Antifungal Activity of Mentha Pulegium Crud Extracts against Alternaria Citri

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Abstract

This research evaluates antifungal activity of different concentration of the plant crud extracts derived from leaves and flowers of Mentha pulegium against Alternaria citri in petri dishes (poisoned food). All extracts revealed obvious inhibitory activity against this fungi, but it varied according to plant parts used as a starting material, different concentrations of extracts and type of solvent.The results showed higher efficiency of ethanol extracts than those of methanol. Ethanol and methanol extracts of leaves were generally better than flowers extracts.MIC value of the leaves ethanol extracts was 0.01 g / ml and 0.015 g / ml for flowers ethanol extracts, while the MIC value was 0.015 g / ml for both leaves and flowers extracts when using methanol as an organic solution.

MFC values of different extracts were slightly higher than MIC values. MFC of leaves ethanol extracts at 0.015 g / ml and 0.02 g / ml for the flowers ethanol extracts, while MFC value for the leaves methanol extracts was at the concentration 0.03 g/ml and at 0.04 for the flowers methanol extracts.

Keywords:*Mentha pulegium, Alternaria citri, Antifungal activity***.**

I. INTRODUCTION

Plants are the main source of food, fiber and many other products that are useful to humans. Plants are attacked at various stages of their growth with many pathogens such as insects, bacteria, fungi, viruses and other pests.This reduces their productivity leading to large economic losses. At least 10% of plant products are lost due to plant diseases[1].

Alternaria is one of the most important plant pathogens. It spreads in many vegetable and fruit crops. *Alternaria citri* is one of the most common and prevalent pathogens among crops, like lemons; where fruit is affected by it directly after the fallingof flower petals and it also affects the newly grown buds. *A. citri*develops with trees in many stages of growth and causes significant economic loss in the crop.

The most important method to protect plants from these pathogens is the use of manufactured chemical pesticides. Many of the available pesticides are poisonous and have adverse effects on soil, water and food, as well as the emergence of resistant strains of pathogens against pesticides[2]. It was,therefore, necessary to usealternative friendly and safe methods to control plant diseases.

Researchers have identified a large number of medicinal plants that have inhibitory activities for the growth of pathogenic fungi. They have proved inhibitory ability of many plant extracts such as *anise*, *rosemary*, *allium cepa*, *garlic* (*Allium sativium*), *thymus*,*ginger*, *cinnamon*,*laurel*, *avocado* and *mentha* against many species of *alternaria* fungi, that causes diseases to many plant species, such as falling fruit. ([3], [4], [5], [6]).

Mentha is an important medicinal and economical plant, and it has many species used as nutrition and in preparing medical and pharmaceutical products. *Mentha pulegium* is a type of *Mentha*, which has a high medical importance, is used in the treatment of gastrointestinal pain, spongy spleen, expectorant for sputum, purifies the chest, headache, and brain tonic. It is also used in the preparation of oral disinfectants. It contains an essential aromatic oil called Polygon used in the preparation of many pharmaceutical products.All parts of *Mentha pulegium*can be used except roots [7].

Medicinal plants have become the focus of intense study in terms of validation of their traditional uses through the determination of their actual pharmacological effects [8]. Plants from the genus *Mentha* are used for antimicrobial, antiviral and insecticidal activity ([9], [10]).

Mentha pulegium is a widespread plant in many regions of the Syrian coast.It can be found on the sides of rivers, ponds and inside the moist sites. *Mentha* plant belongs to Lamiaceae family and is considered one of the medicinal plants that have been traditionally used to treat many diseases, and due to serious side effects of chemical fungicides, this research is dedicated to find safe natural compounds.

II. MENTHA PULEGIUM

*Mentha pulegium*belongs to Lamiacae family, genus *Mentha*. It grows near the watercourse. It is a perennial herbaceous plant with a 8 and 40 cm height. Its leaves are simple oval. The nodes are surrounded by the flowers which are small and violet

(Fig. 1), seeds look oval , brown and smooth with 0.5 - 0.7 mm dimensions (Fig. 2).

