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Screening of the antifungal activity of plant *Mentha longifolia* crude extracts against two fungi *Alternaria citri* and *Fusarium moniliforme*

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Abstract
This research focuses on the antifungal activity of different concentrations of ethanol, methanol and acetone crude extracts derived from plant *Mentha longifolia* against *Fusarium moniliforme*, and *Alternaria citri*. All extracts revealed obvious inhibitory activity against both fungi. But it varied based on plant part used as starting material, different concentrations of extracts and type of solvent. MIC of different extracts ranged from 0.002-0.04 g/ml. It was found that at 0.01 g/ml and at 0.002 g/ml of acetone extract of *M. longifolia* flowers, both fungi, *Fusarium moniliforme*, and *Alternaria citri*. Thus acetone extract of *M. longifolia* flowers was the most effective as compared to ethanol and methanol extracts of *M. longifolia* parts used as starting materials.

Keywords: Inhibitory activity, *Mentha longifolia*, *Alternaria citri*, *Fusarium moniliforme*

1. Introduction
Fungal diseases annually incur huge economic losses worldwide, *Fusarium* sp is considered of the most critical phyto pathogens for several vegetables, grains, and other plants. *Fusarium moniliforme* is one of the permanent, dominant and widespread pathogens which blights grains such as maize, wheat, oats, soybeans. Its invasion may initiate at any stage. Upon favorable conditions, it causes many infections such as root, fruits and seed rots wilt. Besides, it causes decrease in germination and more importantly the production of mycotoxins which lead to serious losses in grain crops especially maize [1, 2].
Alternaria fungi belonging to Hyphomycetes are widespread in nature and pathogenic to agricultural crops whether during their growth or storage due to the production enzymes such as cellulose, lipase, and protease [3].
Despite utilization of fungicides over several years has played a major role in attacking fungi, adversely many problems have appeared such as environmental pollution and resistant strains. Therefore, modern studies were directed to produce and to utilize plant extract in the treatment of fungus lesions. The metabolites in these extracts have proved to be effective and feasible. Potential of plant extract depends on its constituents [4]. Thus, researchers started to isolate, purificate these compounds and screen their activity against fungi [5]. Lately, medicinal plants were used in the treatment of many diseases [6]. And they were the main sources of human and animal nutrients. Therapeutically, many researches proved that some plants *Allium sativum*, *Allium cepa*, *Syzygium* sp have antimicrobial activity against pathogens and infections that blight humans, animals and plants [7, 8, 9, 10, 11].
Various bioactive compounds derived from these plants have been used in Ayurveda or alternative medicine, but they showed many side effects because of poor public knowledge about traditional plants. Therefore, researchers selected a big number of antifungal activity against fungi and mycotoxins. It was reported that the extracts of several plants such as Azadirachta, Garlic, Curcumin, some Cucurbitaceae family plants and some essential oils of clove, citrus, and cinnamon inhibit many pathogenic fungi that can be ingested by humans, animals, plants and cause many diseases [12, 13, 14, 15, 16, 17]. *Mentha* sp plant is medically and economically important because it contains many vital chemical compounds of therapeutic uses such as menthol, menthyl acetate, pulegone, and menthofurane. In Ayurveda *Mentha* has been used in treatment of digestive complaints, nausea, indigestion, colic and cramping. Moreover, *Mentha* is considered an efficient spasmolytic and it works as a CNS relaxant so it alleviates headache. Besides, it's reported that *Mentha* has antibacterial, antifungal and antiviral properties, hence, it can be used to treat influenza, pharyngitis.

Mentha longifolia is a widespread plant in many regions of Syrian coasts. It can be found along rivers, ponds and moist sites. Mentha plant belongs to Lamiaceae family and is considered one of medicinal plants that have been traditionally used to treat many diseases due to serious side effects of chemical fungicides therefore researches are dedicated to find safe natural compounds.

