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# Inhibition effects of *Zataria multiflora, Eucalyptus camaldulensis and Myrtus communis* essential oil on mycelial growth of green mold of Orange

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## Abstract

In this study, *Penicillium digitatum*, the causes of green mould disease of orange fruits, were isolated from infected fruit in Bam city. Antifungal activity of three different plant essential oils *Eucalyptus camaldulensis, Zataria multiflora, Myrtus communis* at 50, 100, 150 and 200 ppm concentration, have been evaluated. MIC (minimum inhibitory concentration) and growth inhibition zone diameters analyzed as factorial experiment with 3 replications in a completely randomized design by MSTATc software. *Essential oil Zataria multiflora Due to having* Thymol and carvacrol as the main component has an important role in the antifungal properties.The essential oil of *Myrtus* has been shown the weakest antimicrobial effect.

**Key words**: anti-fungal, essential oils, *Eucalyptus camaldulensis, Myrtus communis , Penicillium , Zataria multiflora.*

## Introduction

Providing food for the population is required to further development in the agricultural sector, with the growing world population. In this regard, the use of cultivars with high and stable yield can be useful. But limiting factors should also be considered (Bourgaud et al., 2001).One of the main limiting factors is plant disease, So that, 10% of World food production disappears by pests and plant diseases. While more than 800 million people do not have adequate food (Sinclair, 1984).

Citrus rot during storage is one of the major limiting factors. According to the Ministry of Agriculture report, the rate of destruction of citrus during storage in the province of Fars has been estimated 34,383 tonnes. One of the main causes of damage to agricultural crops, are microscopic fungi (Aidoo, 1991). The most devastating citrus fungi are blue and green mould rot of citrus (Zargari, 1995).

The use of chemicals to prevent or delay the destruction of food is well known. The negative effects of industrial chemicals on human, such as carcinogenesis has increased tendency to use of natural materials such as essential oils and antibiotics (Schuenzel et al., 2006). Essential oils are liquid oils that are

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obtained from different plant organs such as seeds, roots, buds, leaves, bark, twigs, buds and flowers. The antimicrobial properties of essential oils are mainly related to their phenolic compounds (Bagamboula et al., 2004), (Kim et al., 1995).

Carvacrol and thymol are the main components of *Zataria* essential oil. Compositions of the essential oils are different based on geography, season harvest, and the growing distance between cultivated plants. The composition of essential oil obtained from different parts of a specific plant may be different. Essential oil of this plant has antibacterial and antifungal properties and also stimulates the immune system.

Major essential oil components of *Myrtus communis* L. contain Depanthin and Myrtenol. Both oils are found in various organs of the plant, especially the leaves. In addition, Tannic acid (tannin) and organic acids were found in this plant. Therapeutic effect of plant is related to these compounds (Aidoo, 1991). Leaves of Eucalyptus yielded 3- 5 % essential oils. The oil of this species was characterized by a high content of *cineol* (70-85%), *linalool*, *borneol*, α*-pinene* and *caryophyllene*. Some *Penicillium* species cause disease in some fruits and plants including *P. expansum*, *P. digitatum and P. allii*.

Based on the distribution and abundance of medicinal plants are high in our country, the studies on these plants in the term of their antifungal properties provide a suitable field that their results are used to replace the fungicides of natural origin to control the post-harvest deseases of crops, and this can result in decreasing the application of fungicides and their effects.

In doing so, the studies were performed to compare the antifungal effects of essential oils of *Zataria multiflura, Myrtus communis* and *Eucalyptus camaldulensis* on penicilium fungus, responsible for green mould of orange fruits.

## MATERIALS AND METHODS

The aerial parts of *Eucalyptus, Myrtus* and *Zataria* plants were pulverized. The oil of samples was isolated by hydro distillation, using a Clevenger type apparatus. The distilled oils were dried over anhydrous sodium sulfate and stored in 10 CC vials in Refrigerator until the analysis. Concentrations of 50%, 100%, 150% and 200% was prepared from the initial solution. GC/MS analysis was performed by using Thermoquest-Finnigan gas chromatograph. The analysis was carried out using fused silica capillary DB-1 column (30 m × 0.25 mm; film thickness 0.25µm). Oven temperature program, 50°–250°C at the rate of 2.5°C/min,and finally held isothermally for 30 min.

Inhibitory effect of Plant essential oil in Mycelium Growth *Penicillium***.**

In the method of combining to culture medium, required concentration in 100 ml culture medium was calculated and was dissolved in 1.5 ml solvent. It was devided in petries after autoclave.

Then fungal discs were prepared with 6m diameters and were stored in 25°C incubation until the fungus occupies the culture medium.

### Testing of growth inhibition of fungi

The paper disc diffusion method was used to screen the antifungal activity of plant extracts

PDA media was prepared. 20 ml of it was poured into Plate-dishes (9cm). After solidification of the medium, Fungus samples were inoculated in sterile conditions. The discs impregnated with different concentrations of essential oil placed on the medium. To prevent the outflow of volatiles Plate was completely blocked by the Para film. The diameters (mm) of the inhibition zones were measured.

**Results**

**The evaluation results of inhibition effect through combining the essential oil to culture medium:**

The results from variance analysis and comparing data mean showed that 50 ppm concentration of *zataria* essential oil has the highest inhibition against fungus with mycelium growth diameter of 3.22 mm among the combinations of *Eucalyptus-Myrtus - Zataria*, *Eucalyptus- Zataria* and *Myrtus- Zataria*.

The lowest inhibition against fungal mycelium is related to *Myrtus* (5.49 mm).

