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REACTION OF SOME PLANT PATHOGENIC FUNGI AGAINST ESSENTIAL OIL OF THYMUS

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Abstract:

In the present study, essential oil of Zataria multiflora was used to inhibit the growth, fresh and dry weight of Fusarium solani, Fusarium oxysporum f.sp. lycopersici and Alternaria alternat.. The above essential oils were extracted by hydro-distillation (using Clevenger). Its effect on radial growth and fresh, dry weights of Fusarium and Alternaria fungi in solid and liquid media was investigated using the Food Poison Technique at 13 concentrations with 6 replications. Fusarium solani, Fusarium oxysporum f.sp. lycopersici and Alternaria alternata in a solid medium in concentrations of 3500, 4000 and 3000 ppm of essential oil the radial growth of the fungi was inhibited by 100%. Also in liquid medium, Fusarium solani, Fusarium oxysporum f.sp. lycopersici and Alternaria alternata in concentrations of 6500, 5500 and 4000 ppm were inhibited from the essential oil of the studied fungi, respectively. The lowest effective concentration in solid and liquid media was reported to be 100 ppm. By increasing the concentration of essential oil of Zataria multiflora, the reduction of gradual radial growth of fungi and the increase of growth inhibitory percentage which indicated a positive effect of thyme essential oil on the control of the studied fungi.

Keywords: *Antifungal effect, Essential oil, Zataria multiflora, Radial growth, Fresh and dry weights.*

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1. INTRODUCTION

Thyme is a plant of the Lamiaceae family. Thymes are perennial bush, cushioned or clumpy with rose crouched, bent, or creeper and herbaceous forms [1]. *Zataria multiflorais* one of the most widely known herbs of the Lamiaceae family .This plant has a limited distribution in the world; it is exclusively observed in Iran, Afghanistan and Pakistan [2]. *Zataria* has a specific species called as *multiflora.* This plant is used more often in the pharmaceutical and food industries [3]. Fusarium fungus is considered as one of the most important soil fungi that has a special economic significance. Many species are pathogenic and cause many diseases in plants [4]. *Fusarium oxysporum* f.sp. *lycopersici* is as an herbaceous agent that causes *Fusarium* wilt especially in tomato. This disease is one of the most important diseases of tomatoes in farms and greenhouses [5] and has a global importance. The diseases caused by *Alternaria* on leaves, stems, flowers and fruits affect mainly annual plants especially vegetables, ornamentals and trees such as citrus and apple. The damage caused by different Alternariaon hosts has the highest position among each of the diseases of the total produced diseases, the fungal diseases have a large share [6]. This research was carried out with the goal of investigating the antifungal effect of *Zataria multiflora*oil on the pathogenic factors of wheat crown rot, *Fusarium* wilt of tomato and wave form spot of tomato in the laboratory condition.

