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# Evaluation of the Antifungal Activity of Two Plant Essence on the Qualitative and Quantitative Characteristics of Strawberry Fruit During Storage Condition

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**ABSTRAC**

**Objective:** The aim of this experiment was to observe and study the effects of two anti-fungus essences. **Methods:** *Thymus vulgaris* and *Thymus kotschyanus*, against two fungi pathogens *Botrytis cinerea* and *Rhizopus stolonifere,* and the quality of strawberries after cropping. This study is performed in two assays, in vitro and in vivo. A treatment consists: control, 200, 400, 600, 800, 1000 µl/l. **Results:** The results of in vitro assay showed both essential oils had inhibitory effect even at low concentration also best inhibitory effect occurred at 1000 µl/l of *Thymus kotschyanus* for *Rhizopus stolonifere.* The in vivo assay showed that the strawberries groomed by the two plants essences have a higher enzymatic level of CAT and SOD. Therefore these plants essences can replace the chemical anti-fungi medicines used to control the fungi related diseases on agricultural crops due to their anti-fungus effects but still a lot more research needs to be done in order to reach a proper formulation.

## INTRODUCTION

In early century with the consideration to population of the world and top Consumption of fruits and vegetables, methods of mmaintenance and ppersistence for most of the products are the important phenomenon, on the other hand methods of protections for this products and material that are to be used is also very important. Some of the chemical material that is used to help to the persistence of these products might have side effect on the health of user. It seems using organic toxins have a Serious problems such as creation of resistance in genetic of insects ,aattacks of pests ,Phytotoxicity, ttoxicity for vertebrates,eextensive damage to the environment, and rising costs of producttt, lead to an increase in the need of finding effective fungicide and being biodegradable Simultaneously (Glenn et al. 1994; Elhaj, 2000; Wang et al. 2007). There is an increase for searching alternative materials, chemicals, pesticides, natural pesticides for the protection and preservation of plants with low toxicity for human's healthy (Ivibjaro, 1983; Raja et al. 2001). In recent years, researchers start to use nnatural essential oil for increasing the persistence of fruits, green vegetable which have no

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adverse effects on human's healthy also have ddisinfection and anti-Fungal activity , and don't destroy the marketability of horticulture crops . Natural essential oils are safe for human being and other mammals they also have nutrient and medicinal properties and will ddecomposes fast in nature (Tamas, 1990). Essential oils start their job by the method of ddisrupting cell membranes, exposure to the enzyme active sites and involving in cellular metabolism (kim and marshal, 1990). The mechanism of action of essential oils can be attributed to its hydrophobicity properties (Brul and Coote, 1999; Cox et al. 2000). It seems existence of pphenolic rings is essential for the antimicrobial activity of thymol and eugenol (Laurence, 1981). Antimicrobial activity of some essential oils related to the sigmoid core and the negative OH groups (Velluti et al. 2003). The existence of High levels of phenolic compounds seems to lead a stronger antimicrobial activity. It seems that phenolic compounds prevent the fungal cell wall enzymes, such as kitin synthase / kitinase α and β-glucanase (Kumar, 2008). Essential oils of thymol, menthol and eujenul reduced mold growing and increased antioxidant activity also increased collecting free radicals (Wang et al. 2007). Strawberry fruit as a horticultural products are popular among customers around the world. Some fungus that leads to reduced storage life of strawberry fruit are *botrytis cinerea* and *Rhizopus stolonifere*. Fruits suffering from Botrytis fungus will become in a bad shape and they will get destroyed too. (Ashkan and Zakaeei, 2005). Strawberries are one of the richest sources of natural antioxidants in fruits (Wang et al. 2007). Most of the essential oils have been reported to inhibit postharvest fungi in vitro conditions. Sharma and Tripathi (2008) found that the essential oils of thyme ,Cinnamon (250 ppm), parsley (2200 ppm) and the mint has completely inhibitory effects against *A.Flovus* and *A.parasiticus* . Thymol is an essential oil component from thyme and has been used as medicinal drug, food preservative, and beverage ingredient (Jain, 1985; Mansour et al. 1986). Carvacrol treatment at all concentrations of 5% and 0.2 and 0.5 and 1 milimol per liter control the Botriyits pathogen on PDA medium (Martínez et al. 2007). Another anti-fungal property of the essential oils of *Thymus zygis* on the *Rhizoctonia solani, Colletotrichum octatum, Fusrium oxiyisporum* has been reported (Bakali et al. 2008). It was reported that the essential oil of *Thymus vulgaris is* stronger than fungicides such as carbendazim, Mankozeb and could stop *Flavus aspergillus* fungal activity (Kumar, 2008). Mohammadi (2011) reported that the thymol-essential oil strawberries treated, showed significant increased for superoxide dismutase and catalase activity. The main purpose of this study was to evaluate the antifungal potential of *Thymus vulgaris* and *Thymus kotschyanus* oils against two important pathogenic fungi (*R. stolonifer and B. cinerea*) in vtiro condition which can reduce the shelf life of strawberry fruit. Also measuring the SOD and CAT activity in in vivo condition.

