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Full Length Research Paper

Effect of essential oils of *Thymus vulgaris* and *Mentha piperita* on the control of green mould and postharvest quality of *Citrus Sinensis* cv. Valencia

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One of the modern techniques used to control pests and diseases, especially to produce organic crops is to use natural or growing material and components with microbial and herbaceous origin. The aim of this study was to evaluate the effect of natural components on green mould control and the improvement of postharvest fruit quality. The experimental design was completely randomized design with 3 replications. Natural essential oils were obtained from two plants, thyme and peppermint, in 5 concentrations of 0 (as control), 100, 300, 500 and 1000 ppm, and performed in inoculated and non-inoculated "Valencia" orange. The results showed that peppermint and thyme essential oils (1000 ppm concentration) decreased decay (27%), compared to the control (100%) in inoculated group. The highest level of vit.C (54.93 mg) was observed in thyme treatment (1000 ppm). TA content had shown significant difference in inoculated and non inoculated fruits affected by treatments. The highest total acid value was observed in non- inoculated fruits of thyme treatment with 100 ppm concentration (1.49 mg). The highest soluble solid content was observed as 11.75Brix in the control without inoculation. Generally, results showed that thymus essential oils have high fungicides to control fungus disease of citrus crops, but need more research to achieve appropriate formulation.

Key words: Essential oils, thyme, peppermint, green mould, shelf life, citrus.

INTRODUCTION

Use of natural components such as natural extract or herbal oils is one of the healthiest and safest methods to control postharvest diseases. Herbal essential oils include extensive secondary metabolites, which in most cases have antimicrobial, fungicidal, allelopathy, antioxidant and bio-regulating properties (Asghari et al., 2009). A number of known essential oils or herbal oils are about 3000, with 300 of them having economic value (Burt, 2004). In the view of chemical, essential oils are complex components with different kinds of chemical substances including: hydrocarbons, alcohols, cetons, and aldehydes (zargari, 1992). Thymus essential oil is known to contain more than 40% of phenolic compositions (thymol and carvacrol), that have strong antiseptics effect. In addition to thymole, caffeic acid and thanin existing in essential oil can effectively prevent growth of bacteria, fungus and viruses. The highest value of thymole exists in *Thymus vulgaris*. According to GC analysis, *Thymus captatus* contains carvacrol that researchers pointed to its anti microbial property and inhibition activity of the existence of these two compounds

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(Karimi and Rahemi, 2009).

Many aromatic materials are against micro organism, insects and herbivorous animals (Harmmer and Karson, 1999). Researchers are able to extract antimicrobial compositions from plants, and spray these on harvested crops to decrease decay. Researchers studied anti fungus activity of 4 types of *Foeniculum vulgaris*, *T. vulgaris*, *Duphni odura* and *Salvia officinalis* essential oils on green mould in laboratory conditions. The result showed that *T. vulgaris* and *D. odura* essential oils had the most inhibitor effect and *S. officinalis* and *F. vulgaris* essential oils had the least effect and can generally replace other treatments for controlling green mould with *T. vulgaris* and *D. odura* essential oils (Yahya-zadeh et al., 2008). Results obtained from the studies showed that the most essential oil in orange rind is limonin and was tested against 10 postharvest fungus disease agents, with 100 to 1000 ppm concentration, of which 700 ppm concentration showed the most inhibition effect in spores germination and 300 ppm treatment showed inhibition effect in most fungi about 70 to 100% (Sharma and Tripathi, 2006).

In another research of studying 26 plants essential oil on the anti fungus activity of gray mould, result showed that *Zingiber officinalis*, *Eucalyptus officinalis*, *Chenopodium ambrosides*, *Ocimum basilicum*, *Lawsonica ineris* and *Pranus persica* showed 100% inhibition on gray mould (Tripathi et al., 2008). In one research, chemical compositions of *T. vulgaris* and *Mentha piperita* were tested by using GC-MS where most of the essential oils of *Thymus* were thymole (44.7%), cymenin (18.6%) and tripenine (16.5%); and also most of the essential oil of menthe was menthole (Porte and Godoy, 2008). Based on the investigation, it is shown that there is positive use of menthe and lemon essential oils on citrus green mould; and chemical essential oils are appropriate replacement for artificial fungicides (Pooly et al., 2009).