Figure1: Mentha Pulegium

Figure2: Seeds of Mentha Pulegium

III. MATERIALS AND METHODS

A. *Plant Samplings*

Plant samples *Mentha pulegium* were collected from Btara river before and after flowering from June to August 2016. In lab, samples were washed by running tap water many times. Parts of plant samples (leaves, flowers) were separated from each other, and were left to dry in open air and in a shadow place. The samples were placed in an oven at 40 °C until constant weight was attained. Then, they were finely ground using electric grinder and the powder was kept in tightly sealed containers in a fridge until use.

B. *Isolated Alternaria citri*

Alternaria citri was isolated from a soil sample that was taken from a citrus tree field . Theisolated fungiwere cultured on a sterilized nutrient medium of potato dextrose agar P.D.A by serial dilution of soil solutions. Then they were incubated at 25°C for seven days. Growth fungi species were isolated and purified. The classification was based on morphological and microscopic criteria according to taxonomy references[11]. The isolated fungus was stored in P.D.A tubes to be enriched again a week before making new cultures.

C. *PreparingOrganic Extracts*

45 g of each powdered plant parts were placed in 500ml flasks in which 300ml of ethanol or methanol were added.The mixture was vigorously stirred for half an hour using electric agitator.The flasks were wrapped with aluminium foil and left in darkness for 20 days with keeping shaking from time to time over the mentioned period. The extracts were filtered using whatman paper no.1 and morcelain filter.The process was repeated three times until the plant material was completely separated from the organic solvent. In order to thoroughly separate the plant extracts, the plant residuals were separatedfrom the aqueous extracts and then it was concentrated using rotary evaporator at 40 °C until cohesive, soft and dough-like extracts were obtained. Then, they were kept in tightly sealed containers at 4 °C until use.

D. *Antifungal Assay*

Antifungal assay was done using petri dish method according to reference [12] with some suitable modification. The crude extracts were diluted in water andadded to P.D.A-contained flasks at concentrations of (0.04 ,0.03, 0.02, 0.015, 0.01, 0.005, 0.0025, 0.001) g/ml. Then the mediawere poured in 9cm petri dishes. After that, 5mm^3 cube was taken from the edge of each 7 days old colony of the study fungus and placed in the middle of petri dishes. Then they were incubated at 25 °C for 7 days. Control petri dishes containedP.D.A free extractson which the study fungus was planted. Antifungal assay for each concentration of extract was performed in triplicates and the culture platesof the fungal colonies were measured.Then the inhibition percentage was calculated according to:

$$\textbf{Inhibition}\,\% = \frac{\textbf{colony diamater in control} - \textbf{colony diameter in treatment}}{\textbf{colony diamater in control}} \times 100$$

IV. RESULTS AND DISCUSSION:

It was obvious from the findings that ethanol and methanol extracts of *Mentha pulegium*revealed antifungal activity against *Alternaria citri* in different rates due to the plant parts, the type of solvent and the extract concentration. All extracts exhibited antifungal activity at different concentrations as shown in Table I and II. Several studies have shown that the ethanol extract of plant leaves, such as *Mentha arvensis* leaves, *Rosemary* and tropical *Mangrove* had the best antifungal activity against a number of fungi, including *Alternaria citri*in comparison to other parts of plant ([13], [14], [15]) . Antifungal potentialdepends on a plant part, whereEtOH extracts of *M. pulegium*leaves were the most effective in comparison to EtOH extracts of flowers, so the diameter of *A. citri* colony was 1.47± 0.15 cm at a concentration of 0.005 g/ml, with an inhibitory rate of 71.6 %, whileat the same extract concentration, it was 2.03 ± 0.18 cm and the rate of inhibition was 61% when treatment with EtOHflowers extracts. (Fig3, 4,TabI) .