## 2. MATERIALS AND METHODS

The test took place in December 2013 in Zakaria Razi Department of Plant Protection and Department of Horticulture Science and Research Branch, Islamic Azad University, Tehran, Iran.

### *2.1. Plant material*

To perform the experiments, strawberry fruit of sleva cultivars in Varamin transported to the laboratory in the early morning hours. For providing yeast strains of *R.stolonifer and B.cinerea*; fungal infected fruits were collected and fungus isolated from them *B. cinerea* and *R.stolonifer* were obtained in mycological collection of department of plant protection, Islamic azad University of science and research.

### *2.2. Extraction of essential oil*

For the treatments of essential oil; leaves of *T.kotschyans* and *T.vulgaris* collected near Qazvin plain Abhar and their essential oils obtained by hydro distillation method using Clevenger apparatus. About 200 g dried material (plant material was dried at ambient temperature and shade condition) of each species were cut in to pieces, ground and then submitted to hydro distillation for 3 h using a Clevenger type apparatus. The obtained essential oils were dried with anhydrous sodium sulphate and stored at 4° C before analysis and use for antifungal study. The composition of the oils was analyzed by GC and GC-MS.

### *2.3. Test plan*

The antifungal tests were carried out in vitro according to the method described by Pitarokili et al. (2003) using Petri dishes 8 cm in diameter containing potato dextrose agar (PDA). The essential .oils were dispersed as an emulsion in water using Tween 20 (0.05%) and added to PDA immediately before it was filled into the Petri dishes at a temperature of 45-50˚C. The concentrations tested were 200, 400, 600, 800 and 1000 µl/L. The controls included the same quantity of Tween 20 mixed with PDA. The phytopathogenic fungi were inoculated immediately after preparation of the Petri dishes by placing in the centre of each plate a 6 mm diameter disk of the test species, cut with a sterile cork borer from the periphery of actively growing cultures on PDA plates. The Petri dishes were incubated in the dark at a temperature of 24˚C. Mean growth rates were calculated from 3 replicates of each fungal species every 24 h. every replicates has 3 petridishes. This calculated until fungi in

the control filled the Petri dishes completely. Then used the following formula:

$$inhibition\,rate\,(\%) = \frac{R-r}{r} \times 100$$

R= fungal growth on control
r =Fungal growth on treated petridish

Measurement of catalase and superoxide disomotase was conducted under the In vivo.. This experiment has two kind of control. Water-immersion and non-water-immersion control.The in vivo experiment has three replications and there were nine fruits for each replicate. The measurement of CAT and SOD tests were carried out in vivo according to the method described by Luck (1974) and Mirsa (1972).

## 3. Results and Discussion

### *3.1.* Analysis of *Thymus kotschyanus and Thymus vulgaris by (GC/MS)*

The chromatographic analyses resulted in the identification of 30 components. *T. kotschyanus* oil has chemical compositions characterized by Thymol (30/27%), phenol, 2-methyl-5-1-methyl ethyl (27/67%) and Benzene (11/96) as the major components while the major constituents of the *T.vulgaris* were thymol (27/63%), phenol,2-methyl-5-1-methyl ethyl (24/93%) and Benzene (11/76%).

### *3.2. The results of the evaluation of inhibition rate on Botritys cinerea*

Growth inhibition at the level of five percent and 10 degrees of freedom. chart comparison between the average rate of growth inhibition on *Botritys cinerea* shows the maximum inhibition at 1000 µl/L *Thymus vulgaris* with 54/96 % then the highest were respectively at the 1000 µl/L *Thymus vulgaris* treated with 47/23 %, 800 µl/L *Thymus kotschyanus* treated with 46/9 % ،treated with 600 µl/L of *Thymus kotschyanus* with 39/5%, treated with 800 µl/L of *Thymus kotschyanus* with 38/4% , which they have no significant difference ttogether but their difference with the other treatments were significant. Lowest growth inhibition rate happened at 200 µl/L of *Thymus vulgaris* with 16/16% and after that it was the treatment with 200 µl/L of *Thymus kotschyanus* with 19/66 % which was not significantly different then it was 600 µl/L of *Thymus vulgaris* treated with 25/38 % and after that it was the treatment with 600 µl/L *of Thymus kotschyanus* with 26/23%.(Figure 1).

