

Antifungal Activity of Some Plant Extracts against Different Plant Pathogenic Fungi

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Abstract—The negative environmental impact of pesticides used for pest and disease control is intensively increasing everyday. For this reason, alternative methods of reducing pesticide are being developed. One of the effective methods is to use plant extracts which incorporating natural antifungal substance. The aim of this study was to determine methanol extracts of different parts (leave, flower, root, fruit and shoots) of *Trachystemon orientalis*, *Smilax excelsa*, *Rhododendron ponticum*, *Phytolacca americana* and *Prunus laurocerasus*, which were collected from the Black Sea region of Turkey, against three economically important plant disease, *Alternaria solani*, *Botrytis cinerea* and *Rhizoctonia solani*. These diseases cause significant product losses on tomatoes, strawberry, potatoes and cucumber. Antifungal activity of plants extracts were determined by agar plate methods. Different dose of plant extracts (50, 100, 200 and 400 mg/ml) were applied against these plant pathogens and the value of mycelial growth inhibition (MGI) and mycelial growth (MG) were recorded. All plant species were showed that significantly distinguished antifungal activities. The highest MGI value (84 %) was recorded on leaf extract of *P. americana* against *B.cinerea*. The highest antifungal activities against all pathogens were observed doses of 400 mg/ml from *P. laurocerasus*, *T. orientalis*, *P americana*, *R. ponticum* and *S. excelsa* respectively. According to this result, plant extracts of natural antifungal substract can be used as a biological pesticide as an alternative management methods against plant diseases.

Keywords—Antifungal activities, plant extracts, plant diseases, Mycelial growth inhibition.

I. INTRODUCTION

THE rich content of antifungal substances in plants are being used biopesticide since up to the beginning of human civilization. Antifungal effects of plant and plant products emerge clearly every day. Antifungal substances which are obtained from plants have no side effect against environment thus, giving a significant advantage. Nowadays, a commercial pesticide used against plant diseases is found to cause damage to environment and human health. Because of that conducting a research of alternative control methods comes into prominence for minimizing used commercial pesticide. Research found that compounds in the structure of plants and essential oil were showed antifungal, antibacterial, insecticidal, nematocidal, herbicidal and antiviral activities [1-8].

Turkish plant fauna is very rich. Some plants are endemic as *Trachystemon orientalis* (L.) G. Don (Boraginaceae) is found only Black Sea Region in Turkey [9]. *T.orientalis* is 30-40 cm length, rhizomatous, latifolius and pilose, blue-violet flowers and herbaceous perennial plants [10-11]. *Smilax excelsa* L. (Smilacaceae) is up to 15 m length, spiny, climber woody plant [12]. *Rhododendron ponticum* (Ericaceae) is also only found Black Sea Region in Turkey, *Rhododendron* genus has 800 species [13]. *Phytolacca americana* is a herbaceous perennial plant, It has simple leaves on green to purplish stems. Fruits are dark reddish and blackish colour, and spheroidal shape [14]. *Prunus laurocerasus* L., (Rosaceae) is an evergreen shrub or medium-sized tree, flowers are creamy-white colour, and leaves are dark green, leathery, shiny, with a finely serrated margin [15].

A. solani, *B. cinerea* and *R. solani* are important plant diseases that causes significantly yield losses in our country and in the world. *A.solani* is very common diseases on tomatoes which are known early blight [16]. *B. cinerea* is caused significant fruit losses on strawberry plants [17]. *R. solani* is known wherever potatoes are grown and reducing yield and quality [18].

The aim of this study was to determine alternative methods for important plant diseases such as *Alternaria solani* (Ell. ve G. Martin), *Botrytis cinerea pers.*:Fr, and *Rhizoctonia solani* Kühn by plant extracts. For this purpose, the methanol extracts of five plants (*Trachystemon orientalis*, *Smilax excelsa*, *Rhododendron ponticum*, *Phytolacca americana* and *Prunus laurocerasus*) tested and their antifungal activities were determined.

