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اثر اسانس‌های گیاهی بر کپک سبز مرکبات

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چکیده

استفاده از فراورده‌های طبیعی گیاهی به‌عنوان روش جایگزین مناسب‌تر برای کنترل بیماری های بعد از برداشت شناسایی شده است. در این تحقیق درصد ممانعت رشدی و خاصیت آنتاگونیستی اسانس چهار گیاه دارویی آویشن، آلوورا، سیر و زیره سبز (۱۰۰، ۲۰۰، ۳۰۰ و ۴۰۰ پی پی ام) در شرایط آزمایشگاه و محیط زنده علیه *Penicillium digitatum* آزمایش شد. اسانس‌های گیاهی به‌روش تقطیر با کلونجر به‌دست آمد. حداقل غلظت ممانعت رشدی (MIC) با استفاده از روش رقیق‌سازی در آگار مشخص شد. بیشترین درصد ممانعت از رشد (۹۹/۹۵ درصد) در شرایط آزمایشگاهی توسط آویشن مشاهده شد و بهترین MIC را بر علیه *P. digitatum* داشت. آنالیز GC-MS آویشن منجر به شناسایی ۲۲ ترکیب متفاوت گردید. بیشترین درصد ترکیب مربوط به تیمول ۶۰/۱۸ درصد، γ -Terpinene 6/39 درصد، P-Cymene 44/15 درصد و کارواکرول ۲/۸۸ درصد بود. کاربرد اسانس آویشن روی زخم‌های آلوده به پاتوژن سبب تحریک پرتقال برای افزایش ترکیبات فنلی و آنزیم‌های پرکسیداز و کاتالاز شد.
واژه‌های کلیدی: آویشن، آلوورا، زیره سبز، سیر، *Penicillium digitatum*

The effect of plant essential oils on citrus green mold

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Abstract

Using natural plant products has become more important as viable alternatives to control postharvest diseases. The effect of four essential oils of medicine plants including *Thymus vulgaris*, *Cuminum cyminum*, *Allium sativum* and *Aloe vera* were tested (100, 200, 300, 400ppm) *in vitro* and in *vivo* (200,300,400 ppm) for their fungal inhibitory percentage and antagonistic properties against *Penicillium digitatum*. Plant essential oil extracted by hydro distillation using a Clevenger-type, and minimal inhibition concentrations (MIC) was determined using agar dilution method. *In vitro* inhibitory effects of the plant extracts showed maximum percent inhibition (99.95%) by thyme extract, which also had the best MIC value against *P. digitatum*. GC-MS analysis of the thyme oils extract led to the identification of 22 different components which carvacrol (2.88%), 60.18% thymol (60.18%), linalool (4.22%), γ-terpinene (6.39%) were found to be the main constituents in *T. vulgaris*. Thyme essential oil inoculated into wounds with the pathogen stimulated the orange to increase production of phenol compound, catalase and peroxidase activity. Thus, different concentrations of the thyme essential oil are effective on controlling the producing agent of the green mold fungus significantly, and induce resistance to orange by effect on defense enzyme.
Key word: *Allium sativum, Aloe vera, Cuminum cyminum, Penicillium digitatum, Thymus vulgaris*.