In an extensive laboratory study, inhibition effects of *Mentha piperita*, *T. vulgaris*, *S. officinalis*, *Rose hybride*, *C. sinences* and lemon essential oils were evaluated on *Asperigillus parasiticus* growth and aflatoxin production was not observed by adding 1% thymus until 30 days (Muftah and Lloyd, 1982). In another research, *T. vulgaris, Saturega hortensis, Mentha piperita*, Lemon, *Ferula gumosa* and *Eucalyptus officinalis* were investigated in different concentrations. Results showed that *T. vulgaris* and *S. hortensis* essential oils had the most effect on growth inhibition with minimum inhibition concentration of 200 and 400 ppm. Due to these results, it can be said that *T. vulgaris* and *S. hortensis* essential oils in the above concentration have the ability to inhibit fungi growth that contaminates food products, orchard and forming products and can replace present chemical fungicides (Maskoki et al., 2005).

Application of *T. vulgaris* essential oil showed successful results both to control soft decay and gray mould agents in strawberry (Reddy et al., 1997). Effect of essential oils of *T. vulgaris, M. piperita, S. hortensis* and *Cuminum basilicum* were investigated on citrus postharvest fungus diseases such as *Penicillium digitatum* and *Penicillium italicum*, *Alternaria citri*. Their results showed that *T. vulgaris* essential oil in 500 ppm concentration inhibits the micellium growth of 2 kinds of penicillium (Azizi et al., 2007). According to other studies, *T. vulgaris* and *M. piperita* essential oils control green mould decay and postharvest quality of Valencia orange.

MATERIAL AND METHODS

This research was performed during 2010-2011 in Islamic University of Esfshan (Khorasgan). This test was performed in split plot with completely randomized design by using *T. vulgaris* and *M. piperita* essential oils in 5 concentrations of 0 (control), 100, 300, 500 and 1000 ppm, on inoculated and non inoculated Valencia orange. Green mold from contaminated oranges was used as spore source to provide fungi. Fungus spores from the surface of contaminated fruits were removed with scalpel and cultured on potato dextrose agar medium after pouring. 4 days after culturing, suspension solution of green mould fungi spore, 1×10^6 spore/m liter was provided by using Haemocytometer. Then spores were sprayed on two skin horizontal scratches of fruit: fruits were kept in this situation for 12 h until fungi penetrate into the scratches and inoculation processes were performed. Essential oils were provided in 25% ethanol with concentrations of 0, 100, 300, 500 and 1000 ppm by using sampler system. And then they were sprayed on fruits by using usual spray system. Then treated fruits were placed into plastic bags. All these treatments were performed usually for both non- inoculated and inoculated fruits. Inoculated fruits were divided into 15 groups (groups include 15 number) for each treatment, according to washing and inoculating factors. At first and before each treatment, 3 groups contain 5 fruits of inoculated and non-inoculated fruits selected as control and after weighing with double zero scale and taking notes; they were packed into plastic bags and then named. They were kept in store with 6°C temperature and 85-90% relative humidity.

The remaining fruits were divided into groups randomly due to inoculation and non- inoculation factors. And treatments were performed according to the type of treatment. Exiting fruits from store, decay percentage according to counted number of contaminated scratches, vitamin c, acidity and soluble solid content were measured and results were analyzed by using SPSS. Means were compared with Duncan's multiple test at 0.05 probability level. Vitamin C was measured by using titration method with Iod/potassium iodine, and calculated by using the following equation:

iod/potassium iodine × 0.88 ×10 was used to measure vitamin C milligram; to measure acidity, neutralize method with 0.3 N NaOH and phenolphthalein reagent was used according to mg citric acid / 100 ml liter fruit juice. To measure soluble solid content in fruit juice, refrectometer was used.

