 grams of plant samples for each replication. The essential oil which was digested with dry sodium sulfate, dewatered and stored in dark glass at 4 ° C in the refrigerator. Essential oils obtained from plant samples were identified by gas chromatography (GC) and gas chromatography with mass spectrometry (GC-MS). First, the essential oils were injected into the GC device. After finding the appropriate thermal design for the column to separate the essential oil components and determine the percentage and retention time of each compound, the essential oils were injected into the GC-MS and the mass spectrum of the compounds was determined [9]. The compounds were identified based on the retention index and their mass spectrum with the suggested combinations of the device library. The percentage of each compound was made according to its curvature level in the chromatogram spectrum obtained from the GC device by the normalization of the surface of the curve without calculating the factor correction.

Investigating of the fungal essential oils effects in vitro

Fungal essential oils effects extracted from Fennel, Peppermint and Lavender on *Penicillium digitatum* was investigated by mixing essential oil with culture media. For this purpose, the essential oils were prepared in 80% (0.05%) tween solution. Tween 80 (0.05%) solution was also considered as control treatment. Flasks containing a PDA medium after autoclave were placed at room temperature until their temperature dropped to 44-45° C. Concentrations of 250, 500 and 1000 μL of essential oil per liter of culture medium were added to the flasks containing the PDA medium and stirred to form a uniform emulsion [9,5]. The samples were divided into 8 cm petri dishes and the pathogen discs with a diameter of 5 mm were placed in the petri dishes middle and were incubated at 25 ° C. The different concentrations inhibitory percentage of essential oils was determined by the Abbot formula and Minimum Inhibitory Concentration of essential oils was calculated to prevent the growth of fungi:

IP= C-T/C ×100

IP=Inhibitory percentage

C=Check: Control

T=Treatment

Preparation and selection of antagonist fungi: 6 *Trichoderma harzianum* isolates Was received from Plant Protection Department at Razi University of Kermanshah and they were named isolates 1 to 6 (Th1 to Th6). A concentration with 10^6 spores per ml of Trichoderma was prepared in a total volume of 6 liters. The suspension final concentration was reached to 5×10^6 spores per milliliter with a hemocytometer [12].

Dual culture assay with Trichoderma isolates

A high suspension of Penicillium digitatum spores mixed to PDA medium and placed in a petri dish. Then, after a few days, a disc was removed from this medium and placed on the edge of the next petri dish and on the PDA medium and on the other side, was placed a circle of the same diameter of three-day culture of Trichoderma isolate. After 5 days, the mycelial growth rate of the pathogen was evaluated and compared [11].

In vivo tests

Antifungal effect of essential oils on orange fruit

The healthy orange samples were disinfected with 1% sodium hypochlorite solution. Then they were washed twice with sterilized distilled water and dried eventually. A suspension of *Penicillium digitatum* fungus was prepared at 10^5 spores / ml of sterile distilled water and the fruits were immersed for one minute. An emulsion 1, 3 and 5 per thousand was prepared from essential oil of lavender and fennel and the fruits were treated with two methods of immersion and spraying and packed in polyethylene containers [2,4]. The samples were kept at 25 ° C. The Control treatments were immersed and sprayed with tween 80 (0.05%) solution only. Three replicates for each treatment and six experimental units (fruit) for each replicate were considered.

Investigation of Trichoderma isolates effects on orange fruit

The isolates 2, 3 and 5 which had the highest inhibitory in laboratory were selected for in vivo tests. The suspension of 10^6 spores per ml sterilized water was prepared and after scarring, the fruits were infected with 25 μL of the pathogen suspension, and then 25 μL of the Trichoderma isolates suspension was sprayed on the fruits and were at 20 ° C. three replicates were used for each treatment and infected control was inoculated with sterilized distilled water [1,2].

The data were analyzed in a completely randomized design with a comparison of means using Duncan test at 5% level. Data analysis was performed using MSTATC statistical software and SAS software was also used for grouping.

Results

Essential Ingredients

The main compounds witch identified in the essential oil of *M. piperita* include mentanol (39.1%), menthon (28.1%) and 1,8-Cineol (6/8%). The main ingredients in the essential oil of *L. angustifolia* are linalool (47.6%), 4-Terpineol (7.2%) and Lavandulyl acetate (6.3%) (Table 1).