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Introduction

Postharvest diseases cause considerable losses to harvested fruits and vegetables during transportation and storage (Sharma *et al*., 2009). Green mold caused by *Penicillium digitatum* is the major postharvest disease of citrus, wherever it is grown and causes serious losses annually (Eckert and Brown, 1986). There are two principal factors which make plant products more susceptible to spoilage: the high water content in fruit which allows pathogen attack and the wounds present on the plant organs during storage, often as a result of harvesting and transportation. Synthetic fungicides are primarily used to control postharvest diseases (Sharma *et al*., 2009) However, postharvest use of fungicides has been increasingly curtailed by the development of pathogen resistance to many key fungicides (Prusky *et al*., 1985). Lack of alternative fungicides, and negative public perception on pesticides hazards to human health and the environment has promoted governmental policies restricting use of fungicides (Ragsdale and Sisler, 1994). Thus, alternative methods to control postharvest diseases are urgently needed. In the past thirty years, there have been extensive research activities to explore and develop strategies based on microbial antagonists to biologically control postharvest pathogens (Droby, *et al*., 2009; Sharma, Singh, and Singh, 2009; Spadaro and Gullino, 2004). Plant essential oils have antimicrobial activity against a variety of plant pathogens and pests. Several studies have explored the potential of essential oils as antifungal agents (Abd-Alla *et al*., 2001; Abdolahi *et al*., 2010). Plant extracts from plant species *Withania somnifera* and *Acacia seyal* led to the inhibition of the growth of fungus *P. digitatum* by up (70%) when used for 21 days under the conditions of storage. The natural citrus Glucosinolates from mustard and horseradish also showed antimicrobial activity against *P. digitatum* (Ismail and Zhang, 2004).

The plant extracts reported effective against the fungus *P. digitatum* include *Allium sativum*, *Azadirachta indica*, *Withania somnifera* and *Acacia seyal (*Mossini, *et al*., 2009). The objective of this study is to evaluate using of botanical pesticides as means to protect citrus from green mold post-harvest diseases and also protect the environment from harmful effects of fungicides. In this study we consider the antifungal activity of four plant essential oils against green mold rot of citrus caused by *P. digitatum* considered as in *vitro* and *in vivo* experiments during storage.

Materials and Methods

Plant material and Extraction of essential oil: The tested plants including thyme (*Thymus vulgaris*), Cumin (*Cuminum cyminum*), garlic *(Allium sativum*) and *Aloe vera* were collected from the market in Gorgan city of Iran. Plant materials were freed from foreign materials and carefully rubbed between soft cloths to remove dust and subjected to shade drying. The essential oils were extracted by hydro distillation, for 4 h, using a Clevenger-type apparatus according to the method recommended in British pharmacopoeia (Tzortzaki, 2009). For this, fresh plant (100 g) were thoroughly washed with sterilized distilled water and cut into small pieces. The plant material was then placed in round-bottom flask of the Clevenger apparatus for distillation (the ratio of plant material to water was 1:3). The collected oil was dehydrated by anhydrous sodium sulfate, then oil essence collected and stored in dark bottles at 5°C until use.

Isolation of Target Pathogen: Five main markets were selected in Gorgan city of Iran. *P. digitatum* was isolated from infected orange fruits showing dark brown rot symptoms. Healthy oranges were also collected to serve as controls. The *P. digitatum* isolate used in this study was obtained from naturally decayed orange fruits. Small pieces of fruit tissue, previously surface-disinfected with 90% ethanol, were aseptically excised from the advancing edge of the rot and transferred to petri plates containing potato dextrose agar (PDA) amended with 1 ml of lactic acid (80%) per liter. After a 4-day incubation period at 25°C, plates were examined under a stereomicroscope to determine colony morphology. The isolate used in this work was the most aggressive one in our collection and produced the largest lesions on inoculated fruit in pathogenicity test. This fungus was purified and maintained on PDA and stored at 4°C, with periodic transfers through citrus fruit to maintain its aggressiveness.

***Penicillium* Conidial Suspension:** To produce inoculum, a strain of *P. digitatum* was cultured on PDA dishes for 8 days at 24 ± 1 °C. The surface of the *P. digitatum* colony was washed with 6 mL of sterile distilled water containing 0.1% (v/v) tween 80. The resulting suspension was filtered through two layers of sterile gauze and spores were counted. A suspension with a concentration of 5×10^4 conidia/ml was used for all *in vitro* and *in vivo* trials.