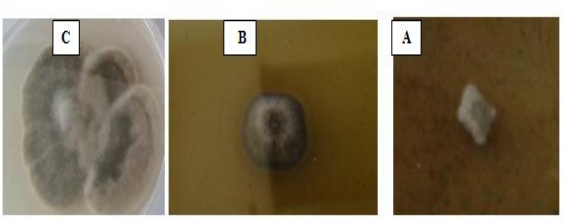

Figure3: ColonyDiameters of A. citri at 0.005 g/ml(A: EtOH Leaves Extract, B: EtOH Flowers Extract ,C: Control)

Minimum inhibition concentration (MIC) value of the EtOH leaves extracts was at 0.01 g/ml, and at 0.015 g/ml for the EtOH flowers extracts. Our study was similar to a study in which the EtOH extract of the fresh leaves of *Mentha arvensis* showed the best effect against the *Alternaria alternata* [16].

Table I: Colony Diameters (cm) of A. citri at Different Concentrations of EtOH Extracts of Mentha pulegium on the 7thDays Incubation.

Mentha pulegium ɔncentration g/ml	Plant Parts	
	Leaves	Flowers
Control=0	5.17±0.41	5.2± 0.43
0.001	2.6±0.19	3.1±0.19
0.0025	1.97±0.11	2.43±0.27
0.005	1.47± 0.15	2.03±0.18
0.01	0	1.37±0.11
0.015	0	0
0.02	0	0
0.03	0	0
0.04	0	0

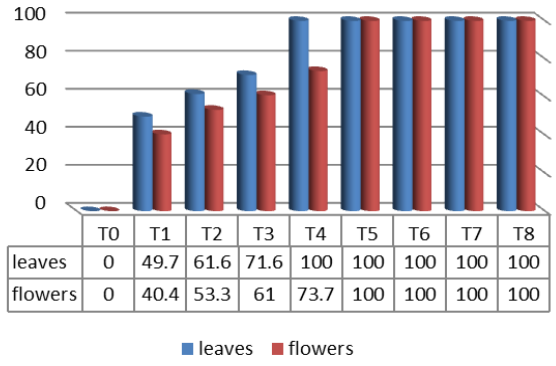

Figure4:Inhibition% of A. citri at Different Concentration of M. pulegium EtOH Extracts. (T0= 0 g/ml, T1=0.001g/ml, T2= 0.0025 g/ml, T3= 0.005g/ml, T4= 0.01 g/ml, T5= 0.015g/ml, T6= 0.02g/ml, T7= 0.03g/ml, T8= 0.04 g/ml.)

Table II shows the average of growth rates of *A. citri* after 7 days of incubation at 25 ° C in different concentrations of MeOH extracts.All concentrations of leaves extracts have affectedthe growth of *A. citri*, and the leaves extracts were the most effective in comparison tothe flowers extracts.So diameter of *A. citri* was 1.1±0.07 cm at concentration of 0.01 g/ml, with an inhibitory rate of 78.64% when it was treated with leaves extracts, while at the same concentration it was 1.77±0.17 cm and the rate of inhibition was 65.83% when it was treated with MeOH flowers extracts. At low concentrations; good antifungal activity was observed at 57.86 % of leaves extracts in diameter of *A. citri* 2.17 cm in comparisonto 51.74% for the flowers extracts in diameter of *A. citri* 2.5 cm at the concentration 0,005 g/ml (Fig 5, 6).

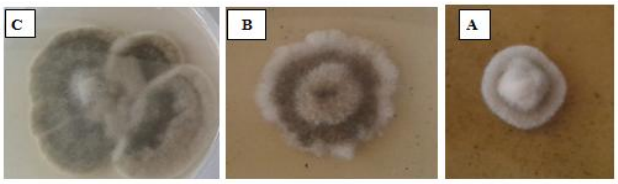

Figure5:colony diameters of A. citri at 0.005 g/ml(A: MeOH leaves extract, B: MeOH flowers extract ,C: control)

MIC value was 0.015 g / ml for both leaves extract and flowers extract when using MeOH as an organic solution (TabII, Fig: 6).

