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COMPARISON OF DIFFERENT METHODS IN THE EFFECT OF VOLATILE OIL OF *ZATARIA MULTIFLORA* ON HERBAL PATHOGENIC FUNGI

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Abstract

What nowadays cause the development of organic farming is the long-term effects of various types of chemical fertilizers and pesticides in agricultural products and on the environment. Therefore, the management of plant diseases using natural herbal products is an important strategy in this direction that is always considered by agriculture sciences researchers. In this research, the antifungal effect of Zataria multiflora was studied for the purpose of optimal use of herbal products in 4 techniques. In all the methods used in this study by increasing the concentration of volatile oil to 1000 ppm, the longitudinal growth was reduced and mutually the growth inhibitory ratio was increased. However, at concentration of 50,150 and 450 ppm of the volatile oil after 48 hours, the highest inhibitory percentage was observed in Food Poison Technique method with 20, 53 and 68.5, then the highest percentage of inhibition in the aforementioned concentrations was visible in surface Technique, volatile Technique, and disc Technique, respectively.

Keywords: *Growth control techniques, Growth inhibitory percent, Volatile oil, Plant disease control*

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1. INTRODUCTION

Antimicrobial properties of essential oils have been known for many years. Besides the antimicrobial properties, the essential oils have anti-fungal, antiviral and anti-parasitic. Despite the recognition of these effects in the past years, the green consumption trend has led to a greater willingness to know the scientific knowledge of these materials [1]. The mature plants produce over 100 hundred thousands of natural secondary metabolites of low molecular weight [2]. Many of these metabolites are effective against plant diseases and insects in plant defense [3]. The antifungal properties of lamiacea family especially thyme has been known to be declared by various sources. Bahaskar reddy *et al.* (1998) [4] used Thyme essential oil to control the *Botrytis cinerea* fungus on strawberry. Shakrami *et al.* (2006) [5] have studies the essential oil effect of five plant species such as Thyme, Mint, Mountain Artemisia, *Myrtus communis*, *Vitex agnus-castus* on the mycelial growth fungi of *Rhizoctonia herbarium*, *Geomonocytes* and *Fusarium*. The results showed that the essential oil of thyme and mint plants caused 100% inhibition of the mycelial growth of the studied fungi. During the research, Lotfi *et al.* (2010)[6] stated that the essential oils of thyme, pennyroyal and Trachyspermum plants have a high effect on the growth of *Fusarium* mycelial growths, so that in the concentration of 300 and 400 ppm of thymus essential oil, the complete inhibition of growth of fungi occurred and an increase in the essential oil percentage caused in the mycelial growth inhibitory effect of the fungus. McConne *et al.* (2016)[7] examined antimicrobial activity of 4 essential oils of thyme, chamomile eucalyptus, rosemary in vitro against several bacteria and fungi,

The results of the experiment showed that the minimum inhibitory concentration of thyme was 15.75 mg/ml in this study and the minimum inhibitory concentration of other essential oils was between 15.56-36.33 mg/ml versus Bacteria. The wild marjoram with scientific name of (*Zataria multiflora*), its synonym is *Zataria Bracteae*. Wild marjoram is one of the most well-known medicinal plants in Lamiaceae [8]. The plant is full of branches with wooden stems of 10 to 30 cm in height which are wild and in the form of thick bushes on dry slopes between boulders and in mountains up to 1200 meters high [9].

This plant is exclusively in Iran, Afghanistan and Pakistan. In Iran, the plant is located in Isfahan, Lorestan, Kerman, Fars, Hormozgan, Sistan and Baluchestan, Yazd, Khuzestan and Khorasan provinces [10]. It has always been one of the effective methods for combating with the plant diseases using natural herbal products. Among them, Wild Marjoram has attracted the attention of many scholars. Many antifungal properties have been reported in various sources, but in the present study, the antifungal effect of this plant's volatile oil in four different methods has been investigated on *Fusarium* fungus as the agent of wilting of tomatoes.