Evaluation of anti-fungal activity of plant essential oil: Five ml of each plant extract, at different concentrations (100, 200, 300, 400 ppm) were added to each of the sterilized petri dishes and mixed with 15 ml of autoclaved PDA medium (Nene and Thapilyal, 2002). The solidified medium was inoculated centrally at the point of intersection of the two perpendicular lines drawn at the bottom of the plate with 5 mm diameter mycelial disc of *P. digitatum* retrieved from one-week-old culture. The plates were incubated at 25°C and daily radial growth measurements were taken until the fungus reached the edge of the control plate. The inhibition zone was measured using the formula of as follows

% Mycelial inhibition =

$$\frac{\text{Mycelial growth (control)-Mycelial growth (treatment)}}{\text{Mycelial growth (control)}}\times 100$$

Determination of Minimum Inhibitory Concentration (MIC): MIC determination was performed using the serial dilutions method. At first, suspension of *P.digitatum* according to standard 0.5 McFarland was provided in Sabouraud Dextrose Broth (SDB) (Merck, Germany). One milliliter of SDB was added to them. In the next step 1 mL of extract was added to tubes according to serial dilutions procedure. Also 20µl of *P. digitatum* suspension was added to tubes. Then they were incubated in 35°C for 24 hours. Tube number 10 was considered as the control tube (1 mL SDB + 20µl *P. digitatum* suspension). After incubation, with observation of turbidity or lack of turbidity in tubes, level of MIC was determined (Alizadeh Behbahani *et al*., 2016).

Effects of plant essential oil on green mold development in oranges: Orange fruits wounded (2-wounds per fruit) at the equatorial side with a sterile stainless steel scalpel where each wound was about 4 mm long and 2 mm deep each wound was inoculated with 20 µl of an aqueous suspension of conidia of P. *digitatum* adjusted to 5×10^4 spores/ ml distilled water with 0.05% Tween 80 (Palou *et al*., 2002). After 2-h incubation at room temperature, each treatment was sprayed with plant essential oil separately at concentration of 200, 300, 400 ppm. Controls were sprayed with the Tween80 and conidia suspension under the same conditions and Tiabendazol treatment used as negative control. Treated fruit were placed on plastic tray at 20°C and ~95% relative humidity (RH) for 10 days. Inhibition percentage of fungi was evaluated using spot measurement by caliper.

Gas Chromatography/Mass Spectrometry (GC/MS): The most effective plant essential oil were analyzed by GC-MS (Varian-3400) column (DB-1, 60 mm 0.25 mm fused silica capillary column film thickness 0.25 µm using a temperature program of 50-250°C at a rate of 4°C min^{-1}, injector temperature 260°C, carrier gas: helium. The constituents were identified by comparison of their mass spectra with those in the computer library and with authentic compounds. The identifications were confirmed by comparison of their retention indices with those of authentic compounds or with literature data. The components of the effective plant essential oil were identified by matching their mass spectra and retention indices with those of the Wiley 275 library in the computer library and literature. The percentage composition was determined from the peak areas of the total plant essential oil composition.

Biochemical analyses

Citrus fruit skin (≈ 1 g) was obtained at different time intervals after treatment (48 and 72 h), and mechanically homogenized in a mortar with 4 ml acetate buffer (100 mM, pH 5.0). Then, the extract was centrifuged (20,000g / 25 min, at 4°C), and the supernatant was collected and used for enzyme activity and protein determination.

Extraction and assay of peroxidase activity: The extraction and assay of POX was carried out using the method described by Lagrimini and Rothstein (1987). The reaction mixture contained 3.5 mL 0.1 M phosphate buffer (pH 6.0), 0.05 mL 12 mM guaiacol, 0.03 mL H_2O_2 and 0.5 mL enzyme. Changes in absorbance were recorded at 470 nm for 1 min with a spectrophotometer. The activity of peroxidase was presented as_OD 470nm /min/mg protein.