II. MATERIAL AND METHODS

A. Plant Materials

Plant species were collected from Düzce, Giresun and Trabzon province in Turkey 2014 (Table 1). The plant parts were air-dried at room temperature for three weeks in dark conditions. The dried plant parts were milled to a fine powder in a mill.

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TABLE 1. LIST OF PLANT SPECIES

Scientific name	Family	Part used	Place of collection
<i>Trachystemon orientalis</i> L. G.Don	Boraginaceae	Leaf, Flower, Root	Düzce, Turkey
<i>Smilax excelsa</i> L.	Smilacaceae	Shoots	Düzce, Turkey
<i>Rhododendron ponticum</i> L.	Ericaceae	Leaf	Düzce, Turkey
<i>Phytolacca americana</i> L.	Phytolaccaceae	Leaf, Fruit	Giresun, Turkey
<i>Prunus laurocerasus</i> L.	Rosaceae	Leaf, Fruit	Trabzon, Turkey

B. Fungi Cultures

The plant pathogenic fungi (Table 2) used in the research were obtained from the stock cultures in laboratory of phytoclinic, Department of Plant Protection, Faculty of Agriculture, University of Ahi Evran University, Turkey. Plants pathogens were grown on Petri dishes (90 mm) containing 20 ml of PDA and incubated at 25±2°C for 7 days, these fungi cultures were used in study.

TABLE 2. LIST OF PLANT PATHOGENS

Plant pathogens	Origin	Place of Isolation
<i>Alternaria solani</i> (Ell. And G. Martin)	Tomato	Antalya, Turkey
<i>Botrytis cinerea pers.:Fr</i>	Strawberry	Antalya, Turkey
<i>Rhizoctonia solani</i> Kühn.	Potato	Antalya, Turkey

C. Plant Extracts

Powdered plant materials (each one was 100 g) were extracted with methanol by incubated orbital shaker (Lab. Corporation Group, Model-SI-300) at 120 rpm for 72 h (30°C). After that it was evaporated to dryness in a rotary evaporator (Heildolph Group, Model-Hei-Vap Presicion). The concentrate was then diluted with 50% Acetone. Each plant extract was used at 50, 100, 200 and, 400 mg/ml doses [19].

D. In vitro Antifungal Activity

The antifungal activities of the plant extracts were determined by agar plate method [20]. Plant extracts were added to PDA at 40 °C to give the concentration of 50, 100, 200 and 400 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 °C for 7 days. Fungus growths were recorded daily [1]. Commercial fungicide [Thiram 80% (Hektaş, group)] was used as a positive control and 50% acetone was used as a negative control. Experiment set up 4 replications and repeated twice.

The percentage of mycelial growth inhibition was calculated accordingly the formula mentioned by [21].

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

I; Mycelial growth inhibition

dc; Is the mycelial growth in control

dt; Is the mycelial growth in treatment

E. 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.

III. RESULTS AND DISCUSSION

In this study, the methanol extracts of *T. orientalis* [leaves (TL), flower (TF) and root (TR)], *S. excelsa* [shoot (SS)], *R. ponticum* [leaves (RL)], *P. americana* [leaves (PAL) and flower (PAF)] and *P. laurocerasus* [leaves (PLL) and fruit (PLF)] were tested. Experiment of plant extracts were conducted by agar plate cultures and identifying antifungal activities.