RESULT AND DISCUSSION

Effect of *T. vulgaris* and *Menthe* essential oils on decay

According to Table 1, in comparison of mean of decay

Table 1. Mean comparison levels of thymol and menthol on characteristics quality and quantify in the fruits.

Treatment	Vit C	TA	TSS	ROT
Thymol100	45.692(10.75197)[a]	1.34667(0.242166)[a]	10.1250(0.80234)[b]	36.102(27.10139)[bc]
Thymol300	43.608(3.46212)[a]	1.32483(0.123341)[a]	10.5833(0.341565)[b]	27.775(27.21506)[c]
Thymol500	42.227(6.5160)[a]	1.22500(0.098314)[a]	10.3333(0.204124)[b]	24.995(29.33693)[cd]
Thymol1000	47.673(9.184669)[a]	1.25683(0.057894)[a]	11.1667(0.801041)[a]	13.887(19.48371)[d]
Menthol100	48.140(12.50472)[a]	1. 28433(0.149205)[a]	10.5147(0.466114)[b]	44.433(32.7664)[ab]
Menthol300	43.408(8.644951)[a]	1.23367(0.083114)[a]	10.5000(0.446307)[b]	36.097(34.00708)[bc]
Menthol500	44.280(2.575795)[a]	1. 31567(0.093906)[a]	10.5417(0.458712)[b]	36.105(22.14556)[bc]
Menthol1000	44.057(1.734516)[a]	1.310500(0.147223)[a]	10.4583(0.600347)[b]	13.887(16.38473)[d]
Control	26.015(28.63847)[b]	0.65950(0.72448)[b]	5. 8750(46.84644)[c]	58.332(46.84644)[a]

Different letters in columns show significant difference based on Duncan's multiple test at level of 5%. Vit C, Vitamin C; TA, titrable acid; TSS, total soluble solid.

rates, significant differences existed among treatments. Most control treatments had 55.38% while other treatments- mint and thyme oil treatments (1000 ppm) had 13.89%; the lowest caries levels were more successful than other essential oils.

The interaction of treatments on the amount of fruit decay

Thyme and mint treatments in fruit inoculated had a significant difference in the 5% level indicated. The largest percentage of caries in the control treatment was 100. Also, the lowest caries in thyme and mint treatment (1000 ppm) were 27.77%; significant difference was observed between treatments as the lowest caries were in thyme treatment of 1000 and 500 ppm, without inoculation. No significant differences were observed in Table 1. This is because after harvesting, to operate these groups of natural compounds is very essential; but probably due to the effects of anti-bacterial and anti fungal properties, essential oils also have active materials such as thymol and menthol. The above results are consistent with the results of the effect of orange fruit oil treatment on microbial reduction (Yahya-Zadeh et al., 2008), where corruption of other fruits was reduced due to phenolic compounds such as thymol and eugenol, respectively (Marjorie, 1999). These appear to control the effects of oil produced. It seems some of them affect the fungal essential oils in the stimulation of plant defense responses which are also relevant. And the mechanism is such that several mechanisms certainly contaminate antimicrobial activity of the fungi set. Perhaps, some combined performance due to the combination of the aforementioned phenomenon is affected. Essential oils are known by nature to escape water, act as a catalyst of common and overlapping activities of different compounds, walls, destroy pathogens and cellular membrane permeability and increase ion leakage of cells. When this is followed by the breaking down of cell wall lipids, proteins and membranes of mitochondria and cytoplasm and draining of clog proton motive force, the damaged cells suffer death (Burt, 2004).

Essential oil of thyme and mint on the amount of vitamin C fruits

As seen in Table 1, comparison of mean showed that significant differences existed between treatments. Most vitamin C with 48 /14 mg in 100 ml of juice treated mint (100 ppm) was the lowest; vitamin C in the control treatment with 26.015 mg in 100 ml of fruit juice, in contrast to all other treatments, had a significant difference.