2. MATERIALS AND METHODS

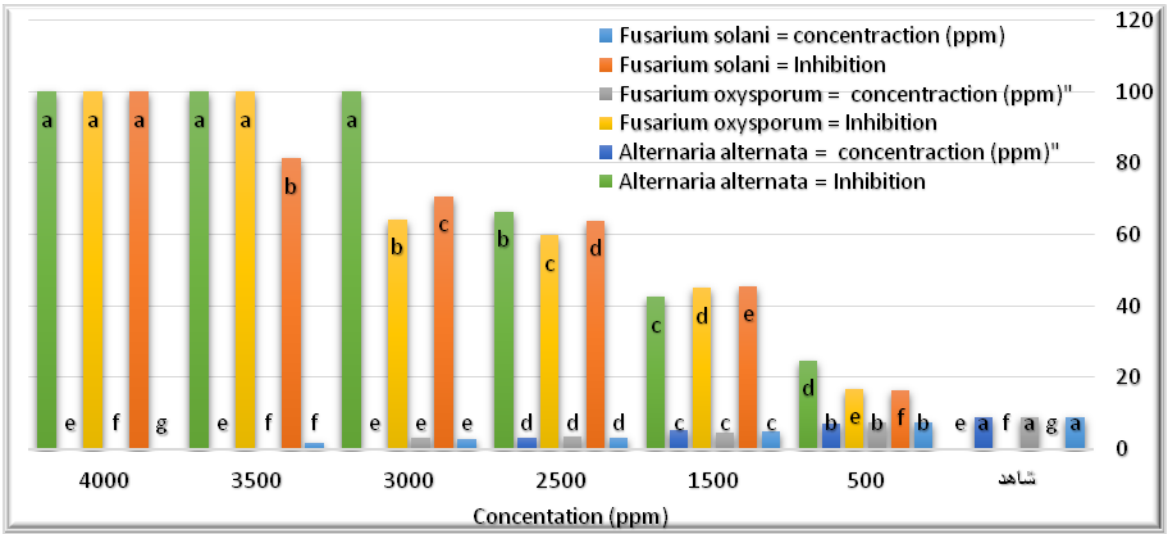
The *Zataria multiflora* was identified by the faculty members of the Payam Noor University in Shiraz. The leaves of the plant were isolated and dried in shadows. The essential oil was taken for 1.5 hours by Clevenger machine.250 g of plant samples and 400 ml of water were utilized for each extraction. The raw oil was digested with sodium bicarbonate and stored in a dark-colored container in a refrigerator at 4 ° C to prevent oxidation. To evaluate the antifungal effect of raw oil, food poisoning technique was utilized by mixing with culture medium. The culture medium PDA (potato, dextrose and agar) was utilized as a solid culture medium which was as commercial powder and dissolved in 42 grams per liter of water. In order to induce raw oil into the culture medium was used of 16 concentrations in 3 replicates. The studied fungus was removed from the main culture with a diameter of 5 mm by loop on the side of the flame and placed in the center of the Petri-plate containing poisoned media. Then the media were kept at a temperature of 25 ± 2 in the incubator [7]. The growth of the pathogen by measuring the longitudinal growth without t daily removing the petri dishes and at a certain time by the coils from the bottom of Petri-plate respectively ,*Fusarium solani*, *Fusarium oxysporum* f.sp. *lycopersici* and wave-form spot of Tomato were measured during the period of 10, 12 and 11 days. In order to calculate the growth inhibitory percentage, the formula I = [(CT) / C] * 100 was utilized where C is the diameter of the mushroom colonization in the control Petri-plate and T is the diameter of the fungus colonization in the poisonous culture medium and I as the percentage inhibits the growth of the fungus (Pandey et al., 1982).Excel data was utilized to record data. Data were analyzed by SPSS software using one-way ANOVA method through Duncan's multiple domain test [8].

