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EVALUATION OF THE PLANT ESSENTIAL OILS TO CONTROL OF DRY BUBBLE DISEASE (*LECANICILLIUM FUNGICOLA* (PREUSS) ZARE) IN THE WHITE BUTTON MUSHROOM

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Abstract

Edible mushrooms are not only a rich source of nutrients and proteins, but also some of the species produce medicinal compounds. The white button mushroom (*Agaricus bisporus* Imbach) was first cultivated in France. Dry bubble disease with *Lecanicillium fungicola* (Preuss) Zare and Gams is one of the most important diseases that cause damage to edible fungi. One of the easiest ways to control edible fungal diseases, especially dry blisters, is to use chemical fungicides such as carbendazim. Given that a small number of fungicides are currently available to control edible fungal diseases. However, the total resistance of fungal pathogen to chemical fungicides is due to their frequent use, so the use of plant essential oils can be an alternative method for controlling fungal diseases. In this study, the effect of plant essential oils including fennel, ferula, harmel, thymus and satureja was studied by two methods as the plant essential oils mixing with the culture medium and using the paper disk on the pathogen caused the bubble disease and edible mushroom. In another section of this research, it was investigated the effect of plant essential oils, including thyme and satureja, on the fungal pathogen in the salon and on edible fungi. The results of the mixing essential oils assay with the culture medium showed that the essential oil of thyme and ferula at a concentration of 1000 ppm inhibited the growth of the pathogen at 90.42% and 78.44%, respectively. These plant essential oils showed the highest inhibition of the growth of edible fungi. The results of the paper disk test showed that all the essential oils used in this study were able to reduce the growth rate of the pathogen. 1000 ppm concentration had the highest inhibition of the pathogen growth among all of the concentrations, and thyme essential oil with 85.41% showed the highest inhibition pathogen growth, followed by fennel, ferula and satureja essential oils with values of 74.78, 73.72 and 70.22%, respectively.

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The results of the study in the salon (*in vivo*), showed that the essential oils of thyme and fennel increased the number of healthy fungi by 77.19 and 55.63%, respectively, compared to the control. According to the results of the *in-vitro* and *in-vivo* (salon) of the essential oils of thyme, fennel and ferula showed a high capacity to control edible fungal diseases, especially bubbles.

Introduction

There are about 1.5 million species of fungi in the world. Edible fungi first lived on fossilized wood about 300 million years ago, when early humans collected and used them as food (HAWKSWORTH 2001). Fungal habitats include soil, water, and organisms that may harbor large numbers of understudied fungi, estimated to outnumber plants by at least 6 to 1. More recent estimates based on high-throughput sequencing methods suggest that as many as 5.1 million fungal species exist (BLACKWELL 2011).

Edible mushrooms were first grown in greenhouses in Sweden in 1754 and have since spread around the world. Edible mushrooms are not only a rich source of protein and vitamins, but some species of these fungi also produce medicinal compounds such as *Ganoderma* sp. In China, more than 700 pharmaceutical products are commercially available as the main active ingredient. According to statistics released by the Ministry of Health (USA), there are at least 106 medicinal fungi including *Ganoderma*, *Cordyceps* and *Shitake*. Mushrooms have all the essential amino acids for the body and are in the nutritional value between meat and vegetables. Besides, other non-essential amino acids and amides are present in the mushrooms, and edible mushroom protein can be added to the diet as a valuable supplement. The water content of edible mushrooms is about 97% (MOHAMMADI GOLTEPEH and POURJAM 2010). White button edible mushroom (*Agaricus bisporus* (Lange) Imbach) was first cultivated commercially in France. The methods that led to the development of mushroom cultivation were its cultivation of composted horse manure and then using the multi--spore technique to produce mushroom seeds. In recent years, mushroom production in Iran has undergone significant changes; production has increased from 7 kg to 20 kg per square meter (GOLAFRA 2008). However, edible fungi are exposed to various diseases and pests that can seriously reduce yields. Several of microorganisms, such as fungal pathogen, bacteria, and viruses, attack edible fungi, and it caused the most damage to edible fungi (FLETCHER et al. 1986). Dry blisters is caused by *Lecanicillium fungicola* (Preuss) Zare and Gams and wet bubbles is caused by *Mycogone perniciosa* (Magnus) and spider webs is caused by *Cladobotrium dendroides* (Bull) in edible fungi. The most practical method for the contro-