The interaction of treatments on the amount of vitamin C in fruits inoculated and not inoculated

As seen in Table 2, comparing the levels of inoculation and non- inoculation suggests that in the inoculated group, there was no significant difference between treatments. Most vitamin C in thyme treatment (100 ppm) with 54.937 mg in 100 ml of fruit juice inoculated had significant difference between treatments. This led to complete decay of fruits that had the lowest vitamin C in the control treatment; most of the Vitamin C in peppermint treatment (100 mg) was 44.67. Increase in storage time decreased the amount of ascorbic acid. This is because vitamin C is generally unstable when it has inclement storage in terms of light, temperature, humidity and diseases. Perhaps, the other reason is the high rate of vitamin C in thyme essential oil treatments due to greater impact of the oil to control decay. This is inconsistent with the results of Karimi and Rahemi

Table 2. Comparison mean, characteristics quality and quantify in the fruits inoculation.

Inculation	Treatment	Vit C	TA	TSS	ROT
No Inoculation					
0	Thymol 100	54.763(4.716952)[a]	1.49467(0.280958)[a]	10.75(0.433013)[abcd]	11.11(19.24308)[ef]
0	Thymol 300	45.877(2.482606)[ab]	1.24767 (0.037899)[a]	10.4167(0.381881)[abcd]	5.55(9.618655)[f]
0	Thymol 500	47.217(2.454513)[ab]	1.23433(0.038553)[a]	10.4167(0.144338)[abcd]	0.00(0)[f]
0	Thymol 1000	54.937(7.154511)[a]	1.22633(0.04596)[a]	10.6667(0.520416)[abcd]	0.00(0)[f]
0	Menthol 100	51.607(7.553021)[ab]	1.23200(0.05658)[a]	10.6667(0.57735)[abcd]	16.66(16.665)[ef]
0	Menthol 300	50.033(6.991082)[ab]	1.22267(0.05658)[a]	11(0.5)[abc]	5.55(9.618655)[f]
0	Menthol 500	46.020(2.615588)[ab]	1.29800(0.00817)[a]	10.3333(0.57735)[abcd]	22.22(19.24308)[def]
0	Menthol 1000	44.157(1.313672)[ab]	1.37067(0.037899)[a]	9.9167(0.144338)[cd]	0.00(0)[f]
0	Control	52.030(4.479297)[ab]	1. 31900(0.85733)[a]	11.75(1.089725)[a]	16.66(16.665)[ef]
Inoculation					
1	Thymol 100	36.620(4.458385) [b]	1. 19867(0.044287)[a]	9.5(0.5)[d]	61.09(9.607108)[abc]
1	Thymol 300	41.340(2.892525) [ab]	1.402(0.136865)[a]	10.75(0.25)[abcd]	50.00(16.6666)[bcde]
1	Thymol 500	37.237(5.041928)[a]	1.21567(0.149721)[a]	10.25(0.25)[bcd]	49.99(16.6666)[bcde]
1	Thymol 1000	40.410(1.200125)[ab]	1. 28733(0.05896)[a]	11.6667(0.763763)[ab]	27.77(16.6555)[cdef]
1	Menthol 100	44.673(17.25742)[ab]	1.33667(0.215658)[a]	10.4167(0.381881)[abcd]	72.20(9.635976)[ab]
1	Menthol 300	36.783(2.508152)[ab]	1. 24467(0.11707)[a]	10(0)[cd]	66.44(0)[ab]
1	Menthol 500	42.540(0.814064)[ab]	1.33333(0.145029)[a]	10.75(0.25)[abcd]	49.99(16.655)[bcde]
1	Menthol 1000	43.957(2.401173)[ab]	1. 25033(0.204671)[a]	11(0)[abc]	27.77(9.624429)[cdef]
1	Control	0(0)[c]	0(0)[b]	0(0)[e]	100.00(0)[a]

Different letters in columns show significant difference based on Duncan's multiple test at level 5%

(2009).

Essential oil of thyme and mint on the amount of total acidity

As seen in Table 1, comparison of the average showed that no significant difference existed between treatments. Results indicate a high amount of acid in the mint treatment (100) having 1.3467 mg in 100 ml of fruit juice; and it (acid) was lowest in control treatment having 0.6595 mg in 100 ml of fruit juice.