In 100ppm concentration, the Lowest growth of fungal mycelium was observed in combined essence of *Eucalypyus*, *Myrtus* and *Zataria* with 2.51 mm and the highest inhibition was observed in 150 ppm concentration in the treatment of combined essence of Eucalyptus, *Myrtus* and *Zataria*, with mycelium diameter of 1.98 and less effective essential oil was *Myrtus* with growth diameter of 3.23 mm. any meaningful difference wasn't observed among the treatments of *zataria*, *Marytus* , *Zataria*, and *Eucalyptus* in 200ppm concentration.

The complete inhibition against fungal mycelium was observed in essential oils of *Zataria*, *myrtus* and *Zataria*, and Eucalyptus, *Myrtus* and *Zataria*.

The essential oil of *Myrtus* in this concentration had the highest fungal mycelium diameter (3.23 mm), with the lowest inhibition property.

The experiment was conducted and analyzed as factorial experiment with 3 replications in a completely randomized design. (Factor A: the factors studied, factor B: concentration of essential oils). The data were subjected to an analysis of variance and the means were separated using Duncan Multiple Range test by MSTATc software.

Table 1: Analysis of variance antifungal activity of essential oils on inhibition zone diameter of Mycelial growth in mm

| | df | Mean-square |
|---|---|---|
| A factor | 6 | 12.068** |
| B factor | 3 | 33.980** . |
| AB | 18 | 0.414** |
| error | 56 | 0.036 |
| Total | 83 | |
| Coefficient of Variation | 6.74% | |

| | df | Sum of Mean-square | Mean-square | sig | error |
|---|---|---|---|---|---|
| A factor | 6 | 72.407 | 12.08 | 336.1409 | 0 |
| B factor | 3 | 101.94 | 33.980 | 946.4881 | 0 |
| AB | 18 | 7.458 | 0.414 | 11.5405 | 0 |

| | | | | | |
|---|---|---|---|---|---|
| error | 56 | 2.01 | 0.036 | | |
| Total | 83 | 183.815 | | | |
| Coefficient of Variation: 6.74% | | | | | |

*,:** mean difference is significant at the 0.05 and 0.01 level . (Factor A includes 7 levels as a1: *Zataria*, a2: *Myrtus*, a3: *Eucalyptus*, *a4: Zataria+Myrtus, a5: Zataria+ Eucalyptus*, a6: *Myrtus+* Eucalyptus, a7: *Zataria+Myrtus+* Eucalyptus. Factor B: concentration of essential: b1:50, b2:100, b3:150, b4:200.

Figure 1. Effect of various essential oils 50ppm concentration on mycelial growth diameter

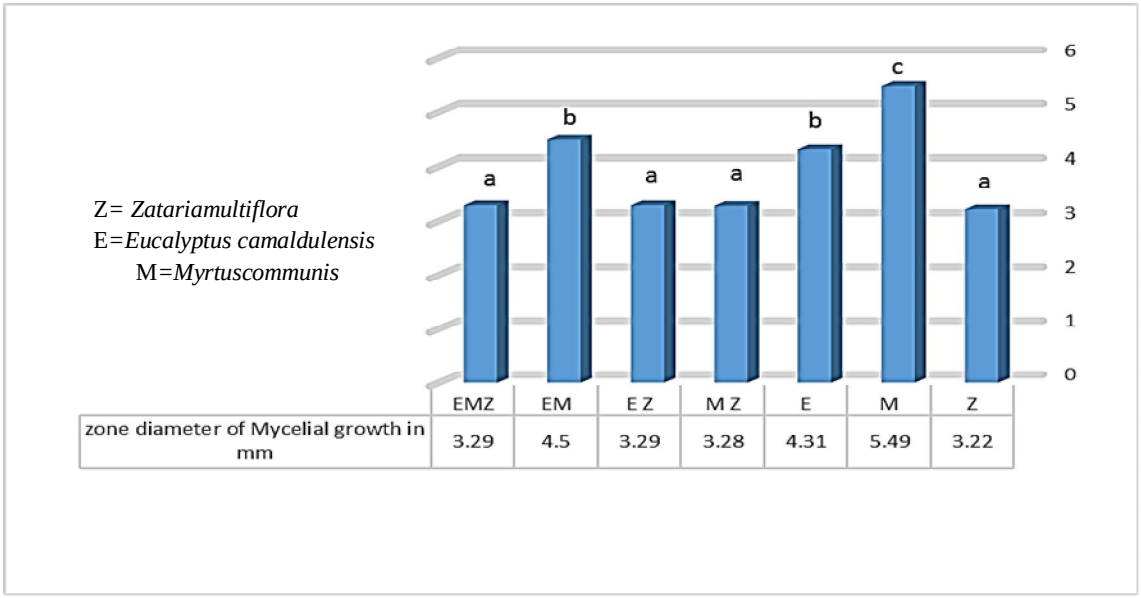


Figure2. Effectof various essential oils at concentrations of 100 ppm by the diameter in millimeters