2. Materials and Methods

2.1 Plant sampling

Plant samples *Mentha longifolia* were collected from Marqia river from March to September 2012. In lab, samples were washed by running tap water many times. parts of some plant samples (leafs, stalk, flowers) were separated from each other. Other plant samples were deposited in whole. The two types of plant samples were left to dry in open air and in a shadow place. The samples were placed in an oven at 40 ºC until constant weight was attained. Then, they were finely ground using electric grinder and the powder was kept in tightly sealed containers in fridge until use.

2.2 Fungal isolation

Tow isolates of the study fungi *Fusarium moniliforme* and *Alternaria citri* were obtained from a soil sample. The fungal isolates were cultured on a sterilized nutrient medium of potato dextrose agar PDA by serial dilution of soil solutions. Then they were incubated at 25 ºC for seven days. Grown fungal species were isolated and purified. The classification was based on morphological and microscopic criteria according to taxonomy references. Isolates of the study fungus were stored in PDA tubes at 4 ºC to be enriched again a week before making new cultures

2.3 preparation of organic extracts

45 g of each powdered plant part were placed in 500ml flasks to which 300ml of ethanol, methanol, or acetone were added the mixture were vigorously stirred for half an hour using electro magnet agitator the flasks were wrapped with aluminum foil and left in dark for 20 days with keeping shaking from time to time over the mentioned period. The extracts were filtered using whatman paper no 1 and champerlaine filter. In order to thoroughly separate the plant residuals from the aqueous extracts which were concentrated using rotary evaporator at 40 ºC the result extracts ware placed in a water bath at 40 ºC until cohesive structure, soft and dough-like extracts were obtained. Then, they were kept in tightly sealed containers at 4 ºC until use.

2.4 Antifungal assay

Antifungal assay was done using petri dish method according to Suarez-Jimenz, 2007 with some suitable modifications. The crude extracts was diluted in distilled water and added to PDA-contained flasks at concentrations of 0.06, 0.04, 0.01, 0.002, 0.001, and 0.0002 g/ml. Then the media were poured in 9cm petri dishes after that $5m^3$ cube was taken from the edge of each 7 day old colony of the study fungi and placed in the middle of each petri dish, then incubated at 25 ºC for 7 days. Control petri dishes contained extract free PDA on which the study fungi were plated. Antifungal assay for each concentration of extract was performed in triplicates and the culture plates and for control dishes. In the end the diameter average of colony was measured and then the inhibition percentage was calculated accordingly

$$\text{Inhibition}\% = \frac{\text{colony diameter in control} - \text{colony diameter in treatment}}{\text{colony diameter in control}} \text{- x 100.}$$

3. Results and Discussion:

It was obvious from the findings that Ethanol, Acetone, and Methanol extracts of *Mentha longifolia* revealed antifungal activity against *Fusarium moniliforme* and *Alternaria citri* at different rates due to the solvent, and to various extracts of the respective plant parts. In general, all extracts exhibited antifungal activity at different concentrations as shown in all tables below. But, ACE was the most active in comparison with EtOH and MeOH extracts Tab1, 2 and 3. Antifungal potential was plant part dependent, viz. ACE of *M. longifolia* plant flowers was the most effective in comparison with that of the other respective plant parts so the diameter of *F. moniliforme* colony was 1cm at a concentration of 0.001g/ml while at the same extract concentration, it was 4.2 cm when treated with leaf ACE and 6.55cm in treatment with the whole plant including the stalk ACEs Tab1

Table 1: Inhibition zone diameters (cm) at different concentrations of Acetone extract of *Mentha longifolia* against of *F. moniliforme* on the 7th day incubation

Study plant part	*M. longifolia* ACE concentrations mg/l mg/l	control	0.0002	0.001	0.002	0.01	0.02	0.04	0.06
whole plant		8.2	6.7	6.55	4.9	0	0	0	0
leafs		7.6	7.4	4.2	0.7	0	0	0	0
flowers		7.6	4.55	1	0	0	0	0	0
stem		8.2	6.7	6.55	4.9	0.7	0	0	0

Table 2: Inhibition zone diameters (cm) at different concentrations of Methanol extract of *Mentha longifolia* against of *F. moniliforme* on the 7th day incubation