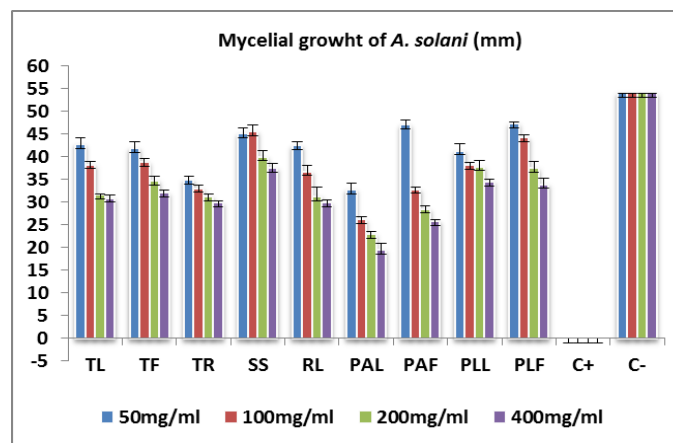


Figure 1. Mycelial growth of *Alternaria solani* against plant extracts

The methanol extracts of all plant parts displayed varied antifungal activity on *A.solani*, *B.cinerea* and *R.solani*. The developments of mycelium of all pathogens have been suppressed by compare with negative control. All plant extract were showed antifungal effect depending on dose increasing. The developments of mycelium in controls were found on *A.solani* and *B.cinerea* at 60 mm, and *R.solani* at 51 mm (Fig. 1, 2 and 3).

All the results of antifungal activities considered together; extracts of PAL, PLL and RL against *B. cinerea* were showed highly effective antifungal activities. In other extracts discernible effect was determined (Fig 2). The highest effect against *A.solani* was observed by extracts of PAL,TR, PAF and TL respectively. The extracts of RL, PAF and SS were displayed the highest effect against *R. solani*.

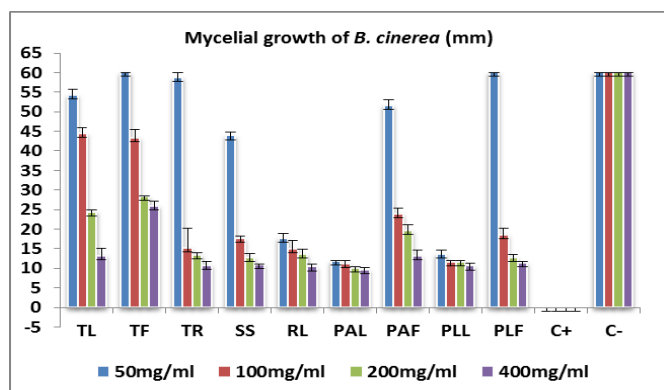


Figure 2. Mycelial growth of *Botrytis cinerea* against plant extracts

B. cinera has been observed the most sensitive diseases against plant extracts. This was followed by *A. solani* and *R. solani*. PLL has shown the highest antifungal effect from all doses against all pathogens. This was followed by the extracts of TF, PAF, TS, RF, PAF, TL, PLF and SS respectively (Fig. 1-3).

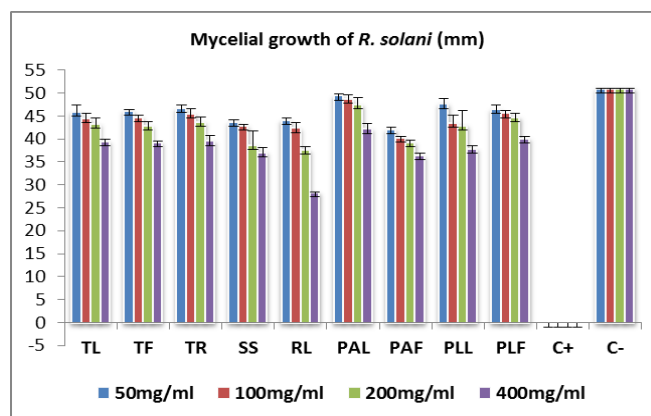


Figure 3. Mycelial growth of *Rhizoctonia solani* against plant extracts

According to the result of mycelium that percent of inhibition rates were found. These rates were displayed different level to compare the type of plants and parts used. Nine methanol extracts against each pathogen were found different level of inhibition mycelium rates (Table 1). Positive control showed that 100% mycelium grown inhibition. As the dose increases, the percent has been shown an increase in inhibition rates.