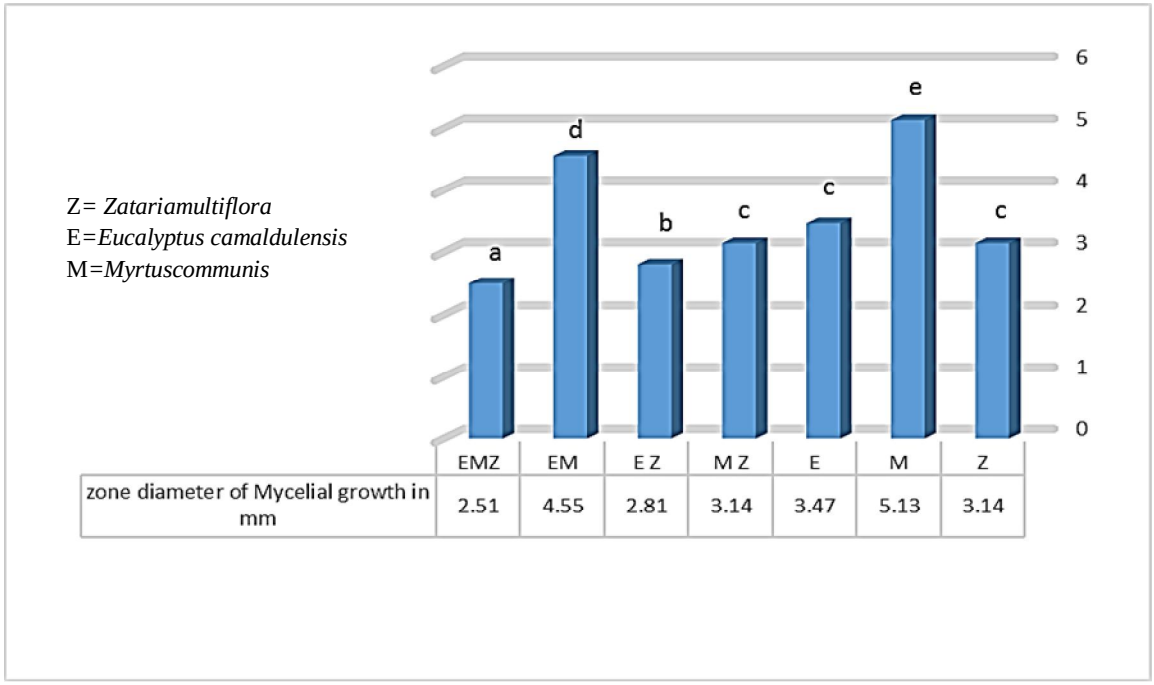



Figure 3. Effect of various essential oils 150ppm concentrations on mycelial growth diameter in mm

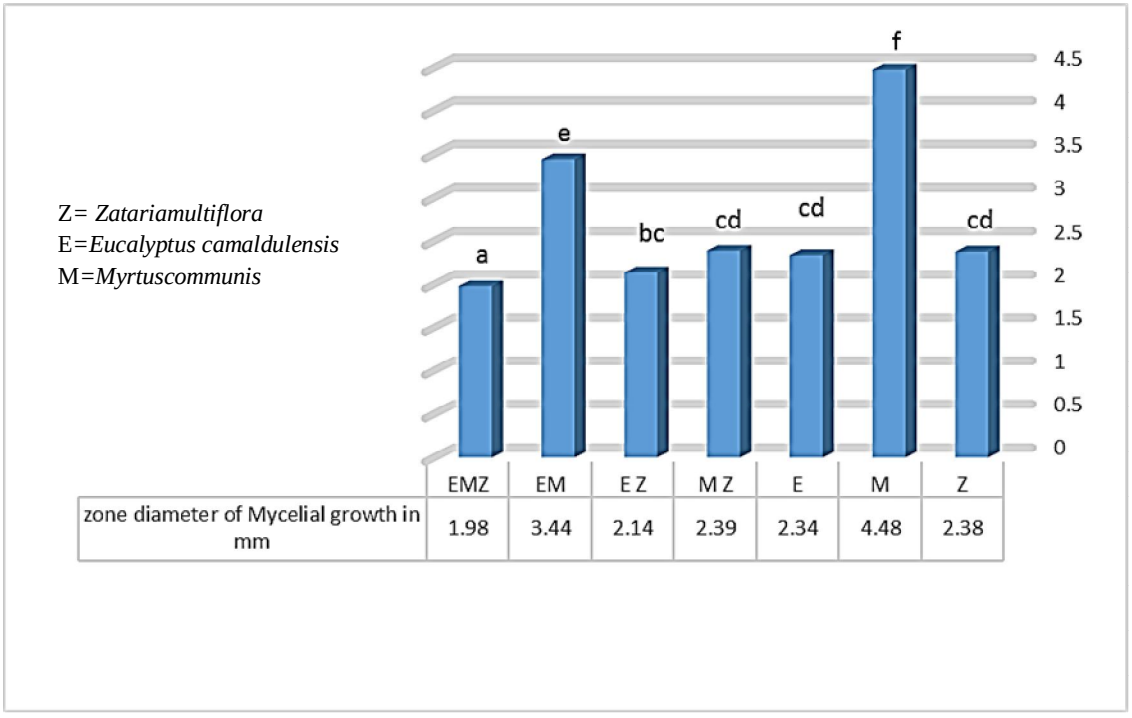
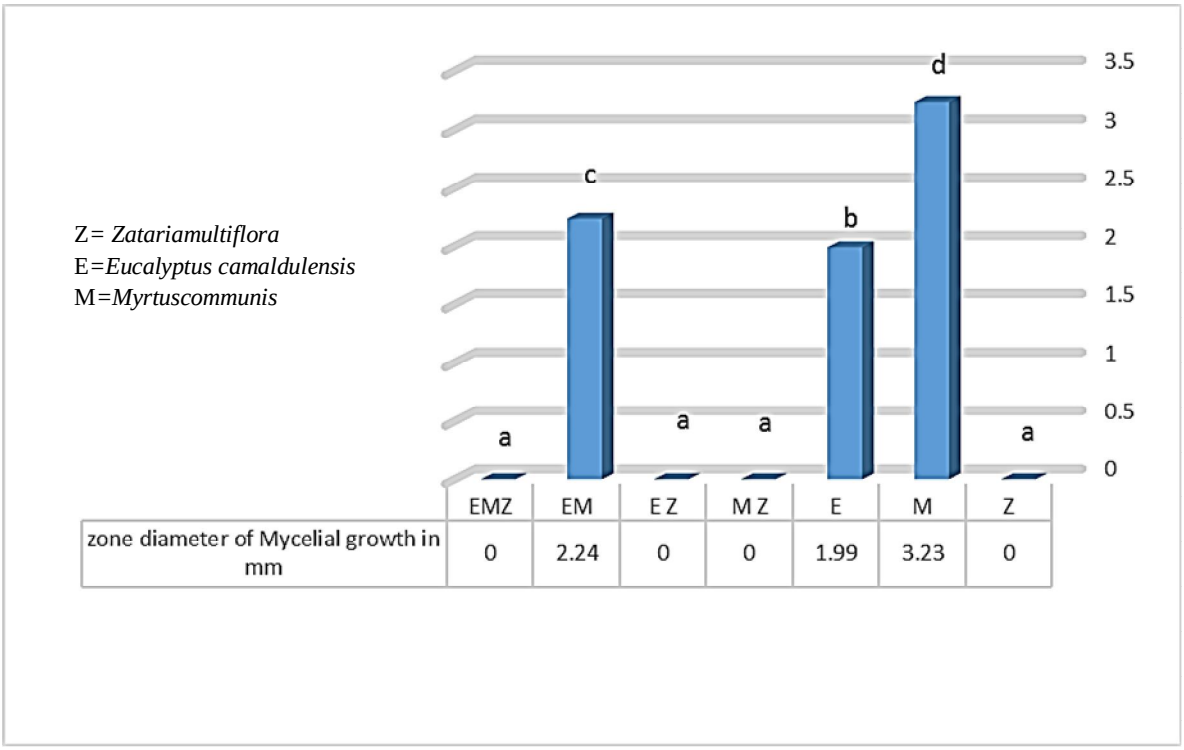


