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In Vitro Inhibitory Effect of Some Plant Extracts Against *Fusarium culmorum* (W.G. Smith) Causal Agent of Foot and Root Rot Disease on Wheat*

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Abstract

Investigations on alternative control methods to chemicals including usage of plant extract for plant fungal disease take important place in current researches. Plant extracts are generally deemed to be less hazardous than synthetic compounds and could be alternative to antifungal treatments.

In this study, the purpose was screening of some plant extracts against *Fusarium culmorum* causing foot and root rot on different small-grain cereals, in particular wheat and barley. In total 6 plant species including levander (*Lavandula angustifolia)*, common mullein (*Verbascum thapsus*), common sorrel (*Rumex acetosa*), wood avens (*Geum urbanum*), poison hemlock (*Conium maculatum*), henbane (*Hyoscyamus niger*) consisted the materials in the study. The antifungal effects of three different concentrations (20%, 40%, and 80%) of each extracted plant, obtained from dried plant parts in methanol, were evaluated on growth of *F. culmorum* by dual test technic on PDA (Potato Dextrose Agar).

The maximum *in vitro* inhibitory efficiencies were obtained from common mullein (*Verbascum thapsus*) at concentrations of 80% and 40% with the ratios of 46.66% and 33.33% respectively, while no repressive activity exhibited by other plant extracts on pathogen growth on PDA.

Keywords: Plant extracts, *Fusarium culmorum, in vitro,* fungistatic activity

Buğdayda Kök ve Kök Boğazı Çürüklük Etmeni *Fusarium Culmorum* (W. G. Smith)'a Karşı Bazı Bitki Ekstraktlarının *In Vitro* İnhibitör Etkileri

Öz

Son yıllarda, bitki fungal hastalıklarına karşı kimyasallara alternatif mücadele yöntemleri üzerine yapılan araştırmalarda bitki ekstraktları önemli yer tutmaktadır. Bitki ekstraktlarının sentetik bileşiklere göre daha az zararlı olduğu bilinmekte ve potansiyel bir antifungal tedavi yöntemi olarak değerlendirilmektedir.

Çalışmanın amacı, başta buğday ve arpa olmak üzere küçük-daneli tahılların kök ve kök boğazı çürüklük etmeni *Fusarium culmorum*'a karşı bazı bitki ekstraktlarının engelleyici etkilerinin belirlenmesidir. Çalışmanın materyali olarak Lavanta (*Lavandula angustifolia)*, sığırkuyruğu (*Verbascum thapsus*), labada (*Rumex acetosa*), meryem otu (*Geum urbanum*), baldıran (*Conium maculatum*), banotu (*Hyoscyamus niger*) olmak üzere toplam 6 bitki türü kullanılmıştır. Kurutulmuş bitki parçalarından metanolde ektraktlar elde edilmiş ve her bitki ekstraktının 3 farklı konsantrasyonunun (%20, %40 ve %80) *F. culmorum'*un PDA (Potato Dextrose Agar) üzerindeki gelişmine etkisi ikili kültür tekniği ile belirlenmiştir.

En yüksek inhibitor etkisi sığırkuyruğu ekstraktının %80 ve %40 oranındaki konsantrasyonlarından elde edilirken, diğer ekstraktların hiçbirisi PDA üzerinde fungusun gelişimi üzerinde engelleyici bir etki göstermemiştir.

Anahtar Kelimeler: Bitki ekstraktları, *Fusarium culmorum, in vitro,* fungistatik aktivite

Introduction

Wheat production is affected by many of plant pathogenic organisms including fungi. *Fusarium culmorum* is a ubiquitous soil-borne fungus able to cause foot and root rot on different small-grain cereals, in particular wheat and barley. It causes significant yield and quality losses. Foot and root rot pathogen *Fusarium culmorum* (W.G. Smith) Sacc. also known as Fusarium crown rot causes some typical symptoms such a seedling blight with death of the plant before or after emergence, brown discoloration on roots and coleoptiles of the infected seedlings, brown discoloration on sub-crown internodes and on the first two/three internodes of the main stem, tiller abortion, formation of whiteheads with shriveled white grains. For controlling such plant diseases, application of fungicides is inadequate in some cases besides their hazardous effects for environment and living organisms.