Study plant part	*M. longifolia* MeOH concentrations mg/l mg/l	control	0.0002	0.001	0.002	0.01	0.02	0.04	0.06
whole plant		8.2	6.95	6.6	6.1	2	0	0	0
leafs		8.5	7.6	7.35	3.15	0.9	0	0	0
flowers		8.5	7.2	2.55	0	0	0	0	0
stem		8.4	7.95	7	6.75	5.75	5.35	4.9	0

Table 3: Inhibition zone diameters (cm) at different concentrations of Ethanol extract of *Mentha longifolia* against of *F. moniliforme* on the 7th day incubation

Study plant part	*M. longifolia* EtOH concentrations mg/l	control	0.0002	0.001	0.002	0.01	0.02	0.04	0.06
whole plant		7.8	7.05	6.45	4.3	0	0	0	0
leafs		8.2	8.05	4.3	2.95	0	0	0	0
flowers		8.1	6.75	3.25	0	0	0	0	0
stem		8.5	7.55	6	5.85	1.2	0.9	0	0

As for *Alternaria citri* fungus, at a concentration of 0.001g/ml derived from leafs the diameter of the colony was 4.7cm while at the same concentration of extract derived from the whole plant and stalk the colony diameters were 5and 6.9 cm respectively Tab4. Ethanol and methanol extracts of the plant flowers exhibited the most efficient antifungal activity against two study fungi; at a concentration of 0.001g/ml of both MeOH and EtOH extracts the diameters of *F. moniliforme* colony were 2.55cm and 3.25cm respectively Tab5,6. While in treatment with the 0.001g/ml EtOH of the whole plant, leafs, and stalk the diameters of colony were 6.6, 7.35 and 7 cm respectively Tab 5,6, the diameters of colony when treated with the same concentration of ethanol extract derived from the whole plant, leafs, and stalk were 6.45, 4.3, 6 cm respectively Tab5. The highest antifungal activity of *Mentha* flowers in comparison with that of the other respective plant parts is attributed to the active acetone, methanol and ethanol extracted metabolites against *F. moniliforme*. As reported by Riahi *et al*, 2013 [13], the active compounds of *Mentha rotundifolia* include phenols, alkaloids, ether butyls, and others

Table 4: Inhibition zone diameters (cm) at different concentrations of Acetone extract of *Mentha longifolia* against of *A. citri* on the 7th day incubation

Study plant part	*M. longifolia* ACE concentrations mg/l mg/l	control	0.0002	0.001	0.002	0.01	0.02	0.04	0.06
whole plant		7.4	5.25	5	3.15	2.5	0	0	0
leafs		7.3	6.75	4.9	1.45	0	0	0	0
flowers		7.1	5.5	4.7	0	0	0	0	0
stem		8.5	7.3	6.9	6.7	4.4	1.6	0	0

Table 5: Inhibition zone diameters (cm) at different concentrations of Methanol extract of *Mentha longifolia* against of *A. citri* on the 7th day incubation

Study plant part	*M. longifolia* MeOH concentrations mg/l mg/l	control	0.0002	0.001	0.002	0.01	0.02	0.04	0.06
whole plant		7.1	6.3	6.05	5.95	2.8	2.5	0	0
leafs		7.4	6.55	6.1	2.85	0	0	0	0
flowers		7.4	5.9	5.25	2.25	0	0	0	0
stem		7.5	6.85	6.35	6.10	4.65	2.20	0	0

Table 6: Inhibition zone diameters (cm) at different concentrations of Ethanol extract of *Mentha longifolia* against of *A. citri* on the 7th day incubation

Study plant part	*M. longifolia* EtOH concentrations mg/l mg/l	control	0.0002	0.001	0.002	0.01	0.02	0.04	0.06
whole plant		7.5	6.5	5.2	2.7	1.6	1.1	0	0
leafs		7.4	6.95	5.7	5	0	0	0	0
flowers		7.1	5.5	4.25	0	0	0	0	0
stem		8.1	7.05	6.6	6.1	2.8	2.3	0	0