3. OBSERVATIONS AND RESULTS:

Solid medium

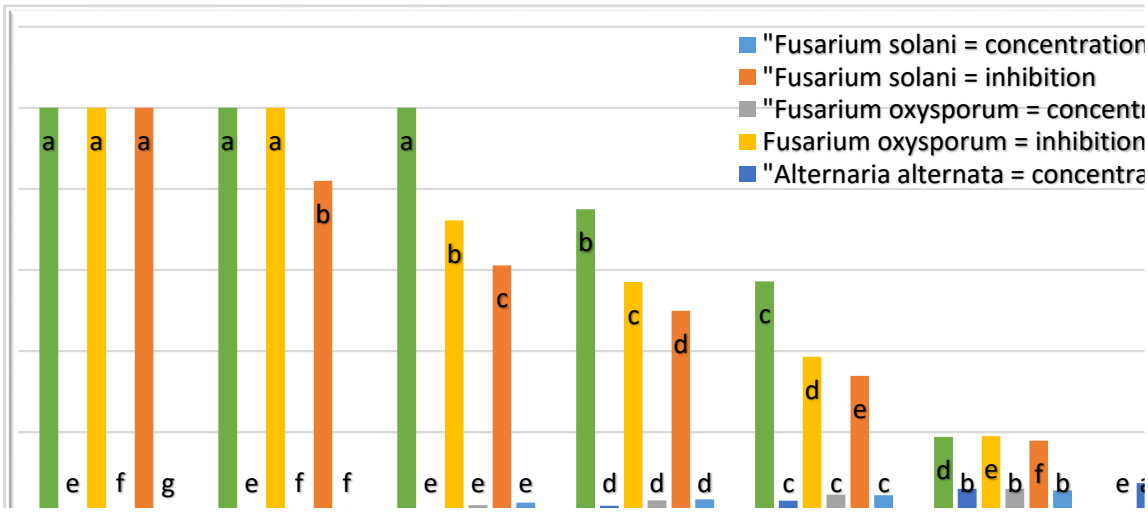
The data obtained from Chart (1) indicate that increasing the concentration of volatile oil in *Zataria multiflora*,,the radial of growth decreased and in contrast due to the increased concentration of volatile oil, the inhibitory growth rate of the pathogens was increasing. So that at the concentration of 4000 ppm and higher, the complete stopping of the long-term growth of the fungus agent of the disease of the croissant rot of wheat at a concentration of 3500 ppm and higher, the complete stopping of the longitudinal growth of the fungus causing the wilting of tomato and at a concentration of 3000 ppm and higher, the longitudinal growth stopping of the fungal disease of *Alternaria solani* of tomatoes was reported. Using the concentrations of 1500 and 3000 ppm of oil, radial growth of *Fusarium solani* (4.89, 2.64), *Fusarium oxysporum* f.sp. *lycopersici* (4.60, 3.22) and *Alternaria alternata* fungi at concentration of 1000 and 2500 ppm, the radial growth was reported as (5.89, 3.03), which were statistically significant. On the other hand, the presence of fungus in the presence of concentrations of 500, 1500 and 2000 ppm of essential oil of *Zataria multiflora* in solid medium of *Fusarium solani* fungi (16.44, 45.59, 63.96), *Fusarium oxysporum* f.sp. *lycopersici* (59.44,66.29,16.92) and *Alternaria alternenta* (24.62, 42.51, 29.66) which the growth inhibitory percentage was increased by increasing the concentration of volatile leaf oil of *Zataria multiflora*,.

(Chart .1): Comparison of different concentrations of essential oil on the radial growth of pathogens in solid medium

