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A COMPARISON OF THE EFFECT ETHYL EXTRACT OF *MENTHE SPICATA* AND *EUCALYPTUSCAMALDULENSIS* AS A FUNGICIDE ON *FUSARIUMOXYSPORUM*

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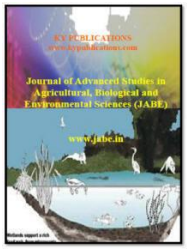

ABSTRACT

The present study aims to evaluate the antifungal activity of ethyl extract of *Menthaspicata* and *Eucalyptuscamaldulensis* as a fungicide on *Fusariumoxysporum*, which isolated from infected cucumbers, chili pepper and eggplant respectively. Three concentration (2, ,4, and 8 mg / ml) was prepared extract of *Menthaspicata*, and *Eucalyptuscamaldulensis* then tested against *F. oxysporum*, , by using food poisoning method and fungal growth inhibition percentage was calculated individually. Sobio-efficiency of fungi after treated with plants extract has studied such as radial mycelia growth. Results exhibited that *Eucalyptuscamaldulensis* crude extracted at 8% concentration had inhibitory growth\actions 100% against *Fusariumoxysporum* . Compared to *M. spicata*crude exhibited that extracted at 8% had inhibitory growth\actions 77.90% the Results has get demonstrated the fungus growth inhibition percentage increases with increasing concentration of ethyl extract. *Fusariumoxysporum* an inhibition of percentage rate was the highest by *Eucalyptuscamaldulensis* extract 30.33at 2% and 57.90 at 4% concentrations, while at 8% concentrations completely, an inhibitory mycelium growth of all Fungus.

Keywords: antifungal activity, ethyl extracts, *Menthaspicata*, *Eucalyptuscamaldulensis Fusariumoxysporum*,

1. INTRODUCTION

Plant diseases are the main cause of the destruction of natural resources in agriculture. Specifically, the plant pathogenic fungi are considered the most damage to agricultural crops because of the high virulence thus,

the main factor to huge economic losses. The dispersal of many phytopathogenic fungi, such as *Phythium*, *Botrytis*, *Rhizoctonia, Phytophthora* and *Fusarium*, have extended during the previous few years because of changes which have introduced in farming, with damaging effects upon crops of economic importance. In addition, not solely growing crops but, stored fruits are prey to fungal infections (Pandya *et al*., 2010).

The intensive and indiscriminate use of pesticides in agriculture has caused many problems to the environment such as water, soil, animals and food contamination; poisoning of farmers; elimination of non-target organisms; and selection of phytopathogens, pest and weed insensitive to certain active ingredients (*Saeed at al.,*2016). That aiming to minimize the negative effects of pesticides are been development the alternative control of plant disease, which includes the biological control, the induction of resistance and the

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use of natural products with induction of resistance and/or with direct antimicrobialactivities. In the latter include the use of extracts and an essential oils from medicinal plants (Rowaished and Moniam, 2006)

The biological control is defined as the use of antagonistic organisms for the control of microorganisms, reducing the amount of inoculum that determines the extent of disease (Cook and Baker,1982).

One of the most encouraging intends to accomplish this objective is by the utilization of integrated disease management which based on the natural plant products which derived from plants to control fungi that cause plant diseases, and these compounds is called botanical pesticides which are biodegradable faster than chemical pesticides which can degrade within a few days, and sometimes within a few hours, as well as most of them are selective against certain pathogenic fungi for the plant more than chemical pesticides, thus are considered to be eco-friendly (Sanjay and Tiku, 2009). Therefore, the study has aimed to evaluate the effect of three concentrations of ethyl extract of *Menthe spicata* and *Eucalyptus*in the inhibition of phytopathogens growth of *Fusariumoxysporum*,

MATERIALS & METHODS

Isolation and identification: A total of 200 samples of infected plants were collected from greenhouses in Baghdad. They were distributed as follows: 100 samples of cucumber plants, 50 samples of chili pepper plants and 50 samples eggplant plants. The infected plants were placed in polyethylene bags marked in the collection area, the glass house number and date of collection. After that, the infected plants were carried to the laboratory to isolate and diagnose the pathogen. *Fusariumoxysporum,*isolated from the infected plants and died seedlings (cucumbers, chili pepper and eggplant respectively). Infected plants and died seedlings were placed under a running water tap for one hour individually, in order to clean them from sticking soil. After that the roots and crown district were sliced to little pieces (5mm) and its surface was sanitized by drenching in sodium hypochlorite (1% free chlorine) for 2min, flushed with sterile distilled water, And then placed in PDA plates and incubated for 4 days at 27±2 Cº. The isolates were diagnosed as *F. oxysporum,* according to the morphological characteristic (Watanabe, 2002). All fungal isolates were cultured and maintained on autoclaved PDA supplemented with 100mg of chloramphenicol and incubated for 7days at 27 ±2C˚. All

Petri dishes used in the experiments had a diameter of 90 mm, were filled with 20 ml PDA, and were singly sealed with Parafilm, also slant culture were prepared and are all preserved in a refrigerator at 4C˚ (Burmeister, 2008).