2. The purpose of the research

As it has been investigated with different methods of antifungal effect of herbal essential oils in this study, we tried to find a solution in order to confirm the fungal effects of thyme on *Fusarium* fungus as the cause of tomato wilting, the optimal use of plant products can be studied in the shortest possible time.

3. MATERIALS AND METHODS

3. 1. Providing a vegetarian sample and volatile oil extraction

In the spring of 1395 *Zataria multiflora* was purchased from the Atari of the Shiraz Vakil market. After identifying and confirming the scientific name of the plant using the keys of identification and coordination with the professors of the Department of Herbalogy of Shiraz University, the raw oil of the plant sample was extracted by water distillation with Clovenger apparatus. For each extraction 300 g of sample were poured in a 1000 ml Cloenger balloon and 250 ml of water was added too. Then, the raw oil was collected after a 2 hour distillation.

The raw oil was dewatered with sodium sulfate and stored in dark containers to prevent oxidation in 4 ° C. It should be noted that the essential oil yield was 0.08%.

3.2. The disease agent and the utilized culture medium

The isolate of *Fusarium oxysporum* f.sp. *lycopersici* was purified and prepared on PED culture medium in the winter of 1394 from the plant pathology laboratory of Payam Noor University of Shiraz.

After several times of re-cultivating of the fungus in the culture medium of P.D.A and the necessary isolation of the required amount were kept as needed and stored in the refrigerator at 4 ° C until the end of the experiment.
In this research, P.D.A (potato dextrose agar) medium was used as a solid culture medium. This medium was commercial powder and dissolved in 42 g/L of distilled water, then heated to completely dissolve and was autoclaved at 121 ° C for 15 minutes and pressure of 1.5 atm.

3.3. The method of using volatile oil in controlling disease agent growth

The inhibitory effect of thyme raw oil deterioration on the growth of fungus agent of tomato wilting was determined by using four techniques in a solid culture medium and finally the results were evaluated to select the best technique. Food Poison Technic: In the mixing method with culture medium, the concentrations prepared from the raw oil are distributed within 9 cm sterile petri dishes and immediately added in each 150 ml of P.D.E container.

And was slowly stirred to provide a uniform environment and allowed to solidify, and then 5 mm diameter fungal discs were prepared by a sterile loop alongside a flame from a one-week cultivation and stored at the center of the petri dish containing the culture medium .

Surface Technic: In this technique, after distributing the P.D.E environment to the petri-plate containers and its coagulation at laboratory temperature, the concentrations achieved from raw oil were distributed at the petri-plate medium containing the culture medium and after 30 minutes, cultivation of the fungus wed done. Volatile Technic: The technique of direct use of raw oil is the same as the addition of raw oil on the surface of the culture medium except that the concentrations produced after the mushroom cultivation, were distributed at Petri-plates's cover and the culture medium was placed in an incubator inversely. Disc Technic: In this technique, the saturated concentrations were added to the Blank Paper Disk and in each petri-plate, 3 paper discs were placed within 2 cm from the pathogen. It should be noted that all of the following steps were taken in the laminar hood and near the flame c and injection of volatile oil concentrations by syringe and disks in a culture medium was performed by sterile pins.

3. 4. Measuring the longitudinal growth of the disease agent and statistical analysis

The growth of the pathogen in the culture medium was measured by measuring the growth without removing the petri dishes daily and at a specific time by the caliper from the petri dish floor. Excel data was used to record data. Statistical analysis of data was done using SPSS software using one-way ANOVA method through Duncan's multiple domain test (Duncan, 1955)[11].