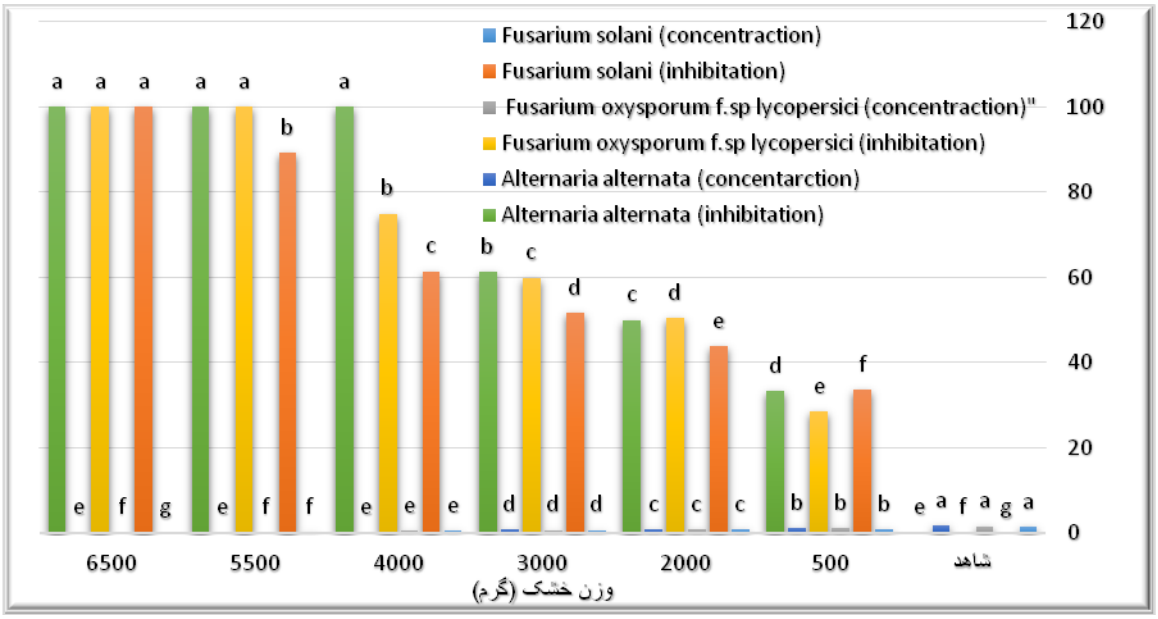


The effect of *Zataria multiflora* on the dry and wet weights of three fungi was studied in liquid culture using nutritional poisoning technique *in vitro*. The aforementioned studies were carried out in a completely randomized design with 3 replications. The effect of this method was determined by weighing fresh and dry weights of the studied fungi after the completion of fungal filling, *Fusarium solani* fungus at a concentration of 6500 ppm, *Fusarium oxysporum* f.sp. *lycopersici* fungus at a concentration of 5500 ppm as well as *Alternaria alternata* a concentration of 4000 ppm, from the essential oil of *Zataria multiflora* , the growth of the studied fungus was 100% under control and fresh and dry weight of it was reported zero. In *Fusarium solani*, by the increase in the concentration of 2000 ppm to 5500 ppm showed an increase in the inhibitory growth rate from 43.73 to 89.66%, and in *Fusarium oxyspoprum* f.sp. *lycopersici* by the increase of concentration of 2000 ppm to 4000 ppm,we observed an increase in the amount of growth inhibitory percentage from 50.32 to 74.82, as well as in *Alternaria alternata*, by increasing the concentration from 500 ppm to 3000 ppm, we observed the increase of growth inhibitory percentage rate from 33.33 to 61.20% .In general, with the increase of *Zataria multiflora*, the fresh and dry weight of the fungus decreased and the growth inhibitory percentage increased (Chart .2 and .3).

(Chart .2): Comparison of different concentrations of essential oil on the fresh weight of pathogens in liquid medium



(Chart .3): Comparison of different concentrations of essential oil on the dry weight of pathogens in liquid medium



4. DISCUSSION AND CONCLUSION:

Due to the increasing interest of people in the consumption of natural ingredients as well as the prevalence of gastrointestinal, respiratory and all types of cancers extensive research has been performed to use a variety of essential oils. The essences in addition to antimicrobial properties have anti-parasitic properties as anti-toxins which are related to the type of effective substance in the essential oil [9] (Bart, 2004).In a study conducted on the effect of antimicrobial effect of German herbs and chamomile medicinal herbs by Izadi et al. (2012) 10]. It has been determined that simultaneous use of the two German and Great Chamomile essential oils has significant inhibitory effects and can be used to optimize the use of essential oils in effective control of microorganisms. Carmen et al. (2014) [11] showed that the obtained oil from *Pelagonium roseum* from the *Geraniaceae* family had a significant inhibitory impact on *Candida albicans* and also antimicrobial activity on negative gram bacteria such as *Pseudomonas aeruginosa*, *Protusmirabilis*, *Escherichia coli* and positive gram bacteria Staphylococcus *aureus* and *Enterococcus faecalis*. In the case of *Candida albicans*, there was observed a complete prevention of fungal growth and about the bacterial inhibition of *Pseudomonas aeruginosa*, *Staphylococcus aureus* , the comparison was made using an appropriate antimicrobial agent. In a study conducted by Khaledi et al. (2015) [12] ,the essential oils of mint, cumin and ‹Thyme had the highest antifungal activity against fungi so that it was determined that the peppermint essential oil had the lowest inhibitory concentration (MIC) for The *Rhizoctonia solani* fungus is among the three tested essential oils, Also, the mentioned pathogen in the presence of mint, cumin and thyme has concentrations of 850, 1200 and 1100 ppm respectively, the essential oil of cumin also has the minimum inhibitory concentration (MIC) for the *Macrophomina phaseoli* fungus, the pathogen in the presence of mint, cumin and thyme have the concentrations of 975, 950 and 1150 respectively. In Iran, by studying the antifungal characteristics of three essential oils of Peppermint, *Zataria multiflora* and gardens thyme on *Phytophthora drechsleri* fungus (fruit decay factor), the experiments showed that by increasing the concentration of essential oils, the growth of the fungus was significantly reduced. It was observed that the values of IC50, MIC and MFC in *Zataria multiflora* were 0.053, 0.1, 0.2 respectively and similarly in the essential oils of mint and garden thyme was 0.4 and 0.8 respectively. The results of this study may contribute to the development of new antifungal agents to help for protecting the crops from pathogenic fungi and many plant pathogens causing product losses [13]. During the research on the essential oils of *Cymbopogon citratus* and Ocimumbasillicum and 2 fungicides such as Mancozeb and (Metalaxyl-Mancozeb) in 6 different concentrations on 3 species of *Phytophthora drechsleri*, *Phytophthora melonis* in pepper ,cucumber and melon in greenhouse and laboratory conditions and laboratory was carried out by Amini et al. (2016)[14] .The severity of the disease