Figure4. Effect of various essential oils 200 ppm concentrations on mycelial growth diameter




| | EMZ | EM | E Z | M Z | E | M | Z |
|---|---|---|---|---|---|---|---|
| zone diameter of Mycelial growth in mm | 0 | 2.24 | 0 | 0 | 1.99 | 3.23 | 0 |

**The evaluation results of growth inhibition zone diameter by using Disc method:**

The results from variance analysis and comparing data mean in the Disc method in the 50 ppm concentration of *Zataria* and Eucalyptus, *Zataria*, showed the growth inhibition zone diameter of 19.52 and 19.24 mm, respectively and the best fungicide state and the essential oil of *Myrtus*, Eucalyptus and *Myrtus*, Eucalyptus werless effective treatment with the lowest growth inhibition zone diameter. In 100 ppm concentration, there was no meaningful difference among the treatments of *Zataria*, *Myrtus-Zataria* and *EucalyptusZataria* and all of them were in a statistical group and were among the most effective treatments. The combined essential oils of Eucalyptus and *Myrtus* had the lowest growth inhibition zone diameter (13.23 mm) with the lowest fungicide property.

In 150 ppm concentration, the highest growth inhibition zone diameter was related to combined essential oil of Eucalyptus and *Myrtus*, *Zataria* and *Eucalyptus*, *Zataria*(42.22 mm and 41.51 mm, respectively) and the essential oil of Eucalyptus was the least effective treatment with the lowest growth inhibition zone diameter.

Its highest diameter was observed in 200 ppm concentration in combined essential oils of *Eucalyptus* and *Myrtus-Zataria*.

The essential oil of *Myrtus* had the lowest fungicide property with 32.01 mm diameter of growth inhibition zone.

Table2. Analysis of variance antifungal activity of essential oils against *P. digitatum.* (Inhibition zone diameter in mm) in Disk method

| | **df** | **Mean-square** |
|---|---|---|
| A factor | 6 | 789.388** |
| B factor | 3 | 4634.123** |
| AB | 18 | 50.277** |
| error | 56 | 3.161 |
| Total | 83 | |
| Coefficient of Variation | 6.46% | |

| | df | Sum of Mean-square | Mean-square | sig | error |
|---|---|---|---|---|---|
| A factor | 6 | 4736.327 | 789.388 | 249.7395 | ٠.٠٠٠٠ |
| B factor | 3 | 13902.369 | 4634.123 | 14661.1025 | ٠.٠٠٠٠ |
| AB | 18 | 904.987 | 50.277 | 15.9062 | ٠.٠٠٠٠ |
| error | 56 | 177.007 | 3.161 | | |
| Total | 83 | 1972.690 | | | |
| Coefficient of Variation: 6.46% | | | | | |

Figure5.Effect of various essential oils 50ppm concentration on the inhibition zone diameterin Millimeters Disk