Preparation of plant extracts:

Crude extract: According to,(Deshmurkh and Borle.1975) 10g of dried leaves and stems of *Menthaspicata*successively extracted in a soxhlet extraction for 24 hours with the solvent (200 ml of 80% ethanol), And then extracted was placed in the oven at 50C° for two days in order to remove the solvent,Then extract the remaining were scraped and saved in refrigerator until utilize

Evaluation of anti-fungal activity of the plant extracts: Food poisoning technique was used to determine inhibitory concentration (IC) {anti-fungal activity} of the studied plant extracts for plant pathogens *Fusariumoxysporum,* based on (Wang *et al*., 2005) as follows: Various volumes of the crude extract were prepared and each of these volumes was mixed apart with 100 ml of autoclaved PDA (Potato Dextrose Agar) when the temperature of autoclaved PDA temperature becomes approximately 45C° under sterile conditions (inside hood), so as to obtain the following concentrations of each extract (0,2, 4,8%), then the mixture was

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shaken well and poured into sterilized petri dishes (90mm) and left to solidify in a sterile conditions. Mycelial plugs 5mm were cut from the growing margin of four days old cultures *Fusariumoxysporum,* (which was the most virulence) and were transferred to the center of PDA plate containing plant extracts concentration (0,2, 4, and 8%) (v/v) individually, with three replicates per concentration and control. Radial mycelia growth rate of the assay fungus was measured after 7days 27 ±2Cº, and the effect of each toxic extracts was expressed as percent inhibition of radial mycelia growth (growth inhibition %) as follows(Burmeister, 2008).

Growth inhibition% = [[Growth in control - Growth in treatment] /Growth in control] × 100

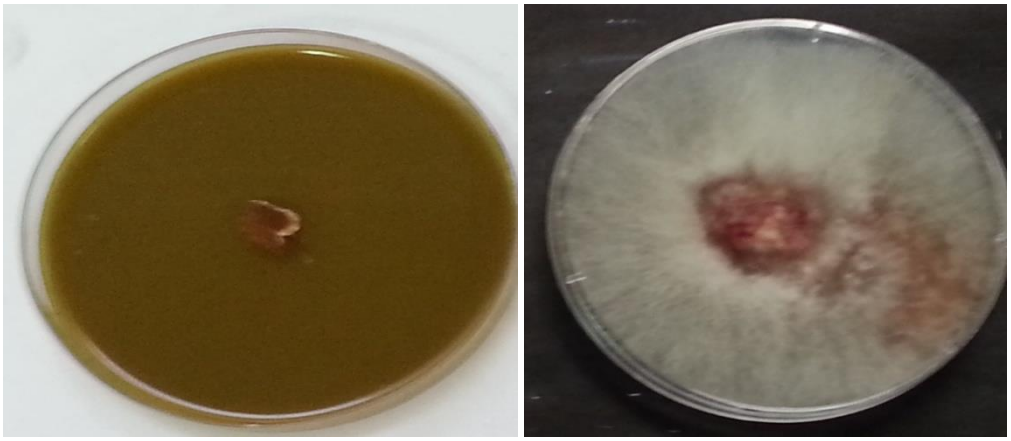
Results and Discussion:

Table 1- Percentage of growth inhibition of *Fusariumoxysporum*by using extracted from *Eucalyptus camaldulensis*at 2, 4 and 8 % concentration

Fungus	*E. camaldulensis*		
	2%	4%	8%
F. oxysporum	30.33	57.90	100

Results showed that percentage of growth inhibition varied according the concentrations which were used in this study. Plant extracts showed inhibitory action against *F. oxysporum*at all concentrations.

Results in the table-1 revealed that concentration at 8% con. was significantly better than others in reduction growth of *F. oxysporum*which recorded 100 %, while *Menthe spicata*. at 8 % was significantly less than others recorded 77.90 % only.



F.CF.E

Figure - Effect of plant extract on Fungi at 8% con.

E=*Eucalyptus*, F=*Fusarium*, C= control.

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Table 2- Percentage of growth inhibition of *Fusariumoxysporum*by using extracted from *Menthaspicata*at 2, 4 and 8 % concentration

Fungus	*Menthaspicata*		
	2%	4%	8%
F. oxysporum	15.55	30.33	77.90

Results in the table-2 revealed that concentration at 8% con. was significantly better than others in reduction growth of *F. oxysporum*which recorded 77.90 %, while. at 2% was significantly less than others recorded 15.55 % only.

