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Antifungal Activity Of Leaf Extract Of Ceratonia Siliqua L. Plant Against Plant Pathogenic Fungi

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Abstract
Antifungal substances obtained from naturally grown plants are among the preferred products because they have a sensitive effect on the environment and on humans. In this study, the antifungal activity of the methanol extract acquired from leaves of *Ceratonia siliqua* plant collected from Demre district of Antalya province was determined. Activity studies have been carried out pathogens of *Fusarium oxysproum f.sp. cucumerium* (FOC) and *Monillia fructigena* plants which cause disease in cucumber and apple plants. Doses of 0.1, 0.5, 1, 2, 5 mg/ml of extract were applied against plant pathogens. Activity studies of test pathogens were carried out using the agar plate method. Mycelial growth, Mycelial growth inhibition, and Lethal dose values (LD_{50-90}) were determined in the experiments for fungi against extracts. *M. fructigena* is more susceptible to the FOC pathogen. MGI ratios of 20% at 0.1 mg/ml and 92% at 5 mg/ml against *M. fuctictigena* were observed. Similarly, these ratios were found at 0.1 mg and 0.5 mg/ml at FOC as 11% and %79 respectively. Lethal dose rates were calculated for LD_{50} = 0.74 mg/ml and LD_{90} = 7.082 for *M. fructigena* and LD_{50} = 1.41 mg/ml and LD_{90} = 19.83 for FOC. According to these results, it has been determined that the extract of the *C. siliqua* has a high level of biological activity against the tested pathogens. Effective results of bio-antifungal substances obtained from the nature are also observed in this study.
Keywords: Antifungal activity, Plant extract, *Ceratonia siliqua*, *Fusarium oxysproum f.sp. cucumerium*, *Monillia fructigena*

INTRODUCTION

Vegetables and fruits are important sources of many nutrients for human and the major consumed products all around the world. Many biotic factors such as bacteria, virus, fungi, insects and nematodes which play major role reducing yield of vegetables and fruits. *Fusarium oxysproum f.sp. cucumerium and Monillia fructigena* are important plant diseases that cause important yield loss in our country and in the world. Fusarium wilt in the cucumber is a pathogen that causes serious economic losses by *F. oxysporum f. sp. cucumerium* in many parts of the World [1]. *Monillia fructigena* that causes brown fruit tissue mildew of different apple cultivars [2].

Synthetically produced pesticides are used to control these diseases. Consequently, using pesticides negative effect on nature and environment and directly affects people [3]. For these reasons, several researchers were conducted to determine alternative methods against plant diseases. One of the effective methods is to use plant extracts which incorporating natural antifungal substance.

The aim of this study was to determine alternative control methods for important plant diseases such as *Fusarium oxysproum f.sp. cucumerium and Monillia fructigena* by plant extracts. For this purpose, the methanol extracts of *Ceratonia siliqua* plant tested and their antifungal activities were determined.

MATERIAL AND METHOD

Plant material

Leaf and fruit parts of *Ceratonia siliqua* were collected from Demre district of Antalya province in Turkey. The collected plants were washed twice and dried in the shade at 25±2 °C.

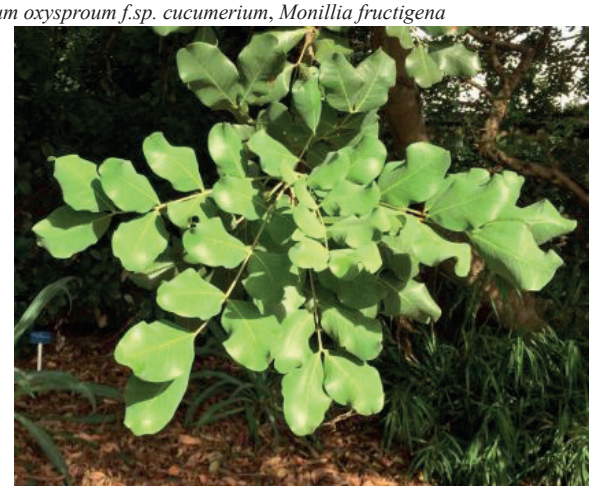

Figure 1. *Ceratonia siliqua leaf parts [4]*

Figure 2. *Ceratonia siliqua fruit parts [5]*

Fungi culture

Fungal cultures have been isolated from different host plants of *Fusarium oxysporum f. sp. cucumerium* (the cause

of fusarium wilt in cucumber) and *Monilinia fructigena* (the cause of brown rot in apple).

Figure 3. *Fusarium oxysporum f. sp. Cucumerium [6]*

Figure 4. *Monilinia fructigena [7]*

Plant extracts

Powdered plant materials (each one was 100 g) were extracted with ethanol by incubated orbital shaker at 120 rpm for 72 h (30ºC). After that it was evaporated to dryness in a rotary evaporator. The concentrate was then diluted with 50% Acetone. Each plant extract was used at 0.1, 0.5, 1, 2 and 5 mg/ml doses [8].

