2011 International Conference on Environmental and Agriculture Engineering
IPCBEE vol.15(2011) © (2011) IACSIT Press, Singapore

Antifungal Activity of Spearmint (Mentha Spicata L.) Essential Oil on *Fusarium oxysporum f. sp. radicis-cucumerinum* the Causal Agent of Stem and Crown Rot of Greenhouse Cucumber in Yazd, Iran

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Abstract. Fusarium oxysporum f. sp. radicis-cucumerinum (Forc) induces root and crown rot of cucumber world wide. Fungicide applications are often needed to control this pathogen. Natural metabolites do not have any indiscriminate hazardous effects like synthetic fungicides. In this study, antifungal activity of 1, 3 and 5 µL of the essential oil of spearmint plants were studied on the mycelial growth of Fusarium oxysporum f. sp. radicis-cucumerinum using a paper disc method. Treated samples were kept at 25 °C and monitored every day up to eight days. All amounts of the essential oil restricted significantly the mycelial growth of the pathogen with maximum activity detected for samples treated with 5 µL of essential oil. The inhibitory effect of the essences was affected by the amounts of the essential oil and the incubation time of the samples. In the samples treated with 1 µL essential oil, the inhibitory effect decreased slightly during the incubation time. While in the samples treated with 3 and 5 µL, the maximum inhibition in the mycelial growth was observed after 2 and 3 days of incubation respectively. This study showed the antifungal activity of spearmint essential oil which can be used in control management of Forc. This is the fist study on Antifungal activity of Spearmint (*Mentha spicata* L.) essential oil on *Fusarium oxysporum* f. sp. *radicis-cucumerinum* the causal agent of stem and crown rot of greenhouse cucumber in Yazd, Iran.

Key words: Essential oil, Mentha, *Fusarium oxysporum,* Yazd

1. Introduction

Pesticide residues in agricultural products and the incidence of resistance in plant pathogens against chemical pesticides demonstrates, the need for non-chemical menthods including natural metabolites. Application of natural compounds on phytopathogens almost returns to the time the plant diseas were ascribed. However, the search for natural products with antimicrobial activity was accelerated from a few past decades.

Kurita *et al.* (1981) studied 40 plant compounds against seven species of fungi and found that some plant metabolites inhibited the growth of the fungi tested. In a similar study (Singh *etal.*1980), were examined five essential oils against 22 species of fungi.Muller-Rhebau *etal.* (1995) screened nine essential oils against four species of plant pathogenic fungi. Pawar *et al.* (2007) examined the effect of essential oils obtained from 75 different plants on *Alternaria porri* and *Fusarium oxysporum f.sp cicer.* This study showed that the most active essential oil found were those of lemongrass, clove, cinnamon bark, cinnamon leaf, cassia,n fennel, basil and evening primrose.

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Gulluce *et a. l*(2007) studied antimicrobial and antioxidant properties of the essential oils and methanol extract from *Mentha longifolia* L. ssp. *Longifolia* and the essential oil showed strong antimicrobial activity against all 30 microorganisms tested whereas the methanol extract almost remained inactive. In contrast, the extract showed much better activity than the essential oil in antioxidant activity assays employed, in the inhibition of free radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and b- carotene/ linoleic acid systems.

Hajlaoui *et al.* (2009) examined biological activities of the essential oils of two species of mentha and showed that essential oil of mentha have strong antimicrobial activite. Studies of Bang (2007) showed positive effect of essential oil of mentha on pathogen of potato. Also studies of Ramesh *et al.* (2006) on various antifungal properties of essential oil of *Mentha spicata* showed that mentha essential oil has fungitoxic effect.

Lamiaceae family consists of more than 4000 species in 200 genera. Many species within this family are medicinal plants that apply in human disease therapy as well as food in row and cooked forms. Many species of lamiaceae contain essential oils that showed biological activity on many fungal plant pathogens.

The Fusarium vascular wilt of cucumber is one of the most important diseases on greenhouse cucumber in Yazd province caused by *Fusarium oxysporum* f. sp. *radicis-cucumerinum* (Forc). The pathogen induces vascular wilt followed by root and crown rot and entire plant necrosis. Several fungicides are being used in cucumber grown in green houses against this disease in Iran. There is no information available about the effect of natural product to decrease the damage of this pathogen. This study aims to study the antifungal activity of essential oil of spearmint plants collected from Yazd province, Iran on Forc in vitro conditions.

2. Materials and methods:

2.1. Preparation of essential oil

Spearmint plants (*Mentha spicata)* were collected from medicinal plant farm of Yazd agricultural and natural resources research center in Mey 2010 and dried at room temperature (22-24°C). The essential oil was extracted from air-dried plant materials using a hydro-distillation device by essential oil maker apparatus in Yazd agricultural and Natural Resources Research Center. The essence was then kept at dark in 4° C until use.

2.2. Preparation of fungi

Fusarium oxysporum was isolated from stem of infected greenhouse cucumber and Stem tissues were surface sterilized in 96% ethanol. The skin was removed using a sterile scalpel and the xylem tissues (2-3 mm) were placed in Petri plates containing acidified PDA (pH 4.2-4.5).