Extraction and assay of catalase activity: The method of extraction as described by Du and Bramlage (1995) was used in the experiment. CAT activity was determined by following the disappearance of H_2O_2 in the enzyme reaction mixture. The enzyme extract (0.25 ml) was added to 2 ml assay mixture (50 mM Tris-HCl buffer pH 6.8, containing 5 mM H_2O_2). The reaction was stopped by adding 0.25 ml 20% titanic tetrachloride (in concentrated HCl, v/v) after 10 min at 20°C. A blank was prepared by addition of 0.25 ml 20% titanium tetrachloride at zero time to stop the enzyme activity. The absorbance of the reaction solutions was read at 415 nm against water. CAT activity was determined by comparing absorbance against a standard curve of $H_2O_2$from 0.25 to 2.5 mm. The activity of CAT was presented as H_2O_2 mm/min/mg protein.

Determination of phenolic compounds: The method of extraction as described by Yamomoto *et al.* (1977) was used in the experiments. Orange fruits (1.0 gr fresh weight of both layers) were ground in a mortar with 10 ml of 80% methanol and filtered through double layers of gauze. The residue was washed twice with 80% methanol (each time with three ml). The filtrate and washing were combined and centrifuged at 4000 g for 5 min at room temperature and the supernatant was assayed. The phenol compound was measured with Folin- Ciocalteu's reagent (Merck, Darmstadt, Germany). 0.5 ml extracts were diluted with distilled water to 7 ml in a test tube. The contents were well mixed. 0.5 ml Folin-Ciocalteu's reagent was added and the tubes were thoroughly shaken again. Exactly 3 min later 1 ml of saturated sodium carbonate solution was added and the mixture made up to 10 ml with good mixing. After leaving the samples for one hour at room temperature, the absorbance was measured at 725 nm. Caffeic acid (Fluka, Germany) was used as a reference phenolic compound. The phenolic compounds of samples were expressed as mg caffeic acid per g of fruit fresh weight.

Statistical analysis: Each treatment was replicated three times. Data were analyzed with Analysis of Variance (ANOVA) using SPSS21. Completely randomized factorial statistical design was used for analysis of experiment. The least significant difference (LSD) was used to test for significant differences between treatments at $P \leq 0.05$.

Results and Discussions

In vitro studies

Effects of plant essential oils on *P. digitatum in vitro:* As shown in Table 1, different plant essential oils varied in their effectiveness in inhibiting percentage *P. digitatum* growth. Indeed, the inhibitory potential of the essential oils was found to vary with the specific plant species as well as the concentration used, inhibition growth percent of plant extract increased with addition of plant essential oil concentration. According to table 2 the most inhibition growth (87.17%) was in 400 ppm. There was no significant difference in *Aloe vera* and Thyme oil in inhibition growth percent at 400ppm concentration but in comparing sum, Thyme was the best plant essential oil and resulted in significant ($P<0.05$) reduction on mycelia growth of *P. digitatum* (99.95%). Thyme essential oil exhibited the strongest toxicity and totally inhibited the mycelial growth of the P. *digitatum.* Thyme and clove essential oils completely inhibited P. *digitatum* growth either when provide an alternative means of controlling postharvest sour rot (*Geotrichum candidum* var. citri-aurantii) on citrus fruit. *Cuminum cyminum* has the least effect on *P. digitatum* growth inhibition percentage (0.25%) in 100ppm concentration. Mohamadifar *et al.* (2012) studied antifungal activities of essential oils from some Iranian medicinal plants that have maximum (100%) inhibition effect on the mycelium growth of postharvest phytopathogenic fungi. Among them *C. cyminum* could stop the mycelium growth of phytopathogenic added into the medium (Yahyazadeh *et al*., 2008).This report is agreed with our result. There were significant difference between thyme and *Aloe vera* essential oil at 300 ppm on inhibition growth percent of *P. digitatum* and thyme oil was more effective. Essential oils from thyme plants showed a significant activity against *P. italicum* and *P. digitatum* (Azizi *et al*., 2008). In particular, *P. italicum* did not show any mycelium growth. According to Liu *et al.* (2009) thyme oil may fungi in 400 ppm concentration on *Aspergillus flavus, Botrytis cinerea, P. italicum, P. expansum, P. digitatum, Rhizopus stolonifer* and *R. lyococcus*, but it has fugistatic effect and doesn't have fungicide effect and it couldn't has maximum inhibition. This result is agreed with our result, *C. cyminum* couldn't be an effective plant essential oil.