Antifungal activity

The antifungal activities of the plant extracts were determined by agar plate method [9]. Plant extracts were added to PDA at 40 ºC to give the concentration of 0.1, 0.5, 1, 2 and 5 mg/ml for each extract and then the PDA with extracts were poured (~10 ml/plate^{-1}) each alone in petri plates (60mm in diameter). Seven-day-old agar discs (5mm in diameter) bearing the desired fungus growth was transferred in the petri plates. These fungus cultures were incubated at 25±2 ºC for 7 days. Fungus growths were recorded daily. Commercial fungicide [Thiram 80%] was used as a positive control and 10% acetone was used as a negative control. Experiment set up 4 replications and repeated twice [10].

The percentage of mycelial growth inhibition was calculated accordingly the formula mentioned by Pandey et al., 1982 [11].

$$I=100\times(dc-dt)/dc$$

I; Mycelial growth inhibition

dc; Is the mycelial growth in control

dt; Is the mycelial growth in treatment

Statistical Analysis

The data were analyses using Analysis of Variance (ANOVA) test. Differences between means were determined by the TUKEY test (at the 0.05 probability level). The software SPSS 13.0 was used to conduct all the statistical analysis. LD doses were calculated by POLO 1.0 (LeOra software).

RESULT AND DISCUSSION

Plant disease and pest cause important yield loss on crop production all around the world. Unconsciously used pesticides are a negative impact on agricultural products, environment and human health. For this reason, alternative pest and disease control methods are needed to develop for minimize the use of chemical pesticides, environmentally friendly and harmless to human and non-target organisms.

In this study, antifungal activities of ethanol extracts were determined obtained from leaf parts of *Ceratonia siliqua.* Experiment of plant extracts were conducted by agar plate cultures and identifying antifungal activities. The methanol extracts of *Ceratonia siliqua* leaf was tested against *F. oxysporum f.sp.cucumerium* (FOL) and *Monillia fructigena* (Mf). Antifugal activity was observed against Foc and Mf plant pathogens. All plant extract were showed activity depending on dose increasing. When compared to the negative control, the average upper and lower bound mycelium growth for leaf extracts of FOC was found to be from 53.46 to 12.74 mm. These rates were observed for Mf from 47.75 to 4.80 mm (Figure 5). Similar studies showed that, antimicrobial and cytotoxic activities of n-hexane, methanol, ethanol, ethyl acetate and water extracts were determined obtained from leaf of *Ceratonia silique*. In this study, antimicrobial activities of the extracts were reported against 10 different bacteria and *Candida albicans* yeast-like fungi by the disc diffusion method. Ethanol, methanol and water extracts inhibited the growth of *C. albicans* (8mm inhibition zone in all) but n-hexane and ethyl acetate extracts had no effect on the growth of *C. albicans* [12]. And one another study showed that the powders extracts *Asteriscus graveolens, Bubonium odorum, Ighermia pinifolia, Inula viscosa, Halimium umbellatum, Hammada scoparia, Rubus ulmifolius, Sanguisorba minor* and *Ceratonia siliqua* were active against *Penicillium italicum,* about 75% greater than the inhibition of mycelial growth [13].

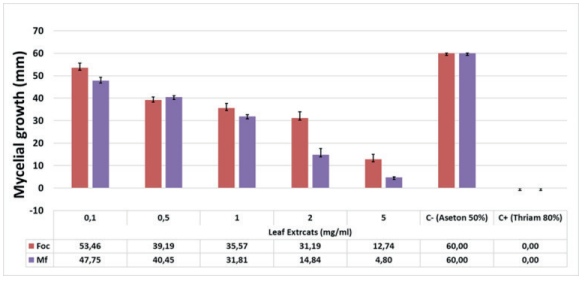

Figure 5. Antifungal activity of plant extracts against test fungi

Mycelial growth inhibition ratios of 20% at 0.1 mg/ml and 92% at 5 mg/ml against *M. fuctictigena* were observed. Similarly, these ratios were found at 0.1 mg and 0.5 mg/ml at FOC as 11% and %79 respectively. In a different study showed that the powders and aqueous extracts of 43 plant species were determined antifungal activities against *Geotrichum candidum*. Powders of *Ceratonia siliqua* and *Halimium umbellatum* completely inhibited (%100) mycelium growth of *G. candidum*. The activity of plant aqueous extracts on spore germination ***varied significantly between tested plants*** [14].

Table.1: Mycelium growth inhibition of plant pathogens against plant extracts

Plant parts	Doses (mg/ml)	Plant pathogens	
		S.s	FOC
Leaf extract	0.1	11	20
	0.5	35	33
	1	41	47
	2	48	75
	5	79	92
C-	Acetone 50%	0	0
C+	Thriam 80%	100	100

Table 2: Lethal doses of plant extract against test fungi

Plant parts	LD values	Plant pathogens	
		FOC	Mf
Leaf extract	LD_{50} (mg/ml)	1.41	0.74
	LD_{90} (mg/ml)	19.93	7.08
	Slope	1.114±0.088	1.302±0.088
	Heterogeneity	1.13	2.26

According to the dose-effect experiments, the LD_{50-90} values of leaf extracts were found 1.41 and 19.93 mg/ml for Foc; 0.74 and 7.08 mg/ml for Mf, respectively.

CONCLUSION

According to these results, it has been determined that the extract of the *C. siliqua* has a high level of biological activity against the tested pathogens. As a result, the plant extracts which used in our study was showed a different level of antifungal activities in a dose depend manner. The extracts determined activities showed that can be used as bio pesticides.

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