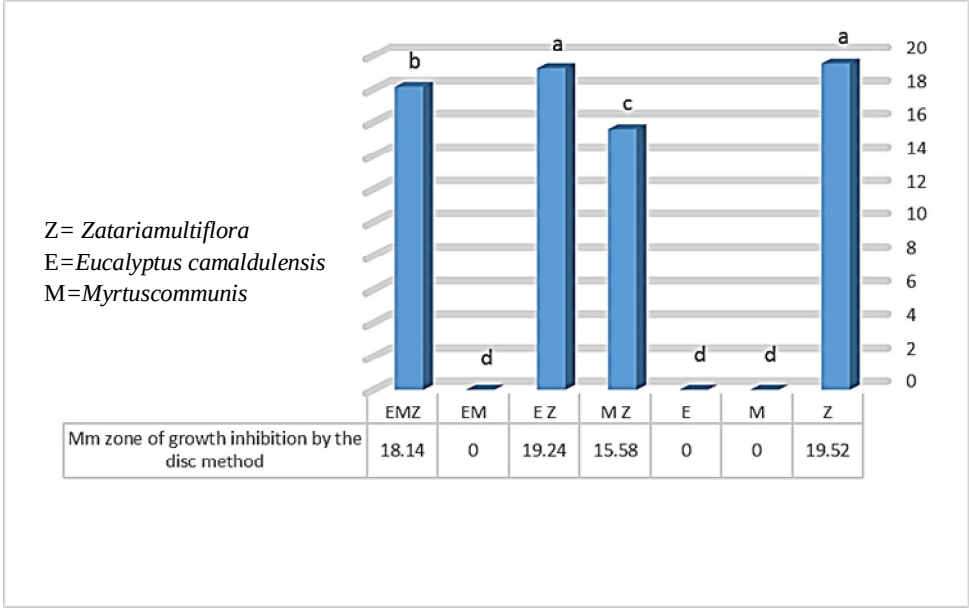


Figure6.Effect of various essential oils 100 ppm concentration on the inhibition zone diameter in millimeters Disk

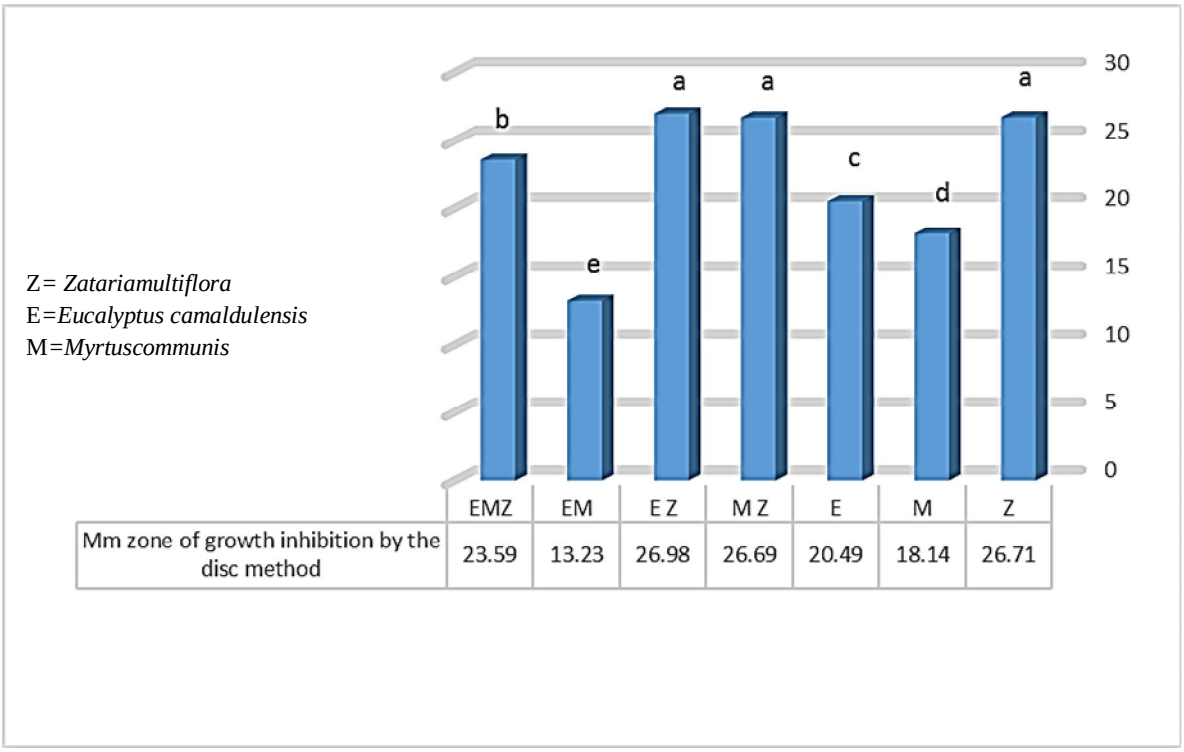



Figure7.Effect of various essential oils 150 ppm concentrations on the growth inhibition zone diameter in millimeters Disk