Garlic was effective in all concentration and in 400 ppm concentration its fungal inhibition percentage was 89.15%. Obagwu and Korsten in 2003 indicate that garlic extracts have a significant effect on the growth (*in vitro* and *in vivo*) of both *P. digitatum* and *P. italicum.* Garlic extracts was more effective in inhibiting *P. italicum* than *P. digitatum*, comparing with our result it shows fungal species has important role on impression of plant extracts.

Table 1. Effect of growth inhibition percentage essential oils on the *Penicillium digitatum* after 7 days of incubation at 25°C.

No.	Plant	Concentration (ppm)	Inhibition percentage (%)
1	**Thyme**	100	67.35g
		200	87.55d
		300	98.55b
		400	99.95a
2	**Garlic**	100	40.17k
		200	59.57h
		300	70.67f
		400	89.15c
3	***Aloe vera***	100	58.40i
		200	71.10f
		300	86.42e
		400	99.82a
4	**Cuminom**	100	0.25m
		200	23.55l
		300	42.38j
		400	59.75h

Values with the same letter are not significantly different (p = 0.05).

Table 2 states all treatments have significant difference on inhibition percentage growth of *Penicillium digitatum* (P<0.05 and P<0.01) in vitro test.

Table 2. Variance analysis effect of growth inhibition percentage essential oils on the *Penicillium digitatum in vitro*.

Source	Mean Square	FD
Plant essence	9918.23**	3
Concentration	6130.10	3
Plant essence×concentration	122.36	9
Error	0.11	
Coefficient of variation	0.5	

MIC

The results in table3 show that MIC for thyme essential oil against *P. digitatum* was 2 mg/mL and for *Aloe vera* essential oil was 2, 4 and 16 mg/mL respectively. According to the results showed in Table 2, minimal inhibitory concentration (MIC) of thyme essential oil found to be the most effective essential oil against P. *digitatum.* The remaining active plant essential oil showed MIC values that ranged between 4 and 8 mg ml^{-1} or more.

Table 3. Minimum Inhibitory Concentration (MIC) of plant extract on *Penicillium digitatum.*

Essential oil	Concentration(mg/mL)										
	2	**4**	**6**	**8**	**16**	**32**	**64**	**128**	**256**	**Positive control**	**Negative control**
Thyme	+	-	-	-	-	-	-	-	-	+	-
Aloe vera	+	+	+	-	-	-	-	-	-	+	-
Garlic	+	+	+	+	+	-	-	-	-	+	-
Cumin	+	+	+	+	+	+	+	-	-	+	-

A Value is expressed as mean ±SD. (+):Penicillium with growth, (-): Penicillium without growth)

Effects of plant essential oils on green mold development in oranges: The efficacy of different active plant extracts in reducing the incidence of green mold on inoculated oranges was shown in Table 4. The inhibitory potential of the plant essential oils was found to be varied with the specific plant species as well as the concentration used, inhibition growth percent of plant essential oils increased with addition of plant essential oils concentration. Treatments of fruits for 2 h before pathogen inoculation by 4 plant essential oils separately show thyme essential oil almost completely inhibited the development of green mold after 10 days of storage at 20° C and there was not significant difference between thyme and Tiabendazol in 400 ppm concentration and both of them are effective against P .*digitatum.* The inhibition percent of mycelial growth on fruit treated by garlic and cumin essential oil were significantly lower than Thyme (Table 4). Thyme was the most effective essence after Tiabendazol and all of treatments have significant

differences (P<0.05) and cumin essential oil has least effect on fungi growth inhibition. *Aloe vera* had a moderate effect on green mold. Plaza *et al*. (2004) reported that thyme and cinnamon essential oils significantly reduced the incidence of green and blue molds of citrus. Also, thyme oil was reported to control most postharvest citrus rots, such as green mold and our result is as the same of this report. In contrast to garlic extract, *Aloe vera* essential oil gave better activity than the dilutions of the same essential oil. Previous studies both support our findings (Gull et al., 2012).