Growth of fungal pathogens has led to considerable economic losses in agricultural crop. As a solution, synthetic fungicides have been used globally since the 1950s to protect major crops from damage by phytopathogenic fungi (Knight et al., 1997; Leroux et al., 2010). As an alternative solution to chemicals, plant extracts are generally deemed to be less hazardous than synthetic compounds and could be appropriate antifungal treatments. Antifungal activity of some isolated principles from plant extracts may be more effective than some commercial synthetic fungicides. The presence of naturally occurring substances in plants with anti-microbial properties have been recognized and tested against a wide range of pathogenic microbes (Tamuli et al., 2014). With the increase of interest in antibiotics plants as a source of potential antimicrobial substances are receiving considerable attention throughout the world. Recently many aqueous plant extracts have been shown to have inhibitory action against some plant as well as human pathogenic microbes. Nowadays some synthetic as well as semi-synthetic antimicrobial agents have been developing, among which very few have broad spectrum activity and most of them are environmentally hazardous in nature. The extensive use of agrochemicals especially fungicides, resulted more carcinogenic risk than other pesticides which may give rise to undesirable biological effects on animals and human beings (Osman and Abdulrahman, 2003).

Many reports are available on the effects of plants on fungal growth and mycotoxin production: thyme, sage, origano, coriander (Yıldız et al., 2001; Yanar, 2014; Mirik and Aysan, 2005), clove (Hitokoto et al., 1980), cinnamon, rosemary, lavender (Bishop and Thornton, 1997; Erdoğan et al., 2014), cumin, pepper (Abdou et al., 1972), garlic, onion (Yıldız et al., 2001; Gandhi and Ghodekar, 1988), basil, saffron, marjoram and anise (Hitokoto et al., 1980), mentha (Erdoğan et al., 2014) are examples. In this study, it was aimed to assess *in vitro* inhibitor activities of 6 plant species including levander (*Lavandula angustifolia*), common mullein (*Verbascum thapsus*), common sorrel (*Rumex acetosa*), wood avens (*Geum urbanum*), poison hemlock (*Conium maculatum*), henbane (*Hyoscyamus niger*), eucalyptus against foot and root rot pathogen *Fusarium culmorum*.

Material and Method

Plant Materials and Pathogen

The leaves, seeds and stem parts of selected plant species including levander (*Lavandula angustifolia)*, common mullein (*Verbascum thapsus*), common sorrel (*Rumex acetosa*), wood avens (*Geum urbanum*), poison hemlock (*Conium maculatum*), henbane (*Hyoscyamus niger*) collected from Konya-Turkey, dried at room temperature (25-30 ºC). The pathogen isolate was obtained from of Prof. Dr. Berna Tunalı (19 Mayıs University, Samsun)'s culture collection.

Preparation of The Extracts from Plant Materials

The extraction of plant materials was made according to the methods defined by Tavares et al. (2009). The dried plant parts were grounded into powder form and packaged stored in polyethylene bags at room temperature until needed. 50 g of each powdered plant sample was weighed and added into a bottle containing 500 ml of methanol (Merck 99.5%). The bottles were closed tightly and incubated at room temperature for 7 days. During incubation period, the bottles were shaken twice a day. After incubation period, each suspension was filtered individually (Whatman Filter Paper No:1), and extracted liquids was transferred to covered glass bottles. The methanol of these extractions was evaporated by vacuum Rotary Evaporator (Heidolphe-VAP Precision) at 42±2 °C. The extractions were kept in water bath at 42 ºC for a day to evaporate the methanol completely. The pure plant extracts then filter sterilized and were transferred to sterile flacon tubes in amber color to avoid from light and kept at +4 ºC to use when needed. The extracts were diluted with methanol (99.5%) at desired volume (v/v%).

In Vitro Inhibitory Activities of Plant Extracts on Fusarium Culmorum

Three concentrations (20%, 40%, and 80%, v/v %) of each extract were included in the study to determine their inhibitor efficiencies against *F. culmorum* growth on PDA. In order to have pathogen inoculum, *F. culmorum* was grown on PDA for a week.

In order to determine the efficiency of extracts, dual test technique was applied (Ulke, 2003). A sterilized filter paper in 0.7 cm diameter was placed on PDA petri dishes as 3 cm from the center and 50µl of extract was soaked. A pathogen disc freshly grown (7 days old) on PDA was placed on opposite of the extract disc as 3 cm from the center in the same petri dish. The petri dishes were sealed with Parafilm. The experiments were carried out in 3 replicates per treatment. As control of the experiment, only sterile distilled water was used instead of extract treatment. Fungal growth was observed and recorded after 7 days incubation at 25±1 ºC. Inhibition percentage of fungal growth was calculated by using the following formula (Bhuyan et al., 2015);

$$\%Inhibition = \frac{100\ (Control - Treatment)}{Control}$$

Results and Discussion

As seen in Table 1, among the extracts the best inhibitory effects (46.66% and 33.33%) were obtained from common mullein (*Verbascum thapsus*) at concentrations of 80% and 40% (v/v) respectively (Figure 1) while no efficiency was observed at concentration of 20% (v/v). It was observed that inhibition percentage of the extract increased with the increase in concentration (Table 1). Besides, none of other extracts did exhibit any *in vitro* inhibitory activity against growth of *F. culmorum* on PDA.