It's been found from **Figures 1, 2, 3, 4, 5, 6, 7, and 8** that acetone extract was most the effective in comparison with ethanol and methanol extracts at different concentrations of *Mentha longifolia* derived extracts,. Significant differences in ratios of inhibition could be noticed between various extracts upon treatment for fungi growth. So as obvious in **Figures 1, 2, 3, and 4** the growth inhibition percentage of *F. moniliforme* was 40% at MIC of 0.0002g/ml in treating with acetone extract of flower while it was 18% at MIC of 0.0002g/ml in treating with acetone extract of the whole plant and leafs and 16% at MIC of 0.0002g/ml in treating with acetone extract of the plant stalk. Similar findings were noticed when extracts of *M. longifolia* whole plant, leafs and flowers except ethanol stalk were used and the MIC was 0.04g/ml. As for MeOH extract

It was found from Fig 1,2,3,4 that at MIC of 0.0002 g/ml the percentage of growth inhibition of acetone flower extract was 40%. On the contrary *F. moniliforme* was less sensitive to acetone extracts of *M. longifolia* whole plant, stem and leaf which were 18, 17, 3% respectively Fig2. Growth inhibition percentage increased with increase in concentration ranged from 0.002-0.06g/ml of acetone extract of *M. longifolia* flower. It was revealed that maximum growth inhibition against 0.01 of acetone extract of *M. longifolia* whole plant and leaves as well as against 0.02 of acetone extract of *M. longifolia* stem. As for ethanol extracts, the same percentages of growth were observed except that of ethanol extract i.e. maximum growth inhibition was at concentration of 0.04g/ml. While methanol extracts of *M. longifolia* whole plant and leaves revealed maximum growth inhibition at 0.02g/ml against *F. moniliforme*, methanol extract of *M. longifolia* flower revealed maximum growth inhibition at a concentration of 0.002. All of the previously mentioned matched with what was reported by Manoorkar and Gachande 20014 [9].

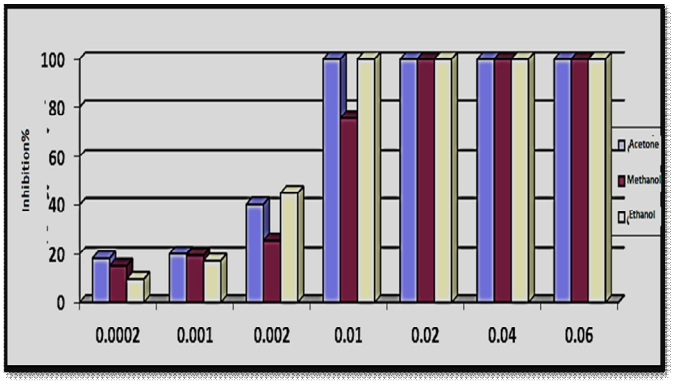

Fig 1: Average growth inhibition of *M. longifolia* whole plant extracts at different concentrations on 7day old colony of *Fusarium moniliforme*

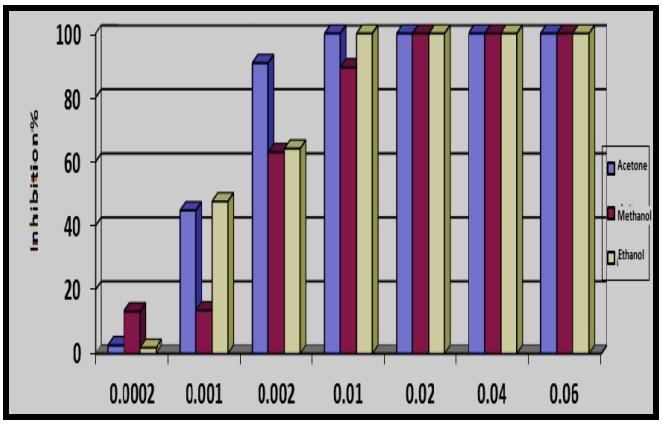

Fig 2: Average growth inhibition of *M. longifolia* leaf extracts at different concentrations on 7day old colony of *Fusarium moniliforme.*