lling of the fungal diseases is the use of chemical compounds and chemical fungicides such as carbendazim and benomyl, which due to the negative effects on the growth of mycelium of edible fungi, reduced yield and the risk of accumulation of toxins in the fruiting organs of the fungus for the consumer and environmental pollution is often obsolete (ZIOMBRA 2001). Considering that a small number of fungicides are currently available and accepted for use in the cultivation of edible fungi, and on the other hand, the total resistance of the fungal pathogen to fungicides due to their use frequently caused by them, it is difficult to find a suitable fungicide that has no adverse effects on edible fungi as well as the environment. However, edible the agricultural mushrooms are crops that cannot be stored and should be consumed quickly, so if a chemical fungicide is used on them, Karen's period is not applied for them. A very safe and environmentally friendly technique can be the use of plant essential oils to control this disease (GLAMOCLIJA 2005, GHOLAMNEZHAD 2017). Recently, much kinds of researches have been done on the antimicrobial properties of medicinal and aromatic plants. The use of these fungicides is very effective in controlling of the growth and proliferation of the fungal pathogen. Medicinal plants are reservoirs rich in secondary metabolites and are sources of active ingredients and many medicinal substances such as thymol and carvacrol (OMIDBEIGI 2006, GHOLAMNEZHAD 2019). Due to the importance of the button mushroom in terms of nutritional and medicinal value and also because the pathogen *L. fungicola* is considered as one of the most important pathogen limiting the production of button mushroom, so in this study the potential plant essential oils are evaluated to control the disease both in the laboratory (*in vitro*) and in storage (*in vivo*) to find the effective compounds.

Material and Methods

Plant Essential Oils

The plant essential oils used in this research were prepared using a Clevenger method. The plants used in this study were all prepared from the rangelands of Lorestan, Yazd, and Isfahan provinces (Table 1). These pastures are pristine and no chemical treatment has been applied to the plants.

Table 1
Plant essential oils used against button edible fungi and dry bubble disease agent, scientific name, common name and partly used in this study

Plant name	Scientific name	Part used
Fennel	*Foenicelum vulgare*	shoot
Ferula	*Ferula assa-foetida*	shoot
Harmel	*Peganum harmala*	shoot
Thymus	*Thymus daenensis*	shoot
Satureja	*Satureia hortensis*	shoot

Pathogen

White button mushroom production centers were surveyed in different parts of Tehran and Alborz provinces, and after observing the infected and suspected specimens of fungi causing dry bubble disease, sampling of infected fungi was performed.

Isolation and Purification of Pathogen

The collected samples were cultured on the PDA (Potato Dextrose Agar) medium and kept in an incubator at 23°C in the dark for two weeks. After the complete growth of the samples in the culture medium, mycelium plaques were removed from the margins of the culture medium and transferred to the water-agar (WA) culture medium for purification. The cultured pathogen were kept in an incubator at 10°C in the dark for 10 days. After fungal mycelium growth, a layer of fungal mycelium was removed from the culture medium and transferred to the PDA culture medium. After two weeks, the pathogen *L. fungicola* was fully grown in the PDA medium and prepared for laboratory tests by plant extracts.

In Vitro Tests

The Effect of Plant Essential Oils on the Growth of the Pathogen by Mixing with Culture Medium Method

To investigate the antimicrobial effect of essential oil, the method of mixing the essential oil with the culture medium method was used for the investigation the antimicrobial effect of essential oils. The PDA medium was used for this assay. The prepared PDA medium was sterilized at 121°C for 15 minutes in an autoclave. The experiment was performed with five essential oils (fennel, ferula, harmel, thymus and satureja) in four

concentrations (250, 500, 750 and 1000 ppm) and three replications. The essential oil obtained from the Clevenger apparatus was considered as 100% concentration, other concentrations were made using the main and base concentrations. The concentrations including of 100, 150, 500 and 1000 ppm (microliters per liter) were poured in medium, before the culture medium was hardened. The culture medium was made with different concentrations of essential oil according to the active substance was prepared by Tween 20 as a completely uniform suspension and applied to the culture medium by sterile sampler at concentrations of 100, 150, 500, and 1000 ppm of the active ingredient was added. In the control (concentration 0), Tween 20 was used instead of essential oil. The culture medium was then poured into nine-centimeter sterilized Petri-dishes. Active disks of the pathogen (*L. fungicola*) with a diameter of 6 mm were placed in the center of the Petri-dishes containing the culture medium. Then the Petri--dishes were placed in an incubator at 23±2 °C, and the diameter of the fungal colonies on the culture medium was reduced after two weeks of filling of the petri dishes during a period of two days and months. The average growth of the fungal colony diameter in each replication was measured for each treatment using the following formula (1) (GHOLAMNEZHAD et al. 2015):

$$\frac{\text{pathogen conoly diameter in the control} - \text{pathogen colony diameter in the treaments}}{\text{pathogen conoly diameter in the control}} = \text{inhibition percentage of mycelial growth}$$