TABLE 3. MYCELIAL GROWTH INHIBITION VALUES (MGD) OF PLANT EXTRACTS AGAINST PLANT PATHOGENS

Plant Pathogens	Doses (mg/ml)	Plant extracts (%)									Controls	
		TL	TF	TR	SS	RL	PAL	PAF	PLL	PLF	C	C
<i>A. solani</i>	50	21	22	35	15	21	39	13	23	12	-	100
	100	29	28	39	16	32	52	39	29	18	-	100
	200	41	36	42	26	42	57	47	30	31	-	100
	400	43	41	45	30	45	64	52	36	37	-	100
<i>B. cinerea</i>	50	9	-	2	27	71	71	14	77	-	-	100
	100	26	28	75	71	75	75	60	81	69	-	100
	200	59	53	78	79	77	77	67	81	79	-	100
	400	78	57	82	82	83	84	77	82	81	-	100
<i>R. solani</i>	50	10	10	8	14	14	3	18	7	9	-	100
	100	13	12	11	16	18	4	21	15	11	-	100
	200	15	16	14	24	26	7	23	16	12	-	100
	400	23	24	23	27	44	17	29	26	21	-	100

For Figure 1-3 and Table 3. (-)= No antifungal activity; TL=Leaf extract of *T. orientalis*; TF=Flower extract of *T. orientalis*; TR=Root extract of *T. orientalis*; SS=Shoot extracts of *Smilax excelsa*; RF=Leaf extract of *Rhododendron ponticum*; PAL; Leaf extract of *Phytolacca americana*; PAF=Fruit extracts of *Phytolacca americana*; PLL= Leaf extract of *Liquidambar orientalis*; PLF= Fruit extract of *Prunus laurocerasus*. C-=Negative control (50% Asetone); C+=Positive control (80% Thiram).

Many articles were published about antifungal properties of target plant pathogenic fungi. Water extracts from leaves, root and flowers of *Trachystemon orientalis* has been reported having antifungal effect on *Ascochyta rabiei*, *Fusarium oxysporum* f. sp. *melonis*, *Fusarium oxysporum* f. sp. *radicis-lycopersici*, *Verticillium dahliae* and *Rhizoctonia solani*. The highest mycelium growth inhibitor showed flower water extracts. This was followed by leaves and root water extracts respectively. All doses of flower water extracts which used for *A. rabiei*, *V. dahliae* and *R. solani*, was completely stopped mycelium growth [1]. Another study was about *Rhododendron ponticum*, *Rhododendron luteum*, *Rhododendron smirnovii* and *Rhododendron caucasicum* displayed antifungal and antibacterial effects on pathogens. Leaves and flower extracts of *R. ponticum* were reported that highly antifungal and antibacterial properties. The highest antifungal effect against tested organisms was showed *R. ponticum* [22]. The methanol extracts of four plant species were tested against seven plant pathogens (*Magnaporthe oryzae*, *Rhizoctonia solani*, *Puccinia recondita*, *Blumeria graminis* f. sp. *hordei*, *Botrytis cinerea*,

Phytophthora infestans, *Colletotrichum capsici*) and displayed antifungal activities. In this study, the methanol extracts of *Phytolacca americana* showed different level of antifungal effect of the plant species used, against *Magnaporthe oryzae*, *Botrytis cinerea*, *Phytophthora infestans* under in vitro and in vivo conditions. In addition, methanol extract of *P. americana* at 3.000 ppm dose was showed antifungal effect on *Magnaporthe oryzae* (62,5%) , *Rhizoctonia solani* (26.7%), *Puccinia recondita* (26.7%), *Blumeria graminis f. sp. hordei* (0.0%), *Botrytis cinerea* (85%), *Phytophthora infestans* (82.1%) and *Colletotrichum capsici* (20%) [23]. In another study, methanol extracts from leaves and fruits of *Cornus mas*, *Morus nigra*, and *Prunus laurocerasus* were tested against *Gnomonia leptostyla* and has identified that showed antifungal activities. Mycelium inhibition was obtained 55.14 % and 49.06% at 750 mg dose from leaves extract and fruit extracts of *Prunus laurocerasus* respectively [24].

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 biopesticides. In addition, first time displayed antimicrobial properties of *Smilax excelsa* by this study.

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