Table 5 states all treatments have significant difference on inhibition percentage growth of *P. digitatum* (P<0.05 and P<0.01) in vivo test.

Table 4. Comparing sum of simple and reaction effect of plant essence level.

No.	Plant	Concentration level (ppm)	Inhibition percentages
1	**Thyme**	200	81.00d
		300	89.67b
		400	99.00a
2	**Garlic**	200	47.17i
		300	62.33h
		400	74.33f
3	**Aloe vera**	200	69.17g
		300	79.33e
		400	87.00c
4	**Cuminom**	200	10.33l
		300	24.33k
		400	38.60j
5	**Tiabendazol**	200	100.00a
		300	100.00a
		400	99.30a

Values with the same letter are not significantly different (p = 0.05).

Essential oil composition: GC-MS analyses of the thyme oils led to the identification of 22 different components. The identified compounds of the volatile constituents of the essential oils (percentage content of each compound, retention index (RI), and structural subclass) are listed in table6, according to their elution order on a HP-5MS column. The GC–MS of the Thymus oil resulted in the identification of 22 compounds. The major *T. vulgaris* compounds were carvacrol (2.88%), thymol (60.18%), linalool (4.22%), and γ-terpinene (6.39%). Volatile compounds investigated in this study were found to be consistent with those of previously published studies in which volatile compounds were identified by different methods (Miladi *et al*., 2013; Mancini *et al*., 2015). According to Šegvić Klarić *et al*. (2007) the thyme essential oil, which contains p-cymene (36.5%), thymol (33.0%) and 1, 8-cineole (11.3%) as main components, and pure thymol exhibited antifungal activities. Pure thymol exhibited approximately three-times stronger inhibition than essential oil of thyme. According to this result the two most antifungal compounds in *Tymus vulgaris* were thymol and carvacrol.

Table 5. Variance analysis effect of growth inhibition percentage essential oils on the *Penicillium digitatum in vivo*.

Source	Mean Square	FD
Plant essence	7884.70**	3
Concentration	1270.16 *	3
Plant essence× concentration	92.21 *	9
Error	0.74	
Coefficient of variation	1.21	

Biochemical analyses

The increase in the activity of catalase, peroxidase enzyme and phenolic compound was demonstrated as result of thyme application in oranges, 24 h after thyme treatment (Fig 1, 2, 3). This change in enzyme activity can contribute for the reduction of orange decay, since these enzymes are involved in defense responses against pathogens. The induction of defense enzymes in fruit tissues by thyme extract can be important in delaying the development of quiescent infections that, typically, become active when the tissue resistance declines. Two days after inoculation the peroxidase, catalase increased but after four days they decreased because they adapted to *P. digitatum* (Fig1, 2). Phenolic compound after 4 days increased (Fig3). This fungi is a stimulate factor to induce resistance in orange, the amount of catalase is more than peroxidase. The most activity of catalase and peroxidase was in P.*digitatum*+ Thyme treatment after 2days. Control treatment has the least amount of catalase and peroxidase activity. The induction of resistance was correlated with the enhanced activities of defense-related proteins such as phenylalanine-ammonia-lyase, peroxidase, and enhanced level of hydroxyproline-rich glycoproteins (Deepak *et al*., 2007). Ballester *et al*. (2006) reported that peroxidases and phenylalanine ammonia-lyase showed higher activity in orange that was more resistant to