The Effect of Plant Essential Oils on Fungal Growth by Paper Disk Method

The antifungal activity of the essential oils was performed based on the "Reverse Petri-dishes" method (SINGH et al. 2006). 20 ml of PDA was poured into Petri-dishes (9 diameters), and after coagulation, a 5 mm disk of the five-day culture of the pathogen was removed from the PDA culture medium and placed in the center of the culture medium. Then, volumetric amounts of 10, 750, 17, and 100 microliters of pure essential oil of each plant were poured separately on a square disk with dimensions of 15 mm of Whatman filter paper number one with a sampler, and this disk was placed inside the lid of a Petri-dishes. By dividing the volume value used by the volume of active space inside the Petri-dishes, concentrations of 250, 500, 750 and 1000 ppm, respectively, was obtained. The Petri-dishes was covered with parafilm and the Petri-dishes was placed upside down at room temperature (24°C). After two weeks, when the mycelium of the fungus reached the edge of the Petri-dishes in the control Petri-dishes, the

effect of essential oils was evaluated. This test was performed with three replications for each treatment and the amount of antifungal activity was measured by formula (1) (SUKATTA et al. 2008).

The Effect of Essential Oils on the Pathogen and Edible Fungi in the Salon of Button Mushroom Cultivation

Thyme and fennel extracts (concentration of 500 ppm) were used for this test, which had good results *in-vitro* tests. Carbendazim as a fungicide (900 ppm) was also used as a chemical control. In the next step, a spore's suspensions with a concentration of 10^6 spores per ml was prepared by a hemocytometer slide from seven-day culture of the pathogen. The prepared spore's suspension was then sprayed on the surface two days after topsoil application. Then, the essential oil was sprayed on the cover soil. For this purpose, according to the inhibitory percentage obtained for each essential oil in the laboratory, an emulsion was prepared for each essential oil (500 ppm concentration) and two days after inoculation of each fungus, it was sprayed on the cover soil. This assay was four replications for each plant essential oils. It should be noted that each of the essential oils was treated alone on edible fungi, so that their effect could be investigated alone. This test had a healthy control and an infected mushroom control without using essential oil treatment. After the edible fungal mycelium completely covered the surface of the cover soil, to create stress for the button edible fungus, the temperature was lowered to 16 to 18°C, which was done by aeration of the hall. After a week, the first symptoms of the disease appeared on the surface of the cover soil and in healthy controls, the first pins of edible fungi appeared.

Measured Characters

Weight of Mushrooms

In the first harvest, all mushroom was collected from the surface of each bed and infected and healthy fungi was weighed and counted separately for each bed and the data were recorded. Ten days after the first harvest, the second harvest took place, like the first harvest, all mushroom was collected and weighed separately from the surface of each bed. All assays were carried out in a completely randomized design. Each treatment consisted of three replicates. Statistical significance was assessed at the level $P < 0.01$. When the analysis was statistically significant, Duncan's Multiple Range Test

Results

The Effect of Plant Essential Oils on the Growth of Pathogen by Mixing with Culture Medium

According to the results of the analysis of variance Table 2, there is a significant difference between the treatments of using different plant's essential oils and also the use of carbendazim fungicide in reducing the growth of pathogen on the culture medium mixed with plant essential oils.

Table 2

Analysis of variance of the plant essential oils effect on the growth inhibition of *Lecanicillium fungicola,* at 23 ± 2°C and dark conditions by mixing plant essential oils with culture medium

Source of variation	df	Sum of squares	Mean	*F*
Essential oil	20	25150.73	1257.53	2609.77**
Error	42	20.23	0.48	–
Total	62	25170.19	–	–

**the difference was significant at 99% probability ($P \leq 0.01$); the data were normal, C.V = 1.17%

The results of the antifungal effects of essential oils of fennel, ferula, harmel, thymus, and satureja obtained from the mixing test showed that different concentrations of essential oils had an inhibitory effect on the growth of the pathogen, and there is a direct relationship between increasing the concentration of essential oil and decreasing the growth of fungi (Figure 1).