The interaction of treatments on the total amount of acid

As seen in Table 2, in the group without inoculation, there was no significant difference in the vaccination group, no significant difference between treatment and control due to the decay of fruit. These results indicate that essential oils of organic acids and other materials such as sugar prevent the amount of soluble solids in fruit vaccinated; and they were lower than the control fruits treated with essential oils. During storage due to oxidation of citric acid, the total amount of acid decreased the citric acid to one of the original materials remaining.

Peppermint essential oil of thyme and soluble solids

Average review showed that there exist significant differences between treatments in the 5 percent level. Accordingly, the lowest in the control treatment was TSS with 5.8750 BriX; the highest TSS was in thyme treatment of 1000 ppm (Table 1).

The interaction of treatments on TSS

The fruit without inoculation had the highest soluble solids in the control treatment (11.75 BriX). In general, treatments which used essential oils had TSS (BriX) fruits decline compared to those that did not use essential oil. This is probably due to reduced loss of juice, soluble solids concentration reduced in control. These results are consistent with the findings of other researchers on increase in fruit soluble solids in tomato and strawberries by Cinnamon and Eucalyptus oil (Tzortzakis, 2007). According to the results in Table 2, it seems quite clear that the essential oils affect the amount of dissolved solids. This finding is consistent with the results of Rapisarda et al. (2003).

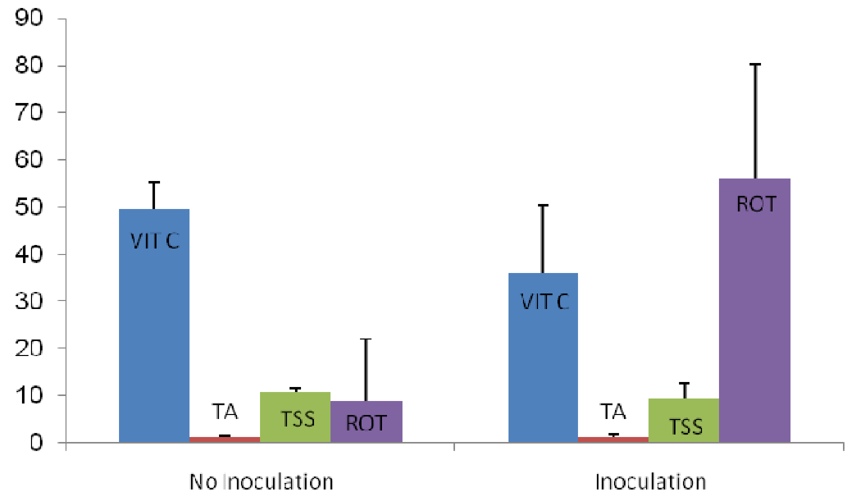

Figure 1. Total comparison group inoculation and no inoculation.

Conclusion

According to Figure 1, it can be concluded that the groups without inoculation were more effective than the inoculation group; and from all indications the group without successful inoculation was more. Results from this study showed that thyme and peppermint essential oil treatments (with concentration of 1000ppm) have been used to reduce decay rates and greatly preserved the amount of vitamin C. No treatment effect on the amount of soluble solids was 100 ppm, but the mint increased acidity of the fruit. In general, oil treatments in maintaining quality and reducing decay was more effective than surgery, but the best treatment in this study is thyme and mint (1000 ppm). As mentioned in essence drawing up mold testing has been proven in many studies, but with little impact on the quality of vegetable oils of fruits and its reports and clear document do not exist. Now, to prevent waste caused by fungi and plant species depending on the circumstances and to protect food whenever it is taken, kept in the garden or farm, or in cold storage and even before or during processing, certain fungicides are used: they include benomyl, thyabendazole, benzoate, and propionate, surbat, all of chemical origin and have health effects such as cancer, with many of them having been proven. So today, while trying to ban their use, many scientists have found suitable substitutes, which are natural and healthy to prevent waste caused by fungi and to make sure organic food products are safe.

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