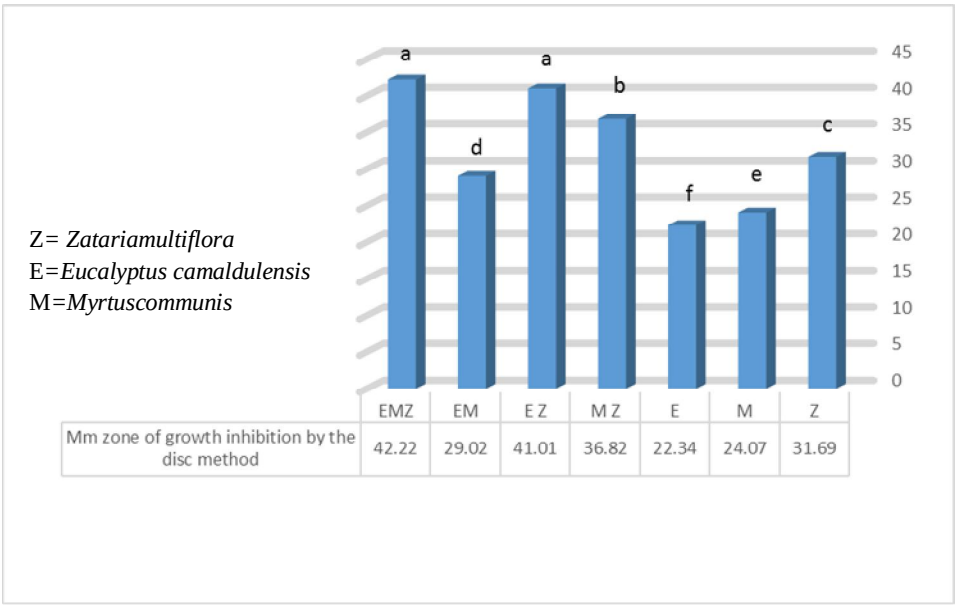
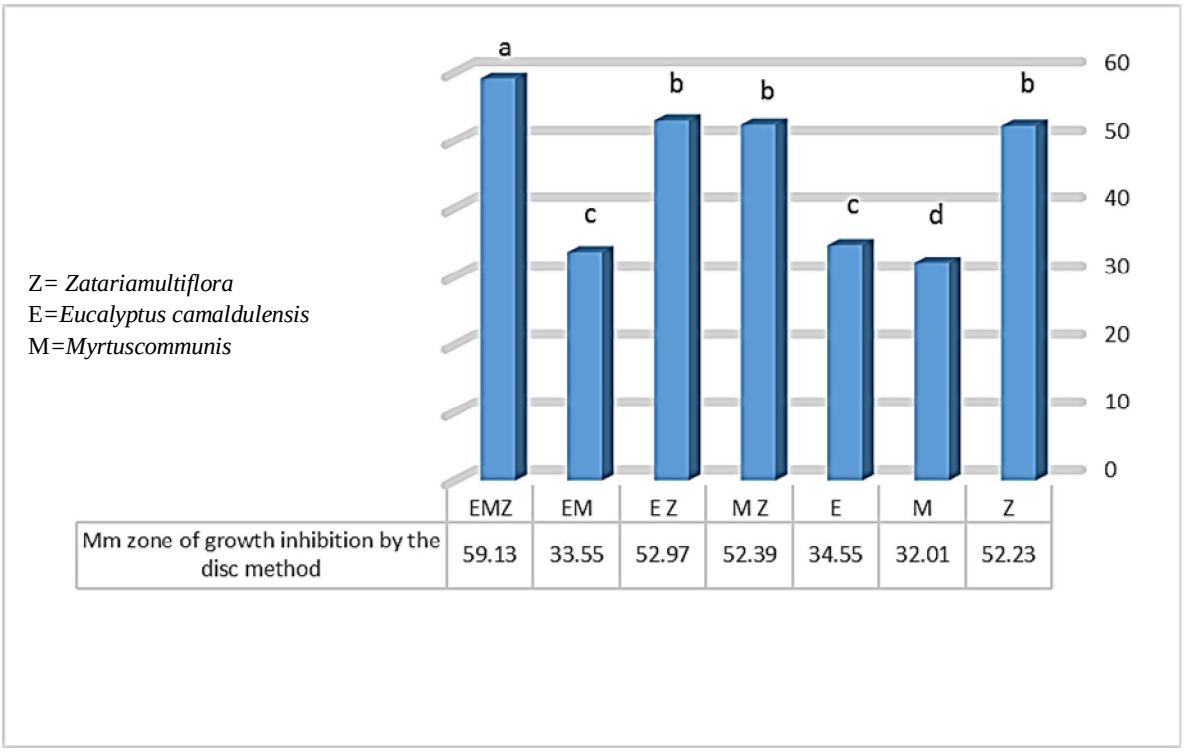


Figure 8. The effect of different oils in a concentration of 200ppm of zone diameter in millimeters Disk




**The results of the combinations of essential oils of *Zataria multiflura*, *Eucalyptus officinalis* and *Myrtus communis*:**

The analysis of *Zataria multiflura* essential oil showed that the it's main compounds (thymol and carvacrol).

Allocate 60 percents of all compounds to themselves. (Table 3), and by indetifying the compounds of *Eucalyptus* essential oil that are 22 compoutls, it was clear that the highest proportion is related to *Eucalyptus* (64%). (Table 4)

In the analysis of essential oils of *Myrtus communis*, 97 percents of compounds were identified so that α-penine, 1, 8- Cinneol, Linalool, α-tripneol, Linalool acetat and Caryophyllene. (Table 5)

**Table3: Analysis of essential oil *Zataria multiflora***

| Name of compound | Percent inhibition | Percent compound |
|---|---|---|
| α-thunjene | 927.27 | 0.08 |
| α-piene | 936.74 | 2.00 |
| Campene | 950.76 | 0.09 |
| 3-octanone | 965.91 | 0.18 |
| β-piene | 973.03 | 0.16 |
| Myrecene | 982.95 | 0.68 |
| p-cymene | 1017.5 | 8.27 |
| β-terpineol | 1026.00 | 0.9 |
| &-terpene | 1053.1 | 2.84 |
| Linalool | 1085.7 | 1.27 |
| p-menth-1-eh-4-ol | 1168.2 | 1.04 |
| p-menth-1-en-8-ol | 1180.3 | 1.12 |
| Carvacrol methyl ethor | 1228.0 | 1.62 |
| Thymol | 1268.7 | 13.10 |
| Carvacrol | 1284.7 | 50.57 |
| Thymylacetate | 1329.7 | 0.68 |
| Carvacryl acetate | 1348.9 | 3.83 |
| Trans-caryophyllene | 1431.0 | 3.5 |
| Eudema-3,7-dien | 1448.0 | 0.1 |
| Aromadendrene | 1451.2 | 2.03 |
| α-humulene | 1463.5 | 0.2 |
| Cyclosativene | 1471.0 | 0.12 |
| Ledene | 1502.9 | 1.07 |
| Spathulenol | 1577.3 | 1.08 |
| Caryophylene oxide | 1584.7 | 1.45 |