Table 1. The type and percentage of identified major compounds in the essential oils of *Mentha piperita* and *Lavendula angustifolia*

Compound	Inhibitory Rate (IR)	*Lavendula angustifolia*	*Mentha piperita*
myrcene	981	1/7	-
1,8-Cineol	1021	-	8/6
linalool	1089	47/6	0/4
menthon	1152	-	28/1
isoimenthone	1158	-	7/1
menthofuran	1162	-	2/9
4-Terpineol	1167	7/2	-
menthol	1180	-	39/1
Lavandulyl acetate	1272	6/3	-
methyl acetate	1342	5/7	-
germacrene D	1458	1/7	4/4

The main components in the essential oil of Foeniculum vulgare were Anethole trans (69.7%), fenchone (9.2%) (Table 2).

Table 2. The type and percentage of identified major compounds in the essential oils of *Foeniculum vulgare*

Compound	Inhibitory Rate (IR)	*Foeniculum vulgare*
α-thujone	935	5/1
β-pinene	975	0/9
α- phellandrene	1000	6/1
p-cymene	1016	0/8
fenchone	1071	9/2
estragol	1221	4/3
Anethole trans	1279	69/7

Antifungal Effects of essential oils

The results showed that Fennel essential oil with 1000 µL/L concentration, had the most inhibitory rate (49.42%) on Penicillium growth. The least inhibitory rate (8.39%) was found on peppermint oil with a concentration of 250 µL / L (Table 3).

Table 3. Inhibitory Rate (IR) of the Penicillium digitatum growth, causal agent of orange fruit rot by essential oil in vitro conditions

Essential oil	Concentration µL / L	Inhibitory Rate%
peppermint	250	8/39
	500	17/19
	1000	29/12
Fennel	250	29/08
	500	34/74
	1000	49/42
Lavender	250	18/14
	500	35/38
	1000	44/24

minimum inhibitory concentration of fennel essential oil on all three fungi was 750 µL/L, but in the case of peppermint oil was more than 1000 µL/L (Table 4).

Table 4. The minimum inhibitory concentration of different essential oils (μL/L) in preventing the growth of the *Penicillium digitatum*, the agent of orange fruit rot in vitro conditions

Essential oil	***Penicillium digitatum***
Peppermint	>1000
Fennel	750
Lavender	>1000

Dual culture results of Trichoderma isolates in vitro

Dual culture of isolates in 5 days after culture showed that the antagonistic isolates effect was significant in preventing the growth of pathogen mycelium against control. The isolate 2 had the highest inhibition rate (51.2%) and the isolate 6 had the least inhibitory rate (33.14%) (Figure 1).

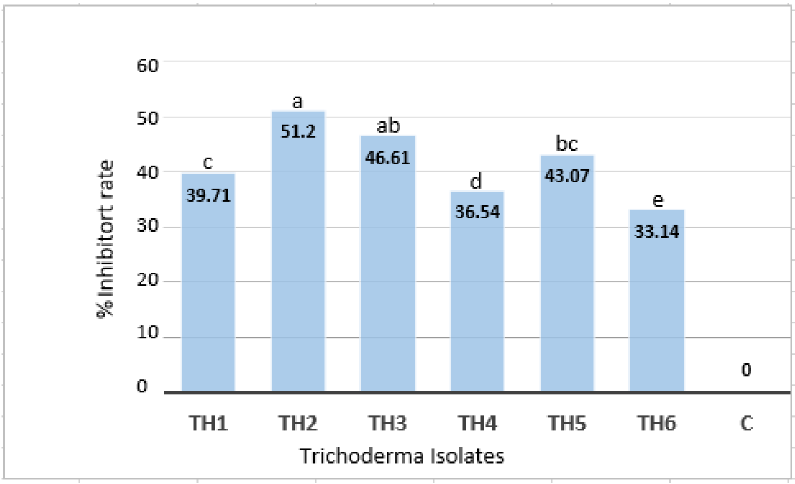

Figure 1. Inhibition rate against Penicillium digitatum hyphal growth by Trichoderma isolates in dual culture under in vitro conditions.

Antifungal effect of essential oils on fruit

Different concentrations investigation of essential oils to rot reducing, showed that rot rate after 10 days of storage at 20 ° C there was a significant difference between two essential oils in fruit rot reducing. The results showed that the antifungal activity increased with increase in essential oil concentration, and the fennel essential oil had the more inhibitory than other essential oils and could prevent the growth of the disease (53/06%) at a 5/1000 concentration. In general, there was a significant difference between the fennel essential oil concentrations and other essential oils. However, there was no significant difference between the inhibitory rate of 3/1000 concentration of fennel and lavender essential oil. There was no significant difference between the two methods of immersion and spray application (Table 5).