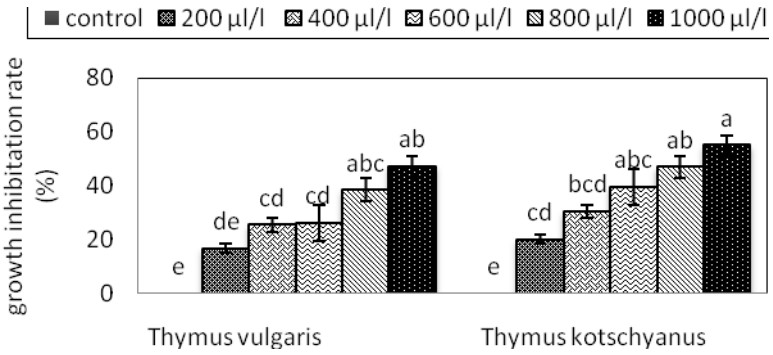


**Figure 1:**

The inhibition rate changes at different concentrations of Thymus *vulgaris* and *Thymus kotschyanus* during the experiment day on B.cinerea

### *3.3. The results of the evaluation of inhibition rate on Rhizopus stolonifer*

Variance Measurement analysis showed a significant difference in the rate of fungal growth inhibition at the level of 1 % and degrees of freedom at 10, the value was 1903/4. Chart comparison between the average rates of growth inhibition shows the maximum inhibition at concentrations of 1000 and 800 µl/L of *Thymus kotschyanus* with 87/9%, and 87/8%, which was not significantly different. Then most inhibition growth rate achieved at 600 µl/L of *Thymus kotschyanus* with the 63/16%. Lowest rate of growth inhibition observed at the control with zero then 200, 400 µl/L of *Thymus vulgaris*, 200, 400 µl/L of *Thymous kotschyanus* , 600 µl/L *Thymus vulgaris* treated with the 54/58 % and 600 µl/L *Thymus kotschyanus* treated with the 63/16%, respectively (Figure 2).

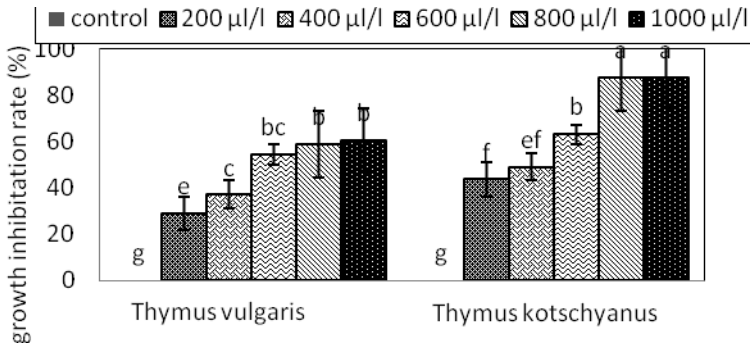


**Figure 2:**

The inhibition rate changes at different concentrations of Thymus *vulgaris* and *Thymus kotschyanus* during the experiment day on Rstolonifere