**Table4: Analysis of essential oil *Eucalyptus camaldulensis***

| Name of compound | Percent inhibition | Percent compound |
|---|---|---|
| α-pinen | 937.2 | 2.87 |
| β-pinen | 974.1 | 1.24 |
| Cymene | 1023.0 | 2.10 |
| Eucalyptol | 1038.6 | 64 |
| Linalool | 1202.3 | 0.32 |
| Pinocarvacrol | 1131.3 | 3.95 |
| Borneol | 1167.0 | 0.70 |
| Terpinen | 1169.2 | 2.53 |
| α-terpineol | 1178.3 | 3.94 |
| Verbenon | 1188.0 | 0.20 |
| Cumene | 1210.7 | 0.37 |
| garaniol | 1256.0 | 0.50 |
| p-cymen-7-ol | 1270.0 | 0.10 |
| 2-isopropyl-5-methylpnenol | 1282.1 | 0.19 |
| β-caryophyllen | 1444.5 | 1.55 |
| Farnesen | 1462.3 | 1.37 |
| Bicyclogermacrene | 1494.1 | 1.10 |
| Spathulenol | 1528.5 | 6.55 |
| Caryophyllene | 1579.4 | 0.46 |
| Globulol | 1584.4 | 1.38 |
| Eudesmol | 1592.0 | 0.50 |

**Table5: Analysis of essential oil *Myrtus communis***

| Name of compound | Percent inhibition | Percent compound | Name of compound | Percent inhibition | Percent compound |
|---|---|---|---|---|---|
| Terpinen-4-ol | 1183.0 | 1.208 | Propyl butyrate | 914.2 | 3.32 |
| α-terpineol | 1198.2 | 8.792 | α-pinene | 934.1 | 16.641 |
| Verbenone | 1261.1 | 0.161 | Camphene | 954.0 | 0.503 |
| Carveol | 1226.1 | 0.342 | β-pinene | 979.0 | 1.605 |
| Nerol | 1234.4 | 0.694 | β-myrcene | 991.1 | 1.5 |
| Carvone | 1248.1 | 0.149 | α-phellandrene | 1007.6 | 0.768 |
| Linaleol-acetate | 1256.3 | 7.743 | Delta-3-carene | 1012.2 | 2.524 |
| Geranial | 1271.1 | 0.153 | Limonene | 1033.1 | 17.771 |
| Lavandulyl acetate | 1300.0 | 0.213 | 1,8-cineole | 1047.1 | 2.144 |
| Carvacrol | 1305.0 | 0.330 | Ocimene(β) | 1051.1 | 1.441 |
| Methyl gerente | 1323.0 | 1.076 | -terpinene | 1063.1 | 1.514 |

| | | | | | |
|---|---|---|---|---|---|
| Pipeitol acetate (cis) | 1354.0 | 4.44 | Linlool-oxide | 1075.3 | 0.384 |
| Neryl acetate | 1362.4 | 0.834 | Terpenolene | 1091.1 | 1.737 |
| Geranyl acetate | 1383.2 | 2.943 | Linalool | 1102.0 | 10.658 |
| Caryophyllene | 1431.0 | 2.407 | Terpin-1-ol | 1125.1 | 0.146 |
| α-humulene | 1465.1 | 1.716 | Neo-allo-ocimene | 1130.1 | 0.145 |
| Caryophyllene-oxide | 1564.2 | 0.922 | Terpinolecis-β | 1146.4 | 0.468 |
| | | | Karhaneone | 1152.2 | 0.282 |

**Discussion and Conclusion**

Because of the complexity, volatility and being insoluble in water, evaluation of antimicrobial action of essential oils is difficult. But by the use of a solvent or substance that facilitates diffusion that has no effect on the growth and differentiation of microorganisms and avoiding prolonged incubation of oils can be greatly overcome the above problems.

Essential oils prevent the synthesis of DNA, RNA, proteins and polysaccharides in bacterial and fungal cells.This substances act Similar to the effects of antibiotics in fungi. Many researchers focus on the antimicrobial activity of essential oils and Combine different plant extract in order to test the possible synergistic effects of them (Kalemba et al., 2003).

According to the results of this study the antimicrobial activities against *Penicillium* were obtained for extracts of *Eucalyptus Zataria and Myrtus*.The extract of *Myrtus* has been shown the weakest antimicrobial effect.

The amount of effective components of plants played an important role in this difference. Thymol and carvacrol as the main component of *Zataria* oil has an important role in the antifungal properties.The main effective material of Zataria multiflura in this research was carvacrol (50.57 percents) that it's antimicrobial effect is shown by researches.( Akgul et al., 1988) (Bouchra et al,. 2003) (Chmai et al., 2004)

These two components do not exist in the essential oil of *Eucalyptus* and *Myrtus*. Carvacrol, interacts with the cell membrane by altering the permeability of the $H^+$ / $K^+$Channel. Change in ion gradients leads to the disruption of cellular functions and cell death. Essential oils interact with each other and it plays an important role in the antimicrobial effect of plant.Thymol and carvacrol have synergistic effects (Aidoo, 1991). Eucalyptus by 80 percent of eucalyptol has a weaker effect than Zataria. It has been proven that thymol and carvacrol possess the stronger antimicrobial activity than eucalyptol (Bagamboula et al., 2004).