Table 5. Effect of Fennel and Lavender essential oils by two methods of spraying and immersion in reducing orange Penicillium rot disease

Inhibitory Disease Percentage	**Lavender Treatment**	**Concentration**	**Inhibitory Disease Percentage**	**Fennel Treatment**	**Concentration**
9/54	immersion	1/1000	11/01	immersion	1/1000
10/36	spraying	1/1000	13/83	spraying	1/1000
24/07	immersion	3/1000	24/19	immersion	3/1000
26/77	spraying	3/1000	27/53	spraying	3/1000
42/76	immersion	5/1000	51/93	immersion	5/1000
44/14	spraying	5/1000	53/06	spraying	5/1000

The inhibitory rate of *Trichoderma harzianum* on disease reduction on orange fruit

The results showed that isolate 2 with 57.6% inhibitory rate had the highest effect in disease reduction on the fruit in immersion method, and isolate 5 by application spraying method and 13.41% deterioration had the least effect on the disease control. However, the results showed that there was no significant difference between immersion and spraying method in any of the isolates (Figure 1).

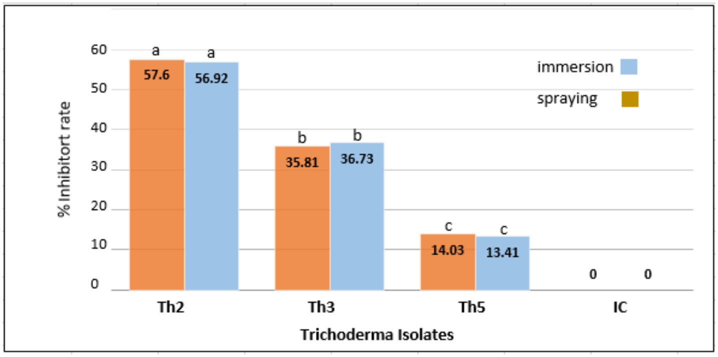

Figure 2. The inhibitory rate of *Trichoderma harzianum* on disease reduction on orange fruit

Among all essential oils and antagonist treatments in laboratory tests and on the fruit, the antagonist isolates 2 with 51.2% and 57.6% showed the most effect on inhibition growth of Penicillium mycelium and reduction of disease.

Discussion

In dual culture method, the Trichoderma isolates due to different mechanisms caused the growth restriction of pathogen. All Trichoderma isolates were able to progress and grow on the pathogen mycelium [3]. The isolate 2 hade the highest inhibitory rate of pathogen growth. Trichoderma fungus has a rapid growth, which has made that a suitable biological control agent against various pathogens. Researchers have mentioned the main antithetical mechanisms of Trichoderma fungus is the parasitism, competition, antibiosis [10,12]. Parasitism is the complex process involves several steps including chemistry, binding, torsion, and secretion of hydrolysis enzymes and cells degradation ultimately. The use of plant essential oils in the control of post-harvest diseases of fruits and vegetables has been described as a biological method in recent years and has attracted the attention of many researchers as an effective and safe way [8,9]. The use of herbal essential oils, while maintaining the health and safety of the product, reduces the fruit loss. The sensitivity of the fungal species varies depending on the essential oil and its different concentrations.

The difference in the antifungal activity of the essential oils depends on their constituents. A compound may cause antifungal activity alone or to be synergist with other compounds [5]. Therefore, it seems that the essential antifungal effects of fennel essential oil are influenced by the dominant composition of the essential oil, but the synergistic effects all of the components are the major determinant of the antifungal activity. Maskuki et al. [13] showed that the essential oils of thyme and zinnia are effective in controlling the growth of the *Aspergillus parasiticus* on pear. Farzaneh et al and Hadian et al. showed that the essential oils of Artemisia, Artemisia, Artemisia and mountain Artemisia plants inhibit the growth of some soil fungi in laboratory conditions. [14,8,15]. Therefore, according to the results of this study, application of Fennel essential oil and *Trichoderma harzianum* isolate 2 reduced the citrus green mould by *Penicillium digitatum*. Batta studies in 2004 showed that the use of Trichoderma isolates can reduce the apple fruit rot after harvest and protect the fruit from mould [16]. Tabe bordbar et al. reported that the biological control of grapefruit mould, depends on the development of resistance time and the antagonist concentration [17].