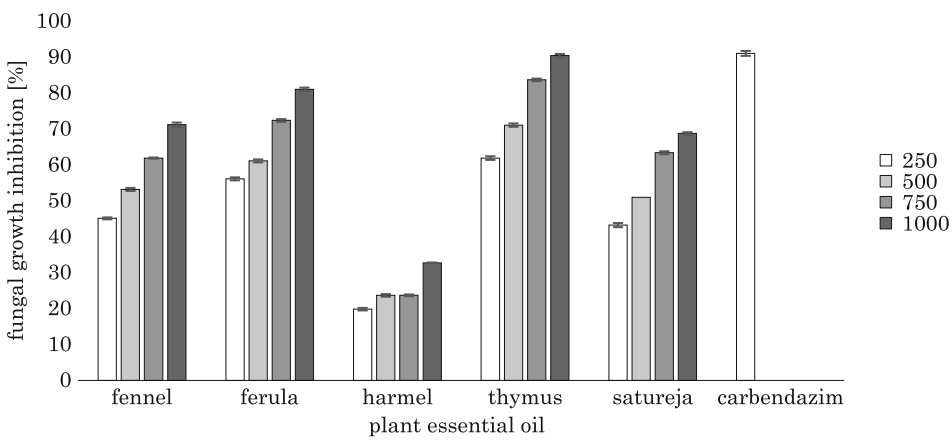

Fig. 1. Effect of plant essential oils on the growth of the pathogen (*Lecanicillium fungicola*) by mixing with culture medium

The highest reduction in fungal colony growth was observed in the treatment of 1000 ppm concentration of thyme essential oil with 90.42%, and two essential oils of ferula and fennel with a concentration of 1000 ppm placed in later levels with values of 81.07 and 71.22%, respectively. However, among the studied treatments, carbandazim treatment with 94.06% had the highest percentage of control, but with 1000 ppm thyme treatment, there was no significant difference. On average, the concentration of 1000 ppm showed the highest control among the concentrations, and this control was observed for all plant essential oils.

The Effect of Plant Essential Oils on the Growth of Button Edible Mushrooms by Mixing with Culture Medium

According to the results of the analysis of variance 2, there is a significant difference between the treatments of using essential oils of different plants and also the use of carbendazim fungicide in reducing the growth of button edible mushrooms on the culture medium mixed with plant essential oils.

The results of studying the antifungal effects of essential oils of fennel, ferula, harmel, thymus, and satureja obtained from the mixing test showed that different concentrations of essential oils have an inhibitory effect on the growth of button edible mushrooms, and there is a direct relationship between increasing the concentration of essential oil and decreasing the growth of fungi (Figure 2). Ferula essential oil showed the greatest effect in reducing the growth of button mushroom at a concentration of 1000 ppm with a value of 78.44%, followed by it was located the satureja and thyme at a concentration of 1000 with values of 73.36 and 69.71%, respectively. Carbendazim fungicide also showed an antifungal effect on edible fungi at 83.26%, which was statistically significant with a concentration of 1000 ppm ferula. Among the studied concentrations, the concentration of 1000 ppm showed the highest inhibition of fungal growth, followed by a concentration of 750 ppm, which always had a significant difference in the inhibition of the growth of fungi with other treatments (Figure 2 and Table 3).

According to this Figure 2, which shows the effect of different concentrations of five essential oils of fennel, ferula, harmel, thymus, and satureja on the average growth of edible mushroom colony growth, the highest inhibition rate was observed for all 1000 essential oils. At all concentrations, Ferula essential oil showed the highest inhibition, and harmel essential oil showed the least inhibition in inhibiting the mycelial growth of edible fungi.

Table 3

Analysis of variance of the plant essential oils effect on the growth inhibition of *Agaricus bisporus,* at 23±2ºC and dark conditions by mixing plant essential oils with culture medium

Source of variation	df	Sum of squares	Mean	*F*
Essential oil	20	15,454.46	1,257.53	1,551.56**
Error	42	20.91	0.48	–
Total	62	15,475.37	–	–