Penicillium digitatum development. The participation of β-1, 3-glucanase in orange in the defense of citrus fruit against *P. digitatum* has also been shown (Porat *et al*, 2001). Resistance plants for pathogen have high level of POD (Percival, 2001). Peroxidases can eliminate the potentially toxic H_2O_2 with concomitant benefits (Liochev, 2013) and hydrogen peroxide, which are highly toxic to many organisms (Wang et al., 2014). In our result it shows application of plant essential oil by effect on catalase, peroxidase and phenolic compound control *P.digitatum*, which are agree with other reports. Catalase expression after inoculation was similar to peroxidase; it was unchanged in control treatment and increased in both layers treated with antagonist, pathogen and combination of them. Peroxidase and catalase convert potentially dangerous to water through their combined action (Gong *et al*., 2001).

The antifungal activity of the essential oils suggests that they may be considered as a potential alternative to the synthetic fungicides for the control of postharvest citrus pathogens. However, despite their potent antifungal activity, commercial implementation of treatments with essential oils is strongly restricted in citrus because of problems related to potential phytotoxicity, intense sensory attributes or technological application as fumigants or in aqueous solutions (Palou *et al.* 2008). Thus, the protective effect of thyme extract in oranges seems to be a combination of the antifungal property and of the ability of the product in inducing defense responses in fruits and thyme extract could be a good replacement of fungicide for prevents citrus post-harvest diseases.

Studies on the efficacy and mechanism of action of new antifungal compounds are always important to provide access to several plant species in order to take advantage of their different biological properties or avoid resistance phenomena, thus making biological control measures more effective. Although further investigations using scaled-up conditions are necessary, the results of this study highlight the potential application of *Thymus vulga*ris essential oil as biological agents for postharvest protection of orange fruits against P. *digitatum*, both to increase the storage time of the fruit and to reduce the economic losses due to green mold decay. Finally, with regard to both ecological and human health concerns, the selectivity of *Thymus vulga*ris essential oil towards catalase and peroxidase and phenol compound, makes the *Thymus vulga*ris essential oil an attractive candidate for a future antifungal compound to protect fresh citrus fruit.

Table 6. List of the compounds present in the Thymus oil analyzed to show the retention time and the area percentage of each compound.

No	Compounds	RRI	% of oil
1	β-Pinene	1104	0.17
2	α-Pinene	1016	0.63
3	α-Thujene	1019	0.50
4	Camphene	1057	0.61
5	Thymol methyl ether	1607	0.51
6	α-Terpinene	1174	1.01
7	Limonene	1194	0.35
8	1,8 Cineole	1202	0.24
9	β-Phellandrene	1203	0.13
10	γ-Terpinene	1242	6.39
11	p-Cymene	1270	15.44
12	Camphor	1521	0.39
13	Linalool	1541	4.22
14	β-Caryophyllene	1596	1.31
15	Terpinen-4-ol	1602	0.93
16	Boroneol	1702	1.76
17	Myrcene	1159	1.17
18	α-Terpineol	1707	0.32
19	δ-Cadinene	1763	0.09
20	Isothymol	2171	0.12
21	Thymol	2225	60.18
22	Carvacrol	2228	2.88
		Total:	99.35