was determined after 28 days. Among the two essential oils ,the essential oil of *Cymbopogon citratus* showed the lowest determined value of EC50 to inhibit the growth of mycelium in *P. capsici* fungi (47.31) *P. melon* (33.09) *P. drechsleri* (69/11), respectively. The average value of EC50 for metalaxyl-mancozeb was in respectively 3 pathogens as *P. capsici* (20.87) *P. melonis* (20.06) *P. drechsleri* (17.70).In the greenhouse conditions of metalaxyl-mancozeb the highest reduction in disease severity was 84.2-86.8-92.1 in melon-cucumber and pepper respectively. The essential oil of *Ocimum basillicum* had the least effect on the pathogens in laboratory and greenhouse conditions. The results showed that the essential oils may help to develop new antifungal agents in *Phytophthora* disease products. In a study by Mansouri et al. (2016) [15] they found that the essential oil of *Ammodaucusleu cotrichus' fruit* ,a causative degradation agent of apple against the fungal pathogen including *Botrytis cinerea* ,*Penicillium expansum* and *Rhizopus stolonifer* was done in preparation method of Poisoned Foods (PF) and volatile Activity in apple after harvesting , In the PF method, MIC for *Botrytis cinerea* and *Penicillium expansum* (0.5 µl) and for *Rhizopus stolonifera* (1 µL) as well as in the VA method, the complete inhibitory of mussel mushroom from *Botrytis cinerea* and (*Penicillium expansum* 125 µl) and *Rhizopus stolonifer* (0.25 µl) was observed in air organs. The overall results indicate that the *Ammodaucus stolonifer* essential oil is used as the antifungal agent for the control of apple after-harvest diseases. Maconen et al. (2016)[16] examined the antimicrobial activity of 4 essential oils of thyme, chamomile, eucalyptus, rosemary *in vitro* against bacteria and fungi which the results of the experiment showed thatف he minimum inhibitory concentration of thyme was 15.75 for most of the bacteria and fungi utilized in this study and the minimum inhibitory concentration of other essential oils in the range of 15.36-75.33 mg/ml were tested versus the bacteria. The results of the experiment indicated that the thyme even had a potent antimicrobial activity in the special conditions for the preparation of antimicrobials. Using the studies of Gaqobi et al. (2017) [17] on the fungicide features of *Eucalyptus camaldulensis* essential oil for investigating the antifungal activity against 5 *Fusarium* species in relation to corn, the control of complete mycelial growth of the pathogens after 5 days of the latent period was at a concentration of 8-7 µl. Also, MIC and MFC were respectively 7-8 µl and 8-10 µl. The findings of this research will confirm that the features of eucalyptus and their components in the utilization is important in economic management and as an alternative for the use of pesticides. In a general summarized form of the total results and by considering the aforementioned points it can be concluded that a suitable method for combating the rot disease of wheat crown, *Fusarium* wilt of tomato and wave-form spot of Tomato is an exploitation of biological control. Therefore, the use of *Zataria multiflira* oil is recommended not only for the management of this disease, but also for other plant diseases, especially malignant diseases caused by pathogenic fungi.

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