According to Batta et al. antagonists can be used as a preventive agent in the fruit ripening process [16]. Trichoderma species can inhibit the pathogen growth by various enzymes production and penetration into the hyphae. Biocontrol activity of *Trichoderma virens* on the effect of apple blue mildew and its ability to induce defense system in apple tissue. After 8 days The *Trichoderma virens* isolates reduced the infection by 57% [17]. The results of the researchers showed that Trichoderma species can also be effective in controlling other pathogen fungi including *Macrophomina phaseolina*, a melon black- shank agent. The survival strength of an antagonist and its ability to adapt to other microorganisms and environmental conditions is one of the important factors of a potent antagonist. Therefore, the preparation of an appropriate formulation of the most effective strains in order to increase their survival can be the subject of future research. In addition to the population of microbial biocontrol agents and their survival and compatibility, other factors are necessary for a successful biological control in natural conditions, which can be accessed by nutrients, the degree of heat and moisture, and the timely application of the antagonist. The host plant and its inhibitory compounds as well as the population and composition of environmental microorganisms may also affect the effectiveness of the selected antagonists.

Refrences

1. Anthony Sk, Abeyvikrama WS, Wilson S. The effect of spraying essential oils of *Cymbopogon nardus, Cymbopogon flexuosus* and *Ocimum basilicum* on postharvest diseases and storage life of Embul banana. The Journal of Horticultural Science and Biotechnology 2003; 78(6): 780-5.
2. Arras G, Usai M. Fungitoxic activity of 12 essential oils against four postharvest citrus pathogens: chemical analysis of thymus capitatus oil and its effect in subatmospheric pressure conditions. J. Food Prot 2001; 64(7):1025-9.
3. Harman GE. Overview of mechanisms and uses of Trichoderma spp. Phytopathology 2006; 96:190-194.
4. Plaza PR, Torres J, Usall N, Lamarca VI. Evaluation of the potential of commercial post-harvest application of essential oils to control citrus decay. J. Hort. Sci. and Biotech. 2004; 79(6): 935-40.

5. Plotto A, Roberts DD, Roberts R.G. Evaluation of plant essential oils as natural postharvest disease control of Tomato (*Lycopersicon Esculentum*). Acta Horticulturae 2003; 628 :737-45.
6. Benitz T, Rincon AM, Timon M. Biocontrol mechanisms of Trichoderma strains, International Microbiology 2004; 7(4): 249-60.
7. Couladis M, Tzakou O, Kujundzi S, Sokovi M, Mimica-Dukic N. chemical analysis and antifungal activity of Thymus striatus. Phytother. Res 2004; 18: 40–42.
8. Farzaneh M, Ahmadzadeh M, Hadian J, Sharifi-Tehrani A. Chemical composition and antifungal activity of the essential oils of three species of Artemisia on some soil-borne phytopathogens. Comm. Appl. Biol. Sci. 2006; 71(3) :1327-33.
9. Ramezani M, Behravan J, and Yazdinejad A. Composition and antimicrobial activity of the volatile oil of *Artemisia khorassanica* Podl. from Iran. Pharmaceutical Biol. 2004; 42(8):1-4.
10. Burmeister L. The antagonistic mechanisms employded by *Trichoderma harzianum* and their impact on the control of the bean rust fungus *Uromyces appendiculatus*. Ph.D.Thesis. University of Hannover. Germany 2008.
11. Nashwa MA, Sallam KAM, Hassan MAE. Evaluation of Trichoderma Species as Biocontrol Agents for Damping-Off and Wilt Diseases of *Phaseolus vulgaris* L. and Efficacy of Suggested Formula Egypt. J. Phytopathol 2008; 36 (2) :81-93.
12. Hajieghrari B, Torabi M, Mohammadi MR, Davari M. Biological potential of some Iranian *Trichoderma* isolates in the control of soil borne plant pathogenic fungi. African Journal of Biotechnology 2008; 7(8): 967-72.
13. Maskuki A, Mortazavi A. Effect of Thymus and Vegan essential oils on controlling the growth of *Aspergilus parasiticus* fungus on pears in cold stores. Science and Technology of Agriculture and Natural resources 2003; 8(2): 207-15.
14. Farzaneh M, Ghorbani-Ghouzhdi H, Ghorbani M, Hadian M. Composition and antifungal activity of essential oil of *Artemisia sieberi* on soil-born phytopathogens. Pakistan J. Biol. Sci 2009; 9(10) :1979-82.
15. Hadian J, Farzaneh M, Ghorbani M and Mirjalili M.H., Chemical composition and antifungal activity of of the essential oil of *Artemisia khorasanica* on soil-born phytopathogens. J. Esse. Oil Res 2007; 10(1):53-8.
16. Batta YA. Effect of treatment with *Trichoderma harzianum Rifia* formulated in invert emulsion on postharvest decay of blue mold. Food Microbiol 2004; 96: 281-8.
17. Tabe bordbar F, Etebarian HR, Sahbani N, Rouhani H. Biological control of apple mildew with Trichoderma virens and induction of defensive responses in apricot tissue at 20 ° C. 18th Iranian Plant Protection Congress 2008;150:37-8.