4. RESULTS AND DISCUSSION:

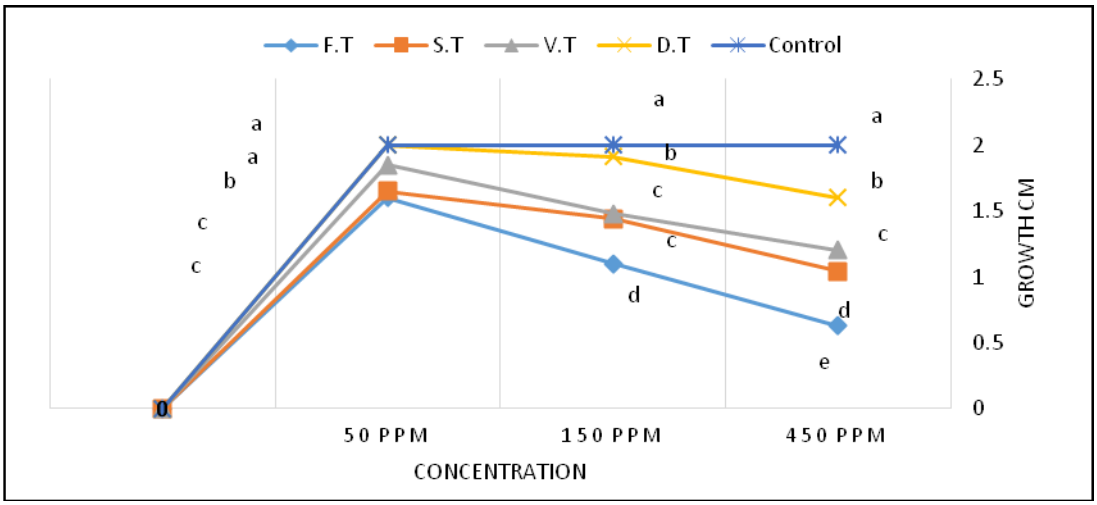
According to the diagrams (1) and (2) as it can be seen, the least longitudinal growth and the highest percentage of inhibition in all concentrations of the volatile oil of Wild marjoram were related to the food poisoning technique and the highest longitudinal growth was related to the examples after the control.

Whose growth is controlled using a paper disc method. The highest growth inhibitory concentration was observed at 450 ppm in all methods. In other word, the longitudinal growth in all methods decreased with the increase in the concentration of volatile oil. In the same concentration, the longitudinal growth of 0.63 and 1.04 in Food Poison Technic and Surface Technic methods was 68.5 and 48% of growth inhibition, respectively. They showed a significant difference in the 5% level of probability. When the disease agent was affected by 150 ppm of Wild marjoram volatile oil, the techniques of Volatile Technic and Surface Technic showed a longitudinal growth of 1.48 and 1.46 cm i.e, respectively 26 and 27% of growth inhibition which statistically did not show a significant difference at the 5% probability level. In other words, the use of volatile oil at 150 ppm concentration had the same effect in both methods in controlling the growth of the studied fungus. As shown in Fig. 1 and 2 the use of volatile oil at a concentration of 50 ppm in a paper disk method did not affect the longitudinal growth control of the fungus which was statistically different from the control sample in 5% probability level. For this concentration, in the techniques of Food Poison Technic, Surface Technic, Volatile Technic, we observed the longitudinal growth of 1.6, 1.65 and 1.85 cm which compared with the control group were significantly different. As noted, many researchers have investigated the antifungal effects of natural plant products in a variety of ways, as listed below. But, the main purpose of this study was to compare the different methods for controlling the longitudinal growth of *Fusarium* fungus caused by the wilting of tomatoes utilizing the volatile oil of Wild marjoram. In a study by Abdolmaleki *et al.* (2009) antifungal effects of 18 species of herbal extracts against fungi causing root and crown rot and sugar beet root rot and causal agents of sugar beet root rot were studied. They said that the most antifungal effect was on Wild marjoram , *Trachyspermum* and Pine which showed a deterrent effect even in the amount of 1 mg per paper disc. The antimicrobial effect of different essential oils of Thyme various species was evaluated by Mohammadpour *et al.* In (2002)[12] using Blank Disc method and finally the essential oil of *Z. multiflora*, Denayi and Mazandaran Thyme have the greatest effects on inhibition of the growth of bacteria and fungi. During a research conducted by Foroughi *et al.*, In 2012, the antifungal effect of the extract of Denayi thyme, *Z. multiflora*, Yarrow and *Ziziphopra capitata* on the *Rhizoctonia solani* was evaluated using a paper disk method and stated that Denayi Thyme extract with a mean diameter of the nimbus of 18.93 mm had the most inhibitory effect on the growth of *Rhizoctonia solani*. The antifungal effect of volatile oil of *Eucalyptus camaldulensis* was evaluated utilizing the Food Poisoning Technique by Akbari *et al.* (2014). The concentration of 8000

ppm of volatile oil was effective in controlling the growth of *Fusarium* fungi.