After the plates were incubated at 25 °C for 4 days, hyphal tips were transferred to fresh Petri plates containing PDA or CMA and incubated at 25 °C for up to 4 days. The isolates were purified using either single spore or hyphal tip method (Singleton et al. 1992). The purified isolates were then used for pathogenicity tests.

2.3. Pathogenicity tests

For pathogenicity tests (Singleton *et al.* 1992), each isolate was grown on a double sterilized mixture of sand: perlite: grounded corn (3:2:1) in 250 mL Erlen Meyer flasks. These cultures were kept at room temperature under 12 h of fluorescent light for up to 5 weeks, and then used to inoculate a variety of cucumber named Soltan. Five grams of each inoculum was mixed with the pot soil when the third leaf of cucumber plants was emerging. The inoculated plants were kept at 25 °C for up to 4 weeks.

2.4. Measurment of antimicrobial activity

The effect of essential oil was assessed on the mycelia growth of *Forc* according to Boyraz and Ozcan (2006) with some changes. Five millimeter mycelia discs taken from the margins of a 7 days old culture were placed on the middle of a PDA plate and then 1,3 and 5 µl of essential oil were added on 5 mm sterile Watman No. 1 paper disc in middle of the each petri covered and rapidly putted on petri contain fungi. The petri plates were sealed with parafilm and incubated at 24 ^{0}c for 8 days. Control plates were treated with the same amount of sterile distilled water. Three replicates of each treatment were arranged according to

Completely Randomized Design (CRD), and the inhibition rate was calculated according to a formula developed by Deans and Svoboda (1990): I=C-T/C×1OO Where I= Percentage of mycelial inhibition, C=the mean of colony diameter of control set, T=the mean of colony diameter of treatment sets. The inhibition rate was measured as described above and subjected to analysis of variance using soft ware Spss17 and Excell2007. Comparison of means was performed by Duncan's multiple ranges test and least significant difference (LSD).

3. Results and Discussion

Fusarium oxysporum caused wilting and yellowing in pathogenicity tests and this is the first report of *Fusarium oxysporum* f. sp. *radicis-cucumerinum* agent root and crown rot of greenhouse cucumber in Yazd, Iran.

The inhibition percentage of essential oil taken from spearmint plants on the mycelia growth of Forc are shown in Table 1. The percentage of mycelium growth inhibition was variable in different days post-treatment. The percentage of growth inhibition was varied from 27.14% in seven day related with concentration 3µl until 65.59 related with concentration 5µl in four days that indicated rate of establishment of different concentration in different days.

All amounts of the essence restricted significantly the mycelial growth of the pathogen compared with the control with maximum activity detected for samples treated with 5 µL of the essence. There was no significant difference between 3 and 5 µL of the essence (Fig 1).

The antimicrobial effects of essential oils depend on the kind of compound and its chemical components. More than twenty kinds of chemical compounds have been reported from essential oil extracted from spearmint. The most important one with antimicrobial activity are Menthol, Menthoforan, Menthon, Pipriton and Polgon.

Additionally, it is known that the compositions antimicrobial effects depend on plant species and regional condition. Some research reported that there is a relationship between the chemical oils and the antimicrobial activity (Deans and Svoboda 1990).

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	Essential oil microliters /Petri dish		
Days	1µl	3µl	5µl
1	37.93	48.28	48.28
2	37.93	50.00	65.52
3	36.25	40.00	63.75
4	36.56	43.01	65.59
5	36.17	42.55	64.89
6	37.17	37.17	49.56
7	27.86	27.14	37.14
8	31.55	33.01	34.95

Table1: Perecentage of growth inhibition of *Fusarium oxysporum* f. sp. *radicis-cucumerinum* treated by Mentha essential oil.

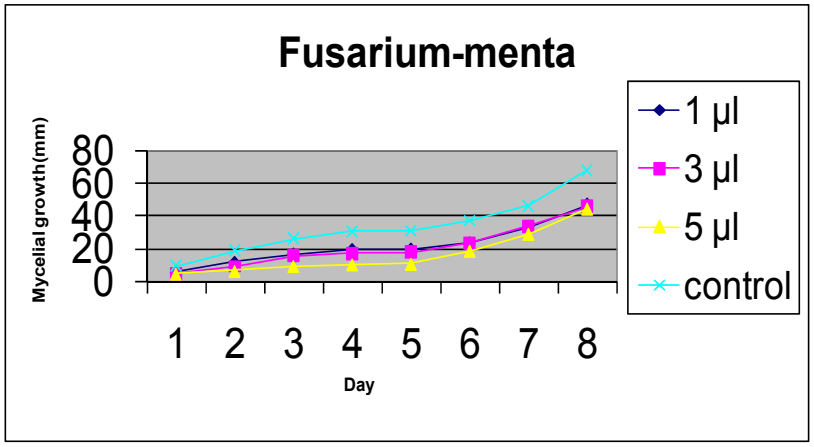

Fig1. Inhibition effect of mentha essential oil on *Fusarium oxysporum* f. sp. *radicis-cucumerinum*

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