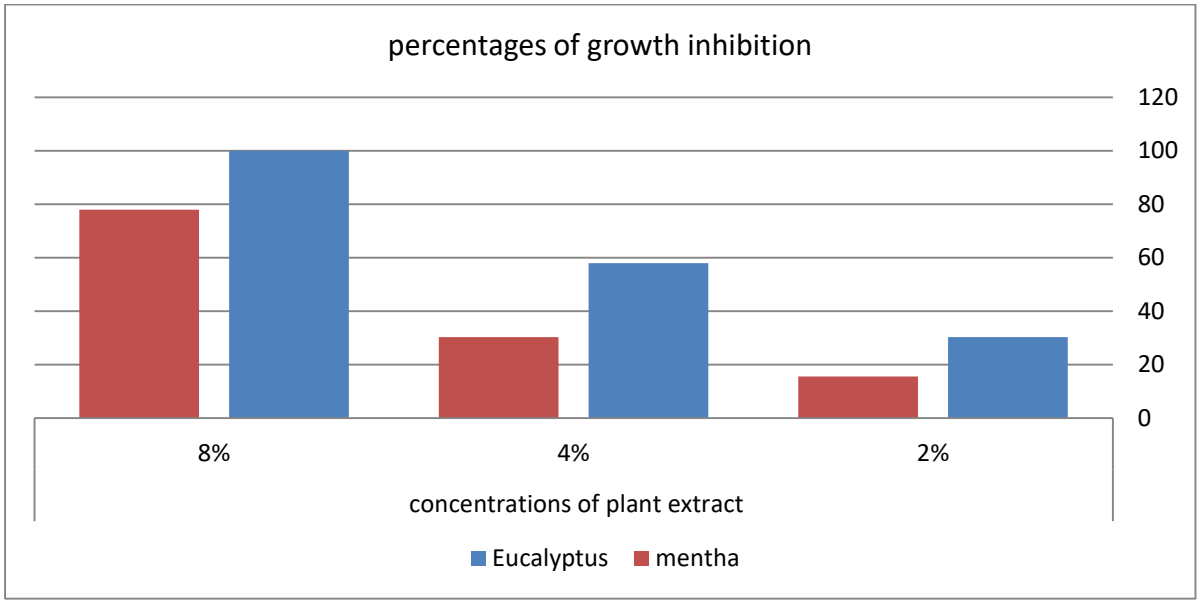

Figure 1: Effect of Ethyl Extract of *Menthaspicata* and*Eucalyptuscamaldulensis* on fungal growth

In other study the effect of *Eucalptus* at 5 % con. on growth of *F. oxysporum*found that inhibition percentage was 100 % that mean Approaching with my results (Ali *et al*.,2013).The inhibitory actions due to crude extract are the additive or synergistic effect of the mixtures (Yazdani*et al*., 2011). In other words, theplantcontainsmore than oneactive compoundseffect together as synergisticallyand complementary against pathogens fungi, this character is notincluded in themanufacturermaterials (Al-Daami, 2001).thescompounds disrupting the lipid-protein interface or by the denaturation of proteins and inactivation of enzymes in the pathogens In addition to inhibition of active transport and Loss of metabolism due to membrane damage(Kaur *et al*., 2011).Treatment with ethanolic extract caused irregular branching in the apical region, loss of linearity, with the appearance of barrel-like structures followed by extrusion of amorphous fibrillar substance (Vijayarathna *et al*., 2012).so the abnormalities observed at this region after treatment suggests the inhibition of fungal growth. The changes in the wall surface indicate an alteration in the normal assembly of the wall components, leading to their incorrect arrangement at the apical dome. (Latha, *et al*., 2011) Also caused degeneration of fungal hyphae, and hyphae appeared empty of cytoplasmic content(Zambonelli, , *et al*., 1996)also induce its fungicidal activity by targeting cell membranes. Since the regulation ofthe main metabolic

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systems depends on the integrity of all the cell organelle membranes, it isclear that the presence of compounds which affect cell membranes may interfere with manybiological processes; in particular, any alteration of the mitochondrial membrane system canreduce energy turn over inside the cell and can result in premature senescence (Afifi, H. 2012). affect the activities of membrane enzymes and interfere with respiratory pathways (Akhgari, *et al*., 2014) More these crude extract compounds effect on fungi is according to concentration. At low concentrations, affect the enzymatic activity but at higher concentrations, they cause protein denaturation (Prindle and Wright 1977).

CONCLUSION

Eucalyptuscamaldulensis contains more active compounds than other plants that can be used as a fungicide against plant pathogenic Fungi

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