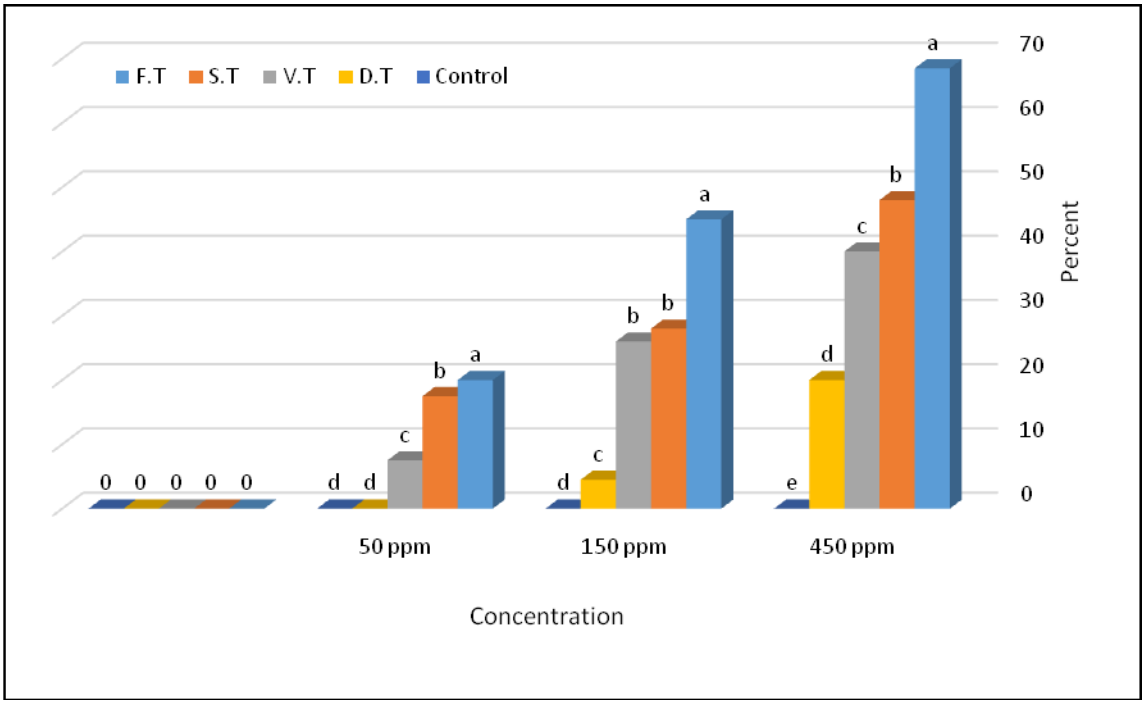
Using the food poisoning technique, the longitudinal growth of Fusarium *solani* fungus with *Rosmarinus officinalis* volatile oil, was controlled in vitro (Farhadi *et al.*, 1395). With the generalization of laboratory findings in the farms, it is hoped that the use of effective plant compounds in the management of fungal diseases will be welcomed more than ever, since the use of compounds will be very effective. In addition to preventing the growth of the pathogen and preventing the damage to the farms, it does not seek to harm the environment and humans.

The chart (1): Longitudinal growth of Fusarium oxysporum f.sp. lycopersici using different methods * In the use of *Zataria multiflora* volatile oil



- F.T:Food Poison Technic, S.T: Surface Technic, V.T: Volatile Technic, D.T: Disc Technic

Chart (2): Inhibition percentage of longitudinal growth of Fusarium oxysporum f.sp. *lycopersici* using various methods * In the use of *Zataria multiflora* volatile oil.



- F.T:Food Poison Technic, S.T: Surface Technic, V.T: Volatile Technic, D.T: Disc Technic

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