**the difference was significant at 99% probability ($P \leq 0.01$); the data were normal, C.V = 1.25%

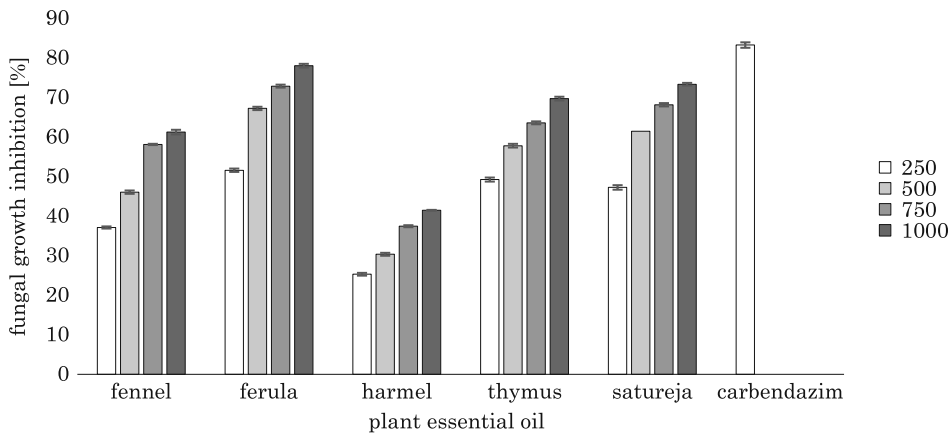

Fig. 2. Effect of plant essential oils on the growth of edible fungus (*Agaricus bisporus*) by mixing with culture medium

The Effect of Plant Essential Oils on the Growth of the Pathogen by Paper Disk Method

The results of the analysis of variance 4 showed that there is a significant difference between the treatments of using essential oils of different plants and also the use of carbendazim fungicide in reducing the growth of the pathogen on the culture medium, in the method of using the paper disk. The results of antifungal effects of essential oils of fennel, ferula, harmel, thyme and satureja obtained from paper disk test showed that different concentrations of essential oils had an inhibitory effect on the growth of edible fungi and between increasing the concentration of essential oil with decreasing Fungal growth has a direct relationship (Figure 3); the highest reduction in the pathogen colony growth was the concentration of 1000 ppm of thyme essential oil of 85.41% and the essential oils of fennel and ferula with a concentration of 1000 ppm were in the next ranks with the values of 74.78 and 73.72%, respectively. Carbendazim fungicide reduced the growth of the pathogen by 44.49%.

Concentrations used in this experiment include 250, 500, 750 and 1000 ppm in the volumetric space of the PDA culture medium. Among the concentrations, the best treatment, for controlling the growth of the pathogen, was the concentration of 1000 ppm, which always showed the highest inhibiting percentage of the pathogen growth, about all of the plant essential oils.

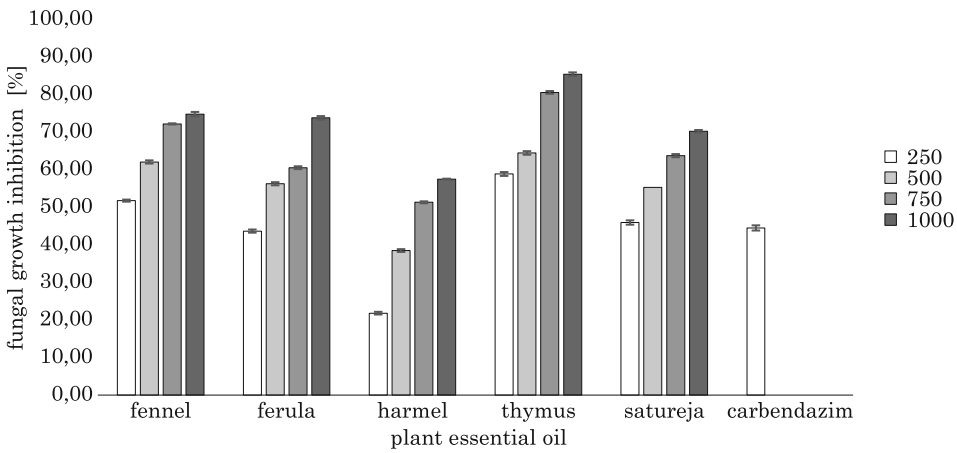

Fig. 3. Effect of plant essential oils on the growth of the pathogen (*Lecanicillium fungicola*) by paper disk method

According to the results of the analysis of variance, there is a significant difference between different treatments at the level of 1 percent probability. The results showed that different concentrations of plant essential oils had an effect on the average growth of the pathogen and reduced the growth of the fungus, which is clearly shown in Figure 3. This figure shows that different concentrations of five plant essential oils have a decreasing effect on the growth of the pathogen's colony and the maximum inhibition for all essential oils was observed in the 1000 ppm concentration. Thyme essential oil had the highest efficiency of 85.41% and harmel had the lowest efficiency of 57.91% in inhibiting mycelial growth of the fungus. After thyme essential oil, fennel, ferula, and saturea essential oils showed 74.71, 73.72, and 70.22% the percentage controlling, respectively.

