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Antifungal Activity Against *Phytophthora capsici leon* Which Causes Root Neck Burn in Pepper Around Kahramanmaras

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In the present study effects of essential oil and extracts obtained from plants on *Phytophthora capsici leon* which is a pathogen fungus for pepper plants (*Capsicum annuum* L.) have been investigated. Essential oils and chloroform, methanol and hexane extracts of various plants including *Pistachia terebinthus* L., *Quercus infectoria* Oliv., *Pinus brutia* TEN., *Juniperus oxycedrus* L., *Cedrus libani* A.Rich., *Pinus nigra* L., *Abies cilicica* Carr., *Salvia aucheri* L., *Phlomis bourgei* Boiss., *Parmelia furfuracea* L., *Crocus chrysanthus* (Herb.) Herb., *Rumex scutatus* L., *Myrtus communis* L., *Eugenia caryophyllata* Thumb., *Smyrnium olusatrum* L., *Ajuga orientalis* L., *Astragalus schizopterus* Boiss., *Papaver hybridum* L. *Salvia multicaulis* Vahl., *Salvia viridis* L., *Satureja cilicica* P.H., *Satureja hortensis* L., have been used. It has been found that essential oils and extracts of some plants have repressive effect on growth of *Phytophthora capsici*.

Key Words: *Phytophthora capsici*, Antifungal activity, Essential oil.

INTRODUCTION

A number of plants in the flora of Turkey have been used as traditional medicines. However, most of them have not been evaluated scientifically and their effects have not yet explained experimentally. In this study, effects of the essential oil and extracts from different plant species were examined on patogen fungi *Phytophthora capsici*, which causes the common disease on root throat burn red pepper (*Capsicum annum* L.).

The pepper has an important role among garden agriculture products in Turkey. The red pepper is grown as a field agriculture product especially in Kahramanmaras, Gaziantep, Urfa, Diyarbakir, Adiyaman, Hatay in Turkey[1].

In recent years there are some diseases in plantation and product quality of red pepper that is economically and traditionally important. One of the causes of this disease is *Phytophthora capsici* which is a fungus and causes root neck burn disease[1-3]. Today, it is extremely difficult to find a solution to the fungal plant diseases which causes economic losses[4]. Yet, there is no cure especially for *Phytophthora capsici* that causes desiccation of the pepper seedlings.

Extensive use of pesticides, fertilizers and plant growth regulators in agricultural area results in pesticide residues on agricultural products. These residues cause harmful effects on soil, water, air and other living organisms. As far as possible, the aim is to keep away these side effects.

Various eradication methods against different pests which are disease factors have been developed for prevention of cultivated plants from diseases and pests thus to obtain quality and adequate product to feed the growing world population. The leading method amongst them is chemical warfare which followed by cultural and biological ones. Additionally, using substances which are obtained from natural plant extracts harmless for other livings and effective in increasing yield and resistivity of the soil is one of the main goals of biological warfare[5].

The pathogen fungus *Phytophthora capsici* L. causes crop loss in Turkey and other countries in which pepper (*Capsicum annuum* L.) is grown[6,7]. In the studies on *Phytophthora capsici*, it was demonstrated that essential oils of *Thymbra spicata* and *Satureja thymbra* were more effective from fungicides such as carbendazim and pentachlornitrobenzene for this fungus[8]. It was reported that taxol obtained from Yew trees was very effective on *Phytophthora capsici* L.[9,10].

This study determine the *in vitro* antifungal activity and investigated of biological warfare possibilities against *Phytophthora capsici* with antifungal effects of various plant extracts.

EXPERIMENTAL

The plants be tested were obtained from at or around Kahramanmaras. The plants used for preliminary work were *Pistachia terebinthus* L., *Quercus infectoria* Oliv., *Pinus brutia* TEN., *Juniperus oxycedrus* L., *Cedrus libani* A.Rich., *Pinus nigra* L., *Abies cilicica* Carr., *Salvia aucheri* L., *Phlomis bourgei* Boiss., *Parmelia furfuracea* L., *Crocus chrysanthus* (Herb.) Herb., *Rumex scutatus* L., *Myrtus communis* L., *Eugenia caryophyllata* Thumb., *Smyrnium olusatrum* L., *Ajuga orientalis* L., *Astragalus schizopterus* Boiss., *Papaver hybridum* L. *Salvia multicaulis* Vahl., *Salvia viridis* L., *Satureja cilicica* P.H., *Satureja hortensis* L.

***Phytophthora capsici* strain:** The fungus *Phytophthora capsici* L. that is used in this study was obtained from Kahramanmaras Sutcu Imam University, Agricultural Faculty. Culture mediums for fungus were prepared as methods defined in the literature[1,11].

Preparation of antibiotic discs from essential oils: The samples were brought to laboratory, identified and processed in sterile conditions. Essential oils of the plants were obtained by Neo-clevenger device. Structures of the plants such as leafs, trunks and seeds were used to extract essential oil[12,13]. The sterile antibiotic discs were saturated with the extracted oils of 1, 2 and 4 µL in volume.

Micro dilution method: Minimum inhibitory concentration (MIC) was determined by the micro-dilution method using serially diluted (2-fold) plant extracts according to the NCCLS[14]. A final concentration from 8.25 to 0.700 mg/mL (for essential oil 2.500 to 0.250 µg/mL) was used for each plant sample. The following

chloroform, methanol and hexane extracts were tested. *Phytophthora capsici* L. Inocula were adjusted to contain *ca.* 10^8 spore/mL. The test plates were incubated at 25 °C for 7 d.