MFC (Minimum Fungicidal Concentration) values of extracts were confirmed and were different from the MIC values. MFC value for the EtOH leaves extracts was at 0.015 g / ml, and at 0.02 g / ml for the MeOH leaves extracts, while the MFC value for the MeOH leaves extracts was at 0.03 g /mL, and at 0.04 g/ml for the MeOH flowers extracts.

Table II: Colony Diameters (cm) of A. citriat Different Concentrations of MeOH Extracts of Mentha pulegium on the 7thDays Incubation.

Mentha pulegium Concentration g/ml	Plant Parts	
	Leaves	Flowers
Control=0	5.15±0.44	5.18±0.41
0.001	2.87+0.11	3.67+0.11
0.0025	2.67±0.17	3.27±0.17
0.005	2.17+0.11	2.5+0.28
0.01	1.1±0.07	1.77±0.17
0.015	0	0
0.02		
0.03		
0.04		

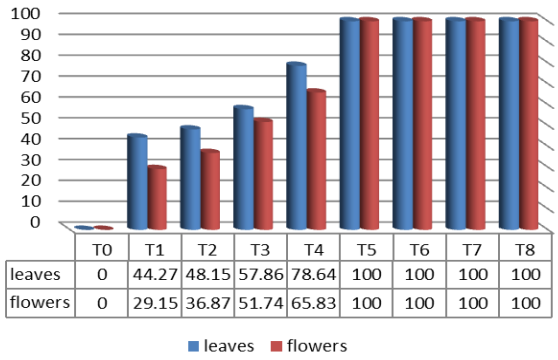

Figure 6: Inhibition% of A. citri at Different Concentration of M. pulegiumMeOH Extracts.

(T0= 0 g/ml, T1=0.001g/ml, T2= 0.0025 g/ml, T3= 0.005g/ml, T4= 0.01 g/ml, T5= 0.015g/ml, T6= 0.02g/ml, T7= 0.03g/ml, T8= 0.04 g/ml.)

In this study, dry parts of *M. pulegium* plants were used and leaves and flowers were selected. The antifungal activity of the plant was tested before and after flowering to determine the best time to obtain the active parts of plant. Several studies indicated that it is preferable to harvest aromatic plants before flowering, that is to get the most benefit from them.([2], [17]).

It has been found in Tab. I,II ; Fig 4,6 that the effect of the leaves extracts was better than the effect of flowers extracts in most concentrations. Many studies have shown the effectiveness of the dry parts of genus *Mentha* in different types against most microorganisms, including fungi. This activity is due to the*Mentha*contains flavonoids and various types of essential oils, especially Menthon, Menthol, Menthe acetate. The *Mentha pulegium* contains the volatile Minton and polygon oils that have antifungal activity [7].

Antifungal activity of *Mentha pulegium* against *A. citri* may be due to the use of raw extracts (mixture of compounds) of various plant parts containing a combination of active compounds rather than a specific substance. Recent research has indicated that the use of raw compounds has a greater benefit in terms of the integrated effect on microorganisms. The use of crud extracts reduce of resistance to these organisms, which occurs when resistant strains are found by repetitive use of chemical pesticides [18].

V. CONCLUSION

1- Ethanol, Methanol extracts of *Mentha pulegium* revealed antifungal activity against *Alternaria citri*in different rates due to the plant parts ,the solvent, and the extracts concentrations.

2- Ethanol extracts exhibited better inhibitory activity in comparison toMethanol extracts.

3- Ethanol extract *M. pulegium* leaves exhibits the highest inhibitory activity against *A. citri.*

4- The study findings suggested to use *M. pulegium* crud extracts to control the spread of *A. citri*.

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