The Effect of Essential Oils on the Pathogen and Edible Fungi in Button Mushroom Cultivation Salon

In this test, the essential oils of thyme and fennel were used at the 500 ppm concentration and the fungicide carbendazim at 1.5 per thousand was concentration. According to the results of analysis of variance in Table 4,

there is a significant difference between the treatments using these two essential oils and the use of carbendazim fungicide in increasing the percentage of healthy fungi and also increasing the weight of the edible fungi at a probability level of one percent. As shown in the comparison Table 5, the tested treatments include the plant essential oils, carbendazim fungicide, healthy control, infected plant control.

Table 4

Analysis of variance of the plant essential oils effect on the growth inhibition of *Agaricus bisporus*, at 23±2°C and dark conditions by the paper disk method

Source of variation	df	Sum of squares	Mean	*F*
Essential oil	20	13,646.45	682.32	654.30**
Error	42	43.79	1.04	–
Total	62	13,690.24	–	–

**the difference was significant at 99% probability ($P \leq 0.01$); the data were normal, C.V = 1.2573%

Table 5

Analysis of variance of the plant essential oils effect on the number of mushrooms and weight of *Agaricus bisporus*, in button mushroom cultivation salon

Specification		Mean of squares (MS)		Sum of squares (SS)		*F*	
Source of variation	df	mushroom weight	number of mushrooms	mushroom weight	number of mushrooms	mushroom weight	number of mushrooms
Essential oil	6	69479.33	17706.68	17369.83	4426.67	1514.81**	2773.53**
Error	12	114.66	15.96	11.46	1.59	–	–
Total	17	–	–	–	–	–	–

**the difference was significant at 99% probability ($P \leq 0.01$); the data were normal, C.V = 1.39% for mushroom weight and C.V = 2.55% for number of mushrooms

The results of antifungal effects of thyme and fennel essential oils, as well as carbendazim fungicide on the percentage of healthy fungi showed that the use of thyme and fennel essential oils increased the number of healthy fungi by 77.99% and 54.63%, respectively, while the percentage of healthy fungi in the treatment of pathogen use alone (infected control) was 7.30%. The use of carbendazim fungicide alone caused 83.60% of the fungi to remain healthy, which was numerically higher than all the treatments (except 94.16% of the healthy control) and showed a significant difference with the other treatments. Of course, both essential oils of thyme and fennel in the presence of the pathogen were able to increase the number of healthy fungi to 62.12 and 32.25, respectively, compared to the infected control at 7.30%.

The use of different treatments caused a significant difference in the weight of the studied fungi (Table 4–6). In this study, the highest weight of

edible fungus was observed in the control treatment at the rate of 322.66 g, followed by the use of carbendazim fungicide with the amount of 295.66 g. The weight observed in edible fungi treated with thyme and fennel essential oil was 253.33 and 215 g, which showed a significant difference at the level of one percent with the infected control (128.33 g).

Table 6

Comparison of the mean of the test for the effect of plant essential oils and fungicide on the percentage of the number of healthy fungi and the weight of *A. bisporus*

Treatment	Concentrations [ppm]	Weight of *A. bisporus*	Healthy *A. bisporus* [%]
Thymus	500	253.33^{c}	77.99^{c}
Fennel	500	215.00^{d}	54.63^{d}
Thymus + pathogen	500	198.22^{e}	62.12^{e}
Fennel + pathogen	500	156.36^{f}	39.25^{f}
Karbendazim + pathogen	900	295.66^{b}	83.60^{b}
Infected plant (positive control)	–	128.33^{g}	7.30^{g}
Control (healthy plant)	0	322.66^{a}	94.16^{a}

Values are the average of three replicates. Values in the same row followed by the same letter are not statistically different in Duncan's Multiple Range Test.

Discussion

The use of plant essential oils can be used as an alternative to pesticides in mushroom breeding centers. Iran is a country rich in important species of medicinal plants that can be used to control plant diseases and disinfect agricultural products, especially storage products as well as edible mushrooms, against pests and diseases (OMIDBEIGI 1995). Edible fungi can absorb a lot of toxins due to their special texture (lack of woodiness) and also their inability to store them for a long time. Avoid making less on edible mushrooms. Plant essential oils are of plant origin and therefore do not have harmful effects on human health and on the other hand is decomposed very quickly, so they do not leave toxic residues in the environment.

In this study, thyme and fennel essential oils showed a very high ability to inhibit the growth of mycelium of the pathogen compared to other essential oils. The results of this study on the very high antimicrobial effect of thyme essential oil with the results of previous research (LEUSCHNER and ZAMPARINI 2002, AMANLOU 2006, HOSEIN et al. 2009, SOLAIMANI et al. 2009) and the very high antimicrobial effect of essential oil ferula confirmed with the results of other studies (NABIGOL and FARZANEH 2010,

GANDOMI et al. 2009). The results of the gas chromatographic analysis for thyme essential oil show 42% carvacrol and 32.34% thymol. There is a direct relationship between the important chemical structures of the compound in the essential oil and its antimicrobial activity (CACCIONI et al. 1998). The very high antifungal power of these two essential oils is due to the presence of phenolic compounds in them. Thymol and carvacrol are phenol terpenoid compounds that have the highest antimicrobial activity compared to other active compounds such as terpenes and terpenoids with other functional groups (VENTURINI et al. 2002, LIU et al. 2002).