Results of in vitro studies showed that both essential oils of *T.kotschyanus* and *T.vulgaris* have antifungal effects on both fungi of *B.cinerea* and *R.stolonifere* .Antifungal properties of these essential oils depending on terms of plant species and concentrations . studies reported Essential oils of *Ocimum basilicum, Thymus kotschyanus, Rosmarinus offcinalis* used To reduce postharvest losses by *Penecillinum expansum* and *Botrytis cinerea* on pear fruit and fungal growth stopped at the concentrations which were above 200 µl/L also *T.kotschyanus* showed the highest antifungal activity (jalili marandi and hasani, 2011). With attention to this results it can be concluded that the antifungal effects of essential oil of *T.kotschyanus* is higher than *T.vulgaris* on the *Botrytis cinerea* and *Rhizopus stolonifere* and it is probably dependent on the effects of antifungal compounds of the essential oil of *Thymus vulgaris* and *Thymus kotschyanus*. In this study *Thymus kotschyanus* had higher values of thymol . As Zambonelli reported *Thymus vulgaris* will prevent fungus growth and its antifungal activity is because of the thymol (zabonelli et al. 1996). Also the plants used in these experiments did not show any inhibition at the concentration of 200 µl/L, perhaps it is because the constituent materials of plants in the area which is harvested. From the other hand plant samples were also tested for isolation of fungi, Pear and strawberry can be important. Nabigol and morshedi (2011) stated that the use of two plant species known as *Thymus denaensis* and *Thymus carmanicus* showed inhabitation against both fungi of *R.stolonifere* and *B.cinerea* even at the low concentrations (300 µl/L ). In this study, using two species of *Thymus vulgaris* and *Thymus kotschyanus* showed inhibition even at low concentrations.Also Asghari Marjanlo (2008), stated that the concentrations of 250, 500, 750, 1000 µl/L *of* basil essential oil which were using by paper disc method to prevent fungal growth show inhibition at all concentrations on gray mold in selva strawberries so it is Recommended to use other in vitro methods, according to the test results. Behdad (2012) stated that the using the concentration of 500 µl/L of *Zataria mulrifolra* as a kind of Thyme *was* effective on inhibiting growth of *Rhizopus stolonifere* (Behdad et al. 2012). As the test results of this research revealed the concentration of 1000 µl/L of *Thymus kotschyanus* can inhibit growth rate up to 87/9 %, which shows species of thyme have different growth inhibition on fungus.

### *3.4. The results of the evaluation of catalase activity*

The results of the evaluation of variance table on catalase activity showed that treatment effects of *thymus vulgaris* and *thymus kotschyanus*, also effects of time of measuring and treatment effects of *Thymus vulgaris* and *Thymus kotschyanus* at the time of measurement was significant at the 1% level . comparing Graph of the average catalase activity levels during treatment, showed that catalase activity reduced during storage time, the highest values of the concentrations were at 1000, 800, 600, 400 µl/L .The highest values of the CAT activity was on the third day of experiment for the *Thymus kotschyanus* treatment and also The lowest value was at the fifteenth day of experiment for the treatment of the control which has no immersion. For *Thymus vulgaris* treatments the highest value was ate the third day of experiment and lowest values was on the fifteenth day of experiment for the immersion control (Figure 3, 4). comparing the average concentration of catalase activity Graph between *Thymus vulgaris* and *Thymus kotschyanus* treatments showed that the highest concentration of catalase activity of *Thymus vulgaris* was at 1000µl/L and The lowest catalase activity achieved at non-immersion control treatments .there was no significant difference between that and the treatment of 200µl/L of *Thymus vulgaris* (Figure 5).

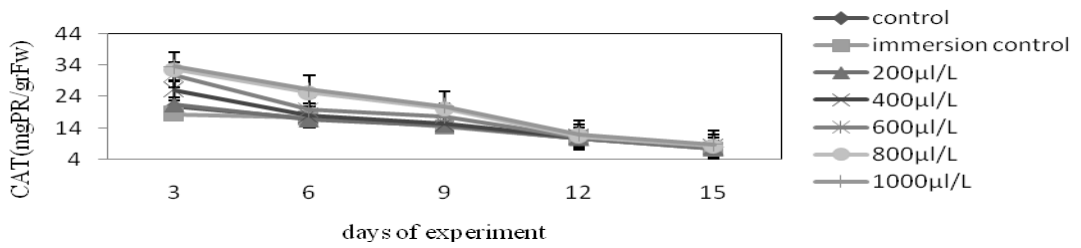


**Figure 3:**

CAT activity comparison of *Thymus vulgaris* treatments means during the storage time

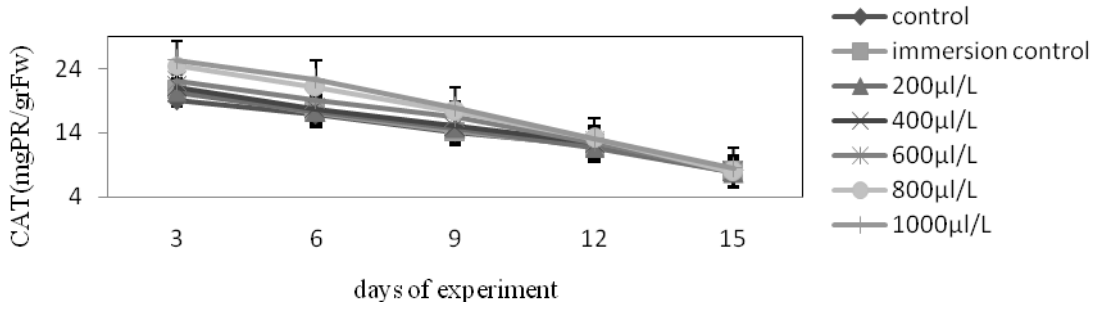


**Figure 4:**

CAT activity comparison of *Thymus kotschyanus* treatments means during the storage time