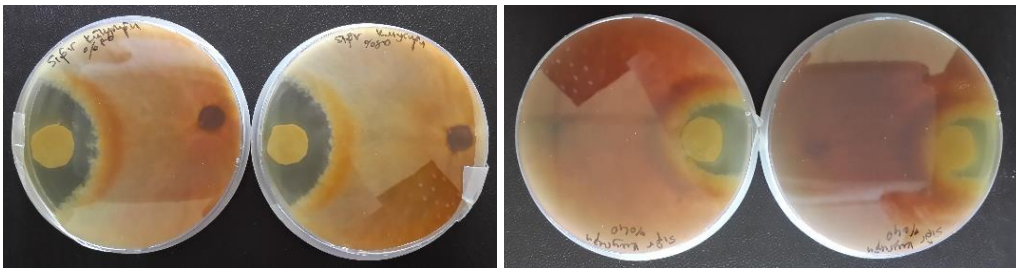

Figure 1. The in vitro efficacies of common mullein extracts at of 80% (left) and 40% (right) (v/v) concentration

Table 1. The inhibitory effects (inhibition percentage) of the extracts on growth of Fusarium culmorum on 7th days of inoculation

Plants extracts	Extract Concentration v/v (%)	Means of Inhibition Zones (cm)	Inhibitory Efficiency (%)
Common mullein	**80%**	**4.2**	**46.66**
(Verbascum thapsus)	**40%**	**3.0**	**33.33**
	20%	0.0	0.00
Levander	80%	0.0	0.00
(*Lavandula angustifolia)*	40%	0.0	0.00
	20%	0.0	0.00
Common sorrel	80%	0.0	0.00
(Rumex acetosa)	40%	0.0	0.00
	20%	0.0	0.00
Wood avens	80%	0.0	0.00
(Geum urbanum)	40%	0.0	0.00
	20%	0.0	0.00
Poison hemlock	80%	0.0	0.00
(Conium maculatum)	40%	0.0	0.00
	20%	0.0	0.00
Henbane	80%	0.0	0.00
(Hyoscyamus niger)	40%	0.0	0.00
	20%	0.0	0.00
Control	**-**	**0.0**	**0.00**
(Sterile distilled water)			

The inhibition of the growth of the pathogenic fungi is due to the active ingredients in the plant (Shetty et al., 1989). Besides, Lubaina and Murugan (2013), indicated that the efficacy of medicinal plant extracts may induce a systemic resistance in host plants against pathogens. The extracts may also retard the development of infections at early growth stages by inhibiting the mycelial growth of pathogens (Krebs and Forrer, 2001), or the toxic effect of active principles may act directly on the pathogens (Amadioha, 2000).

In the present study, the common mullein extract showed a fine inhibition on the hyphal growth of soil-borne pathogenic fungi *F. culmorum* on PDA with increasing effectiveness depending on the concentrations. There is a number of pharmacological activities such as anti-inflammatory, antioxidant, anticancer, antimicrobial, antiviral, antihepatotoxic and anti-hyperlipidemic activity have been ascribed to this plant (Riaz et al., 2013). Dulger et al. (2015), investigated the antibacterial potential of the leaves of common mullein against the pathogens causing complicated urine tract infections. According to their results, the extract showed strong antimicrobial activity against *Escherichia coli*, *Enterococcus faecalis* and *Candida albicans*.

Many studies shows the success of common mullein in pharmacology, however, the number of studies with this plant on plant pathogens seems quite few. The findings reported by Turker and Camper (2002) stated that *Agrobacterium tumefaciens*-induced tumors in potato disc tissue were inhibited by the aforesaid plant extracts. Moreover, the researchers reported antibacterial activity of common mullein (especially the water extract) with *Klebsiella pneumonia*, *Staphylococcus aureus, Staphylococcus epidermidis* and *Escherichia coli*.

The present research are in line with the researches infer that leaf extracts in general have great potentiality in the control of fungal diseases in commercially important crop plants. Soil-borne pathogens such as *Fusarium culmorum* are quite difficult to manage due to there is no chemical to control the disease strictly. The disease may be managed economic and eco-friendly by using an effective plant extracts, since the plants are existing in nature as wild. More detailed studies, however, including characterization bioactive compounds, effect on plants *in vivo* and natural conditions, are needed to be accomplished with the promising plant extracts.

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