Both essential oils (thyme and fennel) are composed of various compounds, but it seems that the commonality of some of these compounds, in the similarity of the effect of these two essential oils on edible fungi and the fungal pathogen. The presence of phenolic compounds such as thymol and carvacrol as the main compounds of these two essential oils can be effective in obtaining antifungal results. In addition to carvacrol, thyme essential oil also contains thymol, which was not found in saturea essential oil in this study, or its amount was very low, which was not specified in the GC analysis. It seems that the inhibitory effect of thyme essential oil more than fennel essential oil is due to the exacerbating effects of compounds such as thymol and other compounds (BULLERMAN 1977).

In a study, the antifungal role of safflower essential oil on *Alternaria citri* was investigated. The results showed that savory essential oil at 400 ppm concentration and above in the culture medium completely affects the growth of the fungus and inhibits its growth. Lower concentrations of essential oil decrease the growth of the fungus but did not stop its growth (YAZDANPANAH GOHARRIZI et al. 2010).

WHITESIDE (1976) reported that consumption of safflower essential oil in addition to controlling the causative agent of *Alternaria citri* disease increases the marketability and quality of the product and due to the effective concentration of 300 ppm safflower essential oil, its consumption is economically viable (WHITESIDE et al. 1976).

NESLIHAN et al. (2008) concluded that carvacrol has a greater inhibitory effect than thymol on the inhibition of the pathogen. In 2008, MUSCOVY et al. reported that thyme and satureja essential oils could inhibit the growth of fungi that contaminate food as well as horticultural crops and could be used as instead of chemical substitutes. RASOOLI et al. (2009) also reported that plant essential oils can control plant fungal diseases (RASOOLI et al. 2009).

However, the essential oils of medicinal plants used in this study were able to prevent the growth of fungi by mixing with the medium to an acceptable level. The mixing essential oils with the culture medium method

exposes the essential oil permanently to the pathogen, and the antifungal compounds in the essential oil will have ample opportunity to penetrate the wall, membrane and subsequently the depth of the target cell. The mechanism of phenolic compounds is related to their effects on cell membranes, and changes in their function and in some cases changes in membrane structure that increase inflammation and permeability of cell membranes (LIU et al. 2002).

The effect of plant essential oils on the reduction of edible fungi was different from the results of the previous test and the essential oils that had the greatest effect on reducing the growth of the pathogen in this part had less reduction in the growth of edible fungi than other essential oils.

In the mixing the essential oils with the culture medium test, the carbendazim fungicide treatment showed the greatest reduction in fungal growth, followed by the essential oils of ferula and satureja with a concentration of 1000 ppm. Thyme, which had the most effect in the previous test, was ranked third here, and fennel also dropped from third to fourth. This difference in the control of the two fungi can be related to the difference in cell wall compositions between the two pathogenic and edible fungi. In other words, the fungicidal compounds of thyme essential oil in the case of edible fungi did not have a high penetration (compared to the pathogen) into the fungus and as a result could not have a high inhibitory effect on this fungus (ALIZADEH-SALTEH et al. 2010).

The results of the paper disk test showed that the process of the mixing the extract with the culture medium was similar with the paper disk test. In the paper disk test, the concentrations of 1000 ppm of thyme, fennel, ferula, satureja and harmel showed the highest control of the pathogen, respectively (TANOVI et al. 2009).

Differences in the effectiveness of plant extracts can probably be related to the difference in the origin of plant essential oils and consequently the difference in the type and composition of the ingredients of plant essential oils (GHOLAMNEZHAD et al. 2016, GHOLAMNEZHAD 2016); also, with increasing the concentration of plant essential oil, the fungicidal effect in all essential oils used to prevent mycelial growth of fungi has increased, these results are consistent with the results obtained by other researchers. LOTFI et al. (2010) studied on *Fusarium oxysporum* and showed that the essential oils of thyme, trachyspermum and mint inhibited the growth of the fungus. SHAHKARAMI et al. (2006) studied the effect of five plant species including the case, mint, five fingers, thyme and artemisia on mycelial growth of plant pathogens *Rhizoctonia*, *Fusarium* and *Pythium*. The results showed that the essential oils of mint and thyme inhibited 100% mycelial growth of the studied fungi.