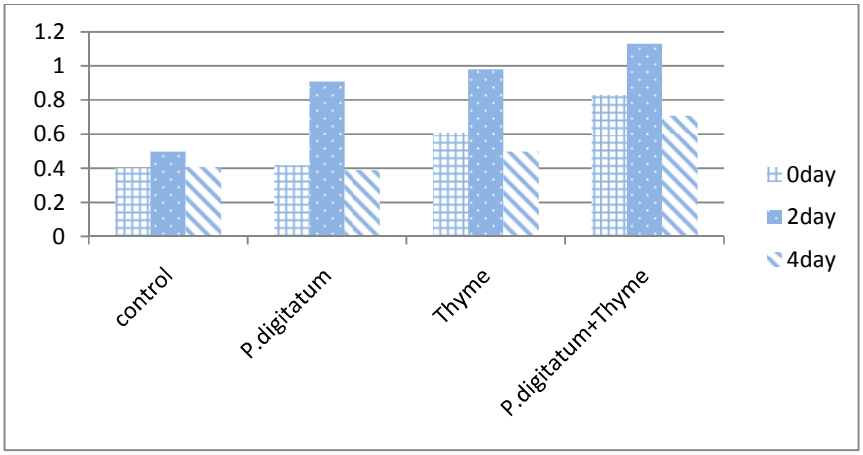

Fig. 1. Effect of thyme oil on peroxidase activity.

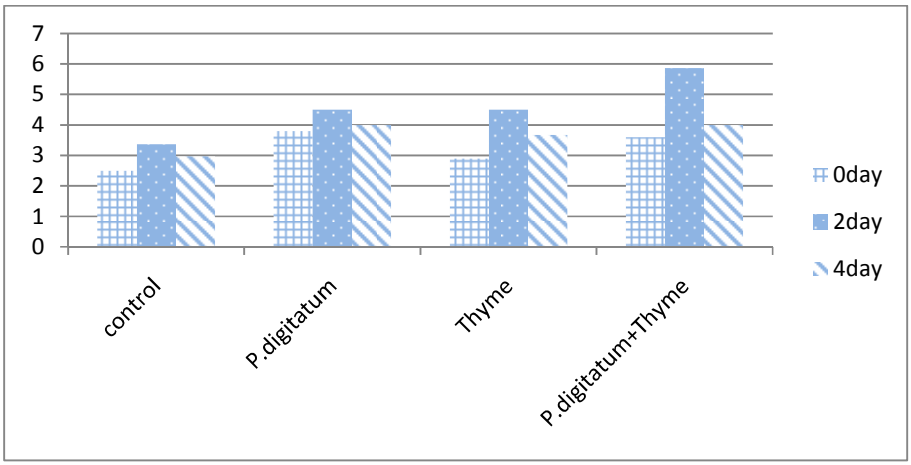

Fig. 2. Effect of thyme oil on catalase activity.

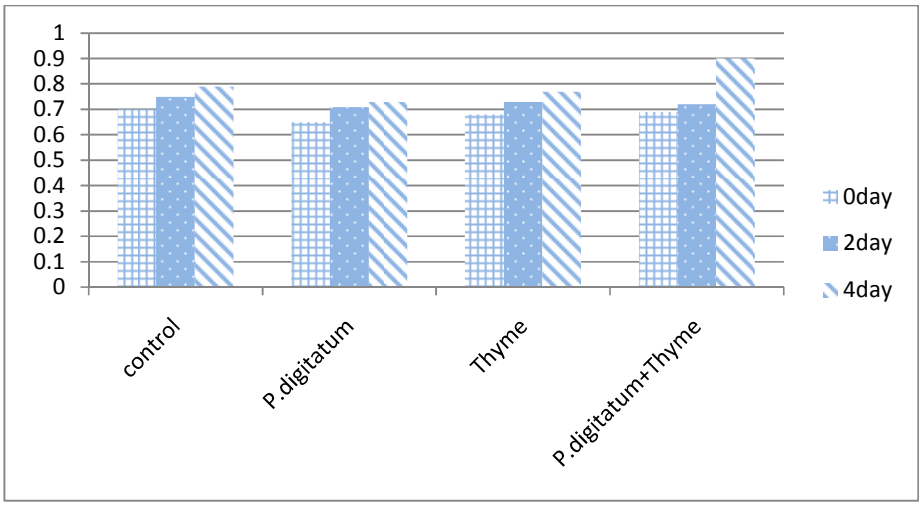

Fig. 3. Effect of thyme oil on Phenolic compound.

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