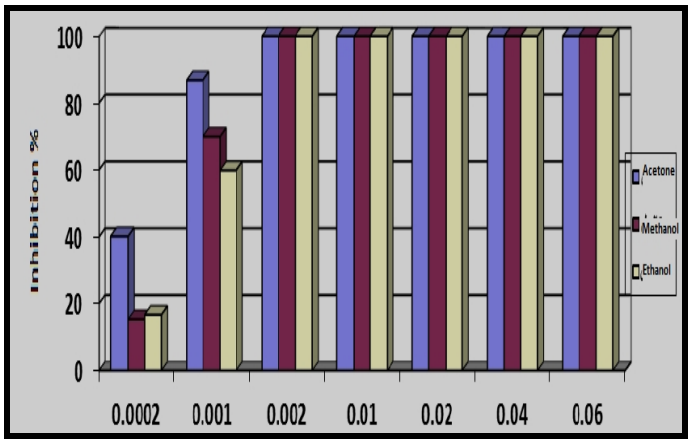

Fig 3: Average growth inhibition of *M. longifolia* flower extracts at different concentrations on 7day old colony of *Fusarium moniliforme.*

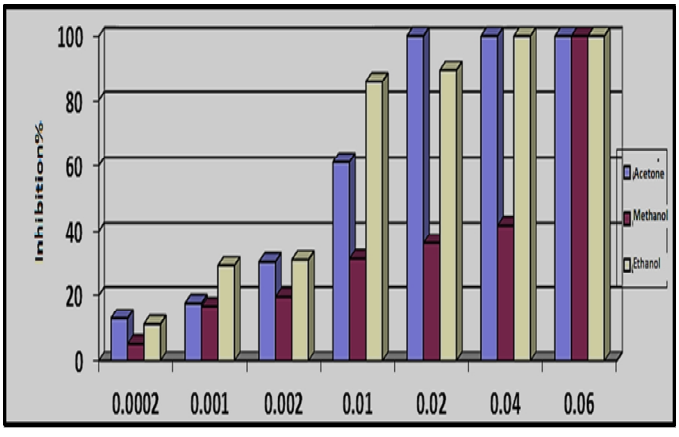

Fig 4: Average growth inhibition of *M. longifolia* stalk extracts at different concentrations on 7day old colony of *Fusarium moniliforme*

It was found from Fig 5,6,7,8 that at MIC of 0.0002 g/ml acetone extract of the whole plant of *M. longifolia* as well as ethanol and acetone extracts of *M. longifolia* flower were most active against *Alternaria citri* in comparison with the same extracts of the other plant parts whereas the percentages of growth inhibition reached 23% for the whole plant and 21% for the flowers. As for methanol extract the crude extracts of leaves and flowers were most active against fungus *A. citri* at MIC of 0.002 g/ml and the percentage of growth inhibition was 20% this value increased with increase in the concentration of methanol crude extract; that is, at concentration of 0.002g/ml the growth inhibition percentages were 70% and 60% respectively for methanol extract of *M. longifolia* flower and leafs. While the maximum inhibition was observed at concentration of 0.01g/ml of methanol extracts of *M. longifolia* flower and leafs, it was at concentration of 0.04g/ml of methanol extracts of *M. longifolia* whole plant and stem. All of the previously mentioned matched with what was reported by Saeed and Tariq, 2005 [16].