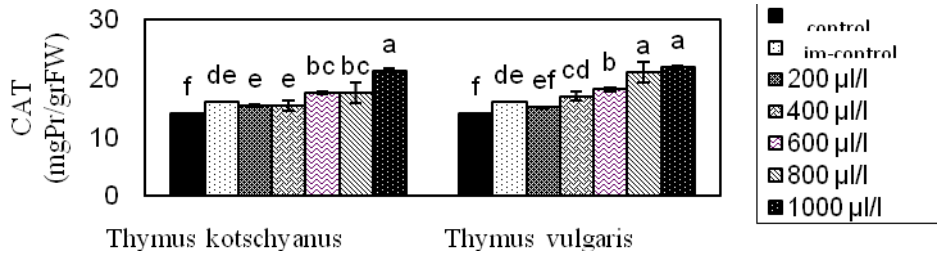


**Figure 5:**

CAT activity comparison of *Thymus kotschyanus* and *Thymus vulgaris* treatments means, among different concentrations

### *3.5. The results of the evaluation of SOD activity*

The results of the evaluation of measuring SOD activity in the strawberry fruit extracts showed that the treatment effect of *Thymus vulgaris* and *Thymus kotschyanus* and time effect were significant at 1% level. The SOD activity during storage time reduced (Table7, 8). The highest values which were measured on each day of testing achieved at 1000, 800, 600, 400µl/L

respectively. The highest value of SOD in both essential oils was at the third day of the experiment for the 1000µl/L treatment and minimum values were at the fifteenth day of experiment for the non-immersion control. Graph comparing the average amount of SOD enzyme activity among the different concentrations of *Thymus vulgaris* and *Thymus kotschyanus* showed the highest activity of SOD was for the 1000µl/L of *Thymus kotschyanus* treatment which was significantly different from other concentrations then the highest activity of SOD belongs to 600µl/L of *Thymus kotschyanus* which has no significant difference form 800µl/L of *thymus kotschyanus* treatment and 1000,800,600 µl/L of *Thymus vulgaris*. (Figure 8). The Importance of nutrition in relation to human health, increasing consumer's attention to use rich-nutrient-foods, especially fruits and vegetables. Antioxidants prevent cell damage which caused by free radicals. Consequently antioxidants as an important nutrition factors are considered. Some Fruits such as strawberries are rich sources of these materials. Antioxidants prevent the formation of reactive oxygen. These antioxidants such as superoxide (SOD) converts superoxide to $H_2O_2$ (Scondalios, 1993). Then Catalase enzyme (CAT) start to convert this $H_2O_2$ to $H_2O$ and $O_2$. Mohammadi reported that the strawberries treated with thymol had significant increasing on SOD and CAT activity superoxide dismutase and catalase activity increased significantly compared with the control (Mohammadi et al., 2012). Increasing the enzymatic antioxidant activity in plant defence against pathogen attack stimulates ROS. According to the results of Wang (2008), the essential oils of thymol, menthol had effects on increasing free radical scavenging capacities (Wang et al. 2008). This study indicated, during the experiment, that SOD levels of treated strawberry fruit were higher than untreated fruit. Statement in 1991 stating that compounds in basil oil, are terpenoid compounds and have antioxidant activity, Antioxidants play an important role in reducing oxidative stress. In addition to oxidative stress with the formation of oxygen free radicals is initiated (Vaughn and Spencer, 1991). In 2010 Gonzalez proposed that superoxide radicals stimulate the sequential reactions, leading to the formation of various types of ROS and consequently antioxidant enzymes such as CAT, SOD are trying to keep the balance of oxidation-reduction reactions. On the other hand, glucose has the ability of the SOD gene expression, also the SOD gene expression needs sufficient sources of key elements of manganese and iron but after post harvest, the crop loses its connection with primary plant and it seems be slightly more difficult to preparing primary compounds for increasing the SOD gene expression (Gonzalez et al. 1999) as this study.