In the evaluation of the effect of essential oils on the pathogen and edible fungi, in the button mushroom cultivation salon, two plant essential oils including thyme and fennel were used in one concentrations of 500 ppm. In the healthy control treatment, we saw the highest number and weight of fungi. In this assay, thyme essential oil showed very good performance compared to fungicide in terms of the number and weight of the fungus. In some ways, fennel essential oil, although less effective than thyme, still had acceptable performance.

Increasing the weight of edible fungi treated with thyme and fennel essential oils compared to the control shows that in addition to antifungal compounds, these essential oils may also contain fungal growth stimulants, which significantly increased the weight of infected fungi. The results showed that there are chemical compounds with different percentages in the essential oils of thyme and fennel. The main composition of essential oil of thyme (*Thymus daenensis*) and thyme (*Thymus vulgaris*) is thymol, which is 43.8% and 45.1%, respectively. Thymol is a phenolic chemical compound. Thymol has antibacterial properties and is found in plants such as thyme.

Despite the great effect of these compounds (thymol and carvacrol) in increasing the growth of edible fungi, but there is no report on the effect of essential oil in increasing the growth of edible fungi as well as plants. The results of this study can be used as a starting point that introduces the use of essential oils as well as plant extracts, in addition to being a very good factor for controlling plant diseases. It can be used as a growth stimulant at least for this fungus (*A. bisporus*).

Measurement of defense gene expression in *A. bisporus* treated with thyme essence showed that the thymus extract increased the expression of two genes, peroxidase and catalase, in the presence and absence of the pathogen. Exactly in treatments which the number of healthy edible mushroom as well as the weight of the mushroom was higher, the activity of these two enzymes was also observed. In other words, these results can be interpreted as the effect of thyme essence has not only been a direct fungicide against the pathogen, but also on the defense mechanism of edible fungi and activating the host defense system against the pathogen. Our research results showed that a part of the disease reduction in *A. bisporus* that with essence was due to the induction of defense genes by these natural compounds.

The results of the Study of defense genes expression profile pattern of wheat in response to infection by *Mycosphaerella graminicola* revealed that by increasing the expression levels of defense genes such as catalase, peroxidase and polyphenol oxidase, the resistance of wheat to septoriosis increases (GHOLAMNEZHAD et al. 2016a,b).

The results of GHOLAMNEZHAD (2019) showed that in the storage conditions, the application of aqueous extract of neem (at concentration of 25%) resulted in 89.11% reduction of disease severity compared with the untreated control. Results of enzymes activity showed the plant extracts can increase the activity of peroxidase, phenylalanine ammonia-lyase, β-1,3-glucanase and polyphenol oxidase in the presence of pathogens, in apple fruits (GHOLAMNEZHAD 2019).

This research showed that the plant essence induced the resistant mechanisms and it can be useful to control edible mushroom disease. On the basis of the results obtained during the experiment and reports of success of plant essence in controlling edible mushroom disease, the tested plant essence hold promise for the organic and eco-friendly management of *A. bisporus*. The findings of these studies may become the foundation for the use of natural agents as a safe and cost-effective control method against *A. bisporus* diseases.

Another notable point that was the secondary results of this study, which was not targeted at the beginning of this study, was the flavoring of edible mushrooms. Edible mushrooms treated with the essential oils of thyme and fennel had the taste of these two essential oils, so that even after cooking they had this taste. The results of this study showed that plant extracts have high fungicidal effects, relatively little effect on the growth of edible mushrooms, as well as flavoring edible mushrooms.

Although in recent years the approach to the study of the effects of medicinal plants on animal and plant diseases has increased, for centuries, medicinal plants as well as plant extracts, essential oils and teas of these plants in Iran, have been used to treat diseases in humans and even animals, and at least in our country, the use of these plants and their products is not strange. The results of this study showed that plant essential oils in addition to good fungicidal effects can have a flavoring effect on edible mushrooms.

Conclusions

Edible mushroom diseases are often controlled with chemical compounds as Carbendazim and Sporgon but the perceived harmful effects of synthetic fungicides currently in use on human and the environment no longer make them attractive to use. Based on the findings of this study, there are great potentials in the control of edible mushroom diseases using naturally occurring substances that are both humanly and environmentally friendly and at the same time affordable at less cost to the users than

the procurement and use of chemically formulated fungicides. The results of this study showed that plant essence, in addition to fungicide effects, they can induce resistance in the edible mushroom.

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