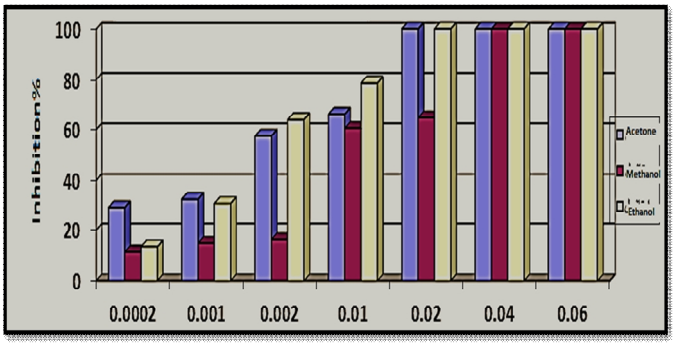

Fig 5: Average growth inhibition of *M. longifolia* whole plant extracts at different concentrations on 7day old colony of *Alternaria citri*

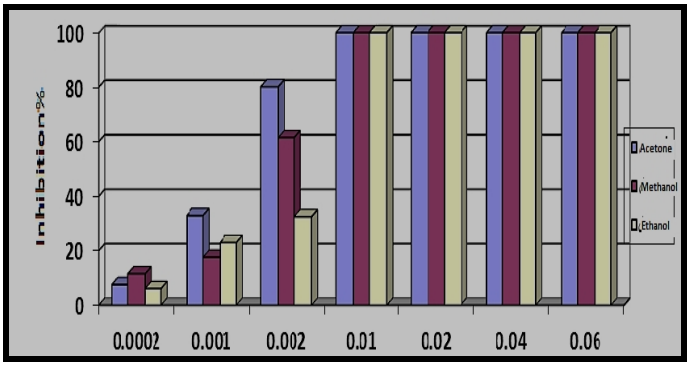

Fig 6: Average growth inhibition of *M. longifolia* leaf extracts at different concentrations on 7day old colony of *Alternaria citri.*

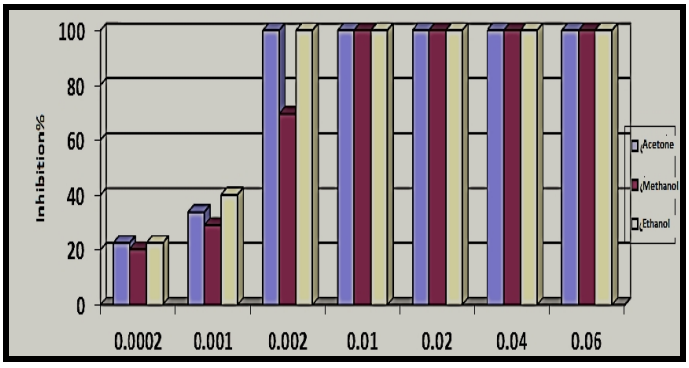

Fig 7: Average growth inhibition of *M. longifolia* flower extracts at different concentrations on 7day old colony of *Alternaria citri.*

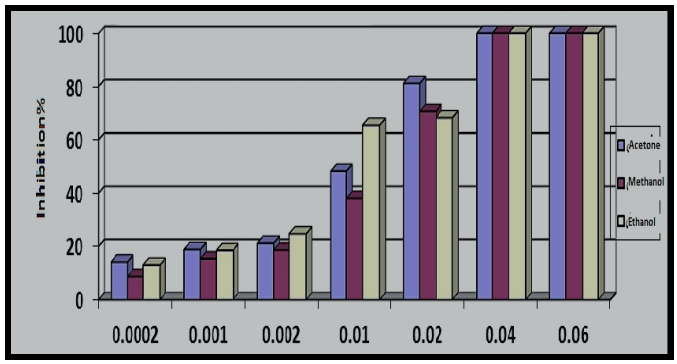

Fig 8: Average growth inhibition of *M. longifolia* stalk extracts at different concentrations on 7day old colony of *Alternaria citri.*

4. Conclusions

1. Ethanol and methanol extracts of *Mentha longifolia* plant inhibit the growth of the two fungi *Fusarium moniliforme* and *Alternaria citri* at low concentrations; significantly against *Fusarium moniliforme*.
2. The efficiency of the crude extracts depends on type of solvent, and Plant part used as starting material.
3. Acetone extract of *M. longifolia* flower exhibits stronger inhibitory activity against the two studied fungi in comparison with Ethanol and methanol extracts.
4. The screening findings suggest to use *Mentha longifolia* crude extracts to control the spread of plant pathogenic fungi which are responsible for poor quality seeds in many crops

5. Acknowledgments

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