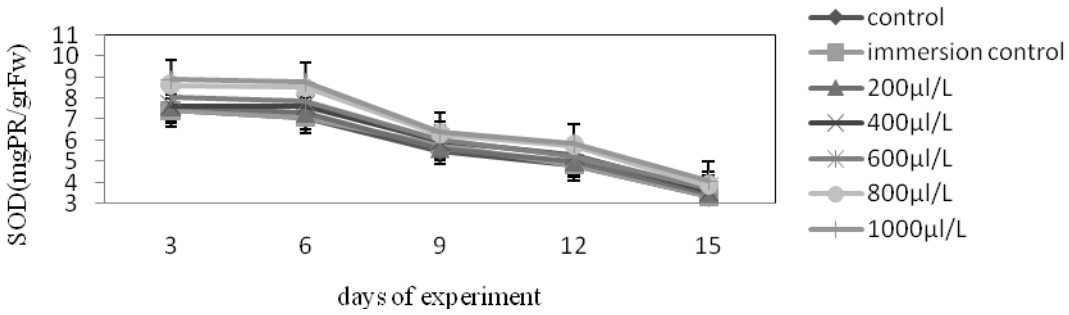


**Figure 6:**

SOD activity comparison of *Thymus vulgaris* treatments means during the storage time

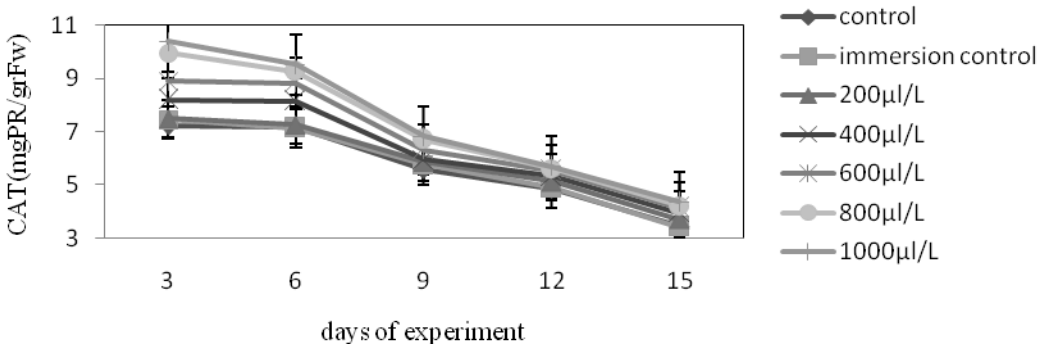


**Figure 7:**

SOD activity comparison of *Thymus kotschyanus* treatments means during the storage time

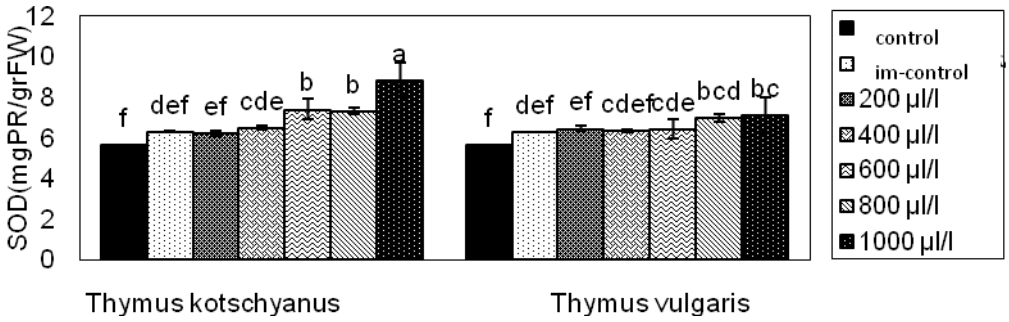


**Figure 8:**

CAT activity comparison of *Thymus kotschyanus* and *Thymus vulgaris* treatments means, among different concentrations

## Conclusion

The results show that the essential oils of *Thymus vulgaris* and *Thymus kotschyanus* have good control effect on *Rhizopus stolonifer* and *Botrytis cinerea.* Also increasing the concentration of the essential oils increases the level of CAT and SOD activity. Essential oils can be used as a source of sustainable eco-friendly fungicides. They have no environmental pollution. So they can be appropriate alternatives for common fungicide. However, for the practical application of these compounds, it is essential to provide a variety of suitable formulations.

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