According to the results of present study, it is concluded that Zataria extract due to having both thymol and carvacrol has strongest effect Compared to Eucalyptus. Synergistic effect of essential oils has beneficial effects on Pathogenic Fungi.

Combined effect of essential oil of plants is not equal the effect of individual oil. It is less than the sum of them. The essential oils have an interaction with each other. In studying the antimicrobial effects of essential oils of *Zataria multiflura*, *Myrtus communis* and *Eucalyptus officianalis* on the bacterial of streptocucus, Hemophylus influenza and katarhalis, yazdi et al (2007) showed that the highest growth inhibition zone diameter of all three bacteria is observed in the essential oil of *Zataria multiflura* and totally, the effects of these three essential oils were good on all three microorganisms. (Yazdi et al., 2007)

Aras and osaie (2001) showed that *Zataria multiflura* essential oil removes the fungal mycelium completely and is able to change the morphology and the kinetics that it's most important antifungal combination was karoecrol that 81 to 83percent of all essential oil is dedicated to it. (Arras et al., 2001)

MIC value of the essential oil of 24 plants determined against 9 bacteria and 7 fungi. All essential oils tested have shown MIC values4000- 62.5 µg/ml against *Mycobacterium Smegmatis*. 13 oils inhibited the growth of *Staphylococcus aureus* and 17 oils inhibited the growth of *Candida albicans.* But only 6 oils were effective on *Aspergillus niger* and 3 of them were effective when the concentration of oil was 4000-250 µg/ml. in the other study, antifungal activity of 11 essential oils were determined against *Aspergillus niger* by ***Broth*** Microdilution ***method (***Kalemba et al., 2003***).***

The results of numerous studies, encourages the use of essential oils as food preservatives. Based on the antifungal activity of these oils the possibility of using these compounds in the medical, pharmaceutical, veterinary, food and cosmetic has been confirmed.Since the plants studied grow abundantly in Iran, the use of these plants is economical. On the other hand, because of the oils in vitro as Compared to their original location is likely to be more active, It is suggested that further research on this subject to be done.

To evaluate the therapeutic properties of essential oils further investigation should be done on postharvest fruits and vegetables.

## References

Aidoo, K.E. (1991). Post harvest storage and pre servation of tropical crops. Mycotoxin and Animal Foods (Smith, J.E. and R.S. Henderson,(eds). CRC pres.), Pp.747-765.

Akgul A and Kivanc M. (1988). Inhibitory effects of selected Turkish spices and oregano components on some food borne fungi. Int. J. Food Microbiol, 69 (3): 263-268.

Arras, G; and usai , M. (2001) . Fungi toxic activity of 12 essntial oils against fouvpost harvest citrus patogens: chernical analysis of *thymus capitatus* oil and its effect in sub atmospheric.pressure conditions. Journal food protection. 64: (1025-1029).

Bagamboula CF, Uyttendaele M and Debevere J. (2004) Inhibitory effect of thyme and basil essential oils, carvacrol, thymol, estragol, linalool andp-cymene towards Shigellasonnei and Shigellaflexneri. J. Food Microbiol, 21: 32 – 42.

Bouchra C, Achouri M, Hassani LMI and hmamouchi M. (2003). Chemical composition and antifungal activity of essential oils of seven Moroccan Labiate against Botrytis cinera pers. Fr J. Of Ethnopharmacology , 89 (1): 165-169.

Bourgaud, F., A. Gravot, S. Milesi and E. Gontier. (2001). Production of plant secondary metabolites: a historical perspective. Plant Sci. 161: 839-851.

Chmai N, Chami F, Bennis S, Trouillas J and Remmal A. (2004). Antifungal treatment With carvacrol and eugenol of oral candidiasis in immune suppressed rats. Braz. J. Infec. Dis. 8 (3): 217-226.

Hernandez, M. M., C. Heraso, M. L. Villarreal, I. Vargas-Arispour and E. Aranda. (1999). Biological activities of crude plant extracts from Vitextrifolia L. (Verbenacae). J. Ethnopharmacol. 67: 37-44.

Kalemba D, Kunicka A.(2003). Antibacterial and antifungal properties of essential oils (A review article).Current Medicinal Chemistry, 10: 813-829.

Kim JM, Marshall JA, Cornell JA and Preston JF. (1995). Antibactrial activity of carvacrol, citral and geraniol against Salmonella typhimurium in culture medium and on fish cubes. Food Sci., 60 (6): 1364 - 8.

Schuenzel KM and Harrison MA (2006). Microbial antagonists of food borne pathogens on fresh, Cambridge press: 324 P.

Sinclair, W.B. (1984). The Biochemistry and Physiology of the Lemon and Other Citrus Fruit. University of California Press: Oakland. CA. 946 P.

Yazdi, MH; Bayat, M; Jafari, shahin, A (2007). the evaluation of antimicrobial effects of essential oils of *Zataria multiflura*, *Myrtus communis* and *Eucalyptus officinalis* on streptocucus, Hemophylus influenza and Muraxelakatarhalys by invitro method.

Zargari A. (1995). Medicinal plants. Tehran university publication, second volume, pp: 301 - 6.