Cultivation of *Phytophthora capsici*: *Phytophthora capsici* was incubated on potato dextrose agar (Difco Laboratories, Detroit) at 25 °C for 7 d. At the end of this period, 5 mL saline was added and mixed thoroughly thus spore suspension was prepared. The spore suspension prepared as described above was incubated at 10^8 spore/mL concentration in potato dextrose agar which was sterilized in Erlenmeyer flask and cooled down to 45 to 50 °C. After the mixture shaked sufficiently, samples were inoculated into 9.0 cm petri dishes.

The discs saturated with oil were placed on hardening agar with pushed gently. Petri dishes were incubated at 4 °C for 2 h. Then the plaques inoculated with fungus spores were incubated at 25 ± 0.1 °C for 4-7 d. At the end of this period average diameters of inhibition zones on culture mediums were measured as millimeter[15,16].

Preparation of the plants used and determination of their effectivity against fungus: The collected plant samples were macerated in a blender. Then macerated plant samples 20 g in weight in 150 mL solvent (*e.g.* chloroform, hexane, methanol) were placed separately into Soxhlet extractor device and extracted for 24 h[17]. The resins were extracted directly by various solvents. The prepared extracts at various concentration were injected into empty sterilized 6 mm. antibiotic discs with a micropipette. The discs injected with only chloroform, hexane, methanol alone were used as control. The standard antibiotic discs were used for comparison. At the end of the period inhibition zones on culture medium were measured in millimeters.

RESULTS AND DISCUSSION

Phytophthora capsici L., a pathogen fungus, causes product loss in red pepper agriculture in Turkey and many other countries around the world. The extent of antifungal activity of various plant essential oils and extracts on this fungus growth was shown in Tables 1 and 2. The results compared in the tables showed that essential oils were more effective as antifungal activity. While the essential oil of *Pinus brutia* TEN produced 11 mm inhibition zone. There was no antifungal activity for chloroform, methanol and hexane extracts as shown in Table-1. Generally, the essential oils of the genus *Pinus* contained α-pinene, β-pinene, 1-limonene, β-caryophyllene and germacrene-D. These compounds in monoterpene and diterpene group were thought to cause antifungal activity[18]. *Juniperus oxycedrus* essential oil was effective at high concentrations (10 mm inhibition zone). There was similarity especially at 4 μL concentration when compared with a standard antibiotic, nystatin. Generally, the genus *Juniperus* were known to contain α-pinene, myrcene and germacrene-D were thought to cause antifungal activity[19].

Essential oil of *Ajuga orientalis* produced 7, 10 and 14 mm inhibition zone at 1.0, 2.0 and 4.0 μL concentration, respectively. The essential oils of *Ajuga orientalis* contain mainly germacrene-D, α, β-cucubene and β-caryophyllene that were considered to have antifungal activity[20].

TABLE-1
INHIBITION ZONES OF DIFFERENT PLANT ESSENTIAL OIL OF ON *Phytophthora capsici*

Plant species	Essential oil (µL)			Standard Nystatin 100U
	1	2	4	
Pistacia terebinthus L.	-*	-	9**	11
Querqus infectoria Oliv.	-	9	11	11
Pinus brutia TEN	-	-	11	11
Juniperus oxycedrus L.	-	-	10	11
Cedrus libani A.Rich.	-	-	-	11
Pinus nigra L.	-	9	15	11
Abies cilicica Carr.	7	12	16	11
Salvia aucheri L.	-	9	15	11
Phlomis bourgei Boiss.	-	-	-	11
Parmelia furfuracea L.	-	-	-	11
Crocus chyranthus (Herb.) Herb.	-	-	-	11
Rumex scutatus L.	-	-	7	11
Myrtus communis L.	-	7	10	11
Eugenia caryophyllata Thumb.	-	-	9	11
Smyrnium olusatrum L.	-	10	16	11
Ajuga orientalis L.	7	10	14	11
Astragalus schizopterus Boiss.	-	-	9	11
Papaver hybridium L.	-	8	15	11
Salvia multicaulis Vahl.	-	-	9	11
Salvia viridis L.	7	12	16	11
Satureja cilicia P.H.	8	13	19	11
Satureja hortensis L.	-	10	16	11

*Not determined inhibition zone, **Inhibition zone, mm

On the other hand, essential oil of *Ajuga orientalis* L. produced 7, 10 and 14 mm inhibition zone at 1.0, 2.0 and 4.0 µL concentratin, respectively.

It was demonstrated that the extracts of *Artemisia annua* prevent the growth of *Gaeumannomyces graminis* var. *tritici*, *Rhizoctonia cerealis, Helminthosporium sativum, Fusarium graminearum, Gerlachia nivalis* and *Phytophthora capsici*[21]. Present findings were consistent with these findings.

The essential oil obtained from *Salvia multicaulis* Vahl. plant produced 8-9 mm inhibition zone only concentration of at 4.0 µL as shown in Table-1. There was no inhibition zone at 1.0 and 2.0 µL concentration. The essential oil of *Salvia viridis* L. produced 7, 12 and 16 mm inhibition zone at 1.0, 2.0 and 4.0 µL concentration, respectively.

The essential oil obtained from *Satureja cilicica* P.H. plant supressed the growth of *Phytophthora capsici* with 8, 13 and 19 mm inhibition zone at 1.0, 2.0 and 4.0 µL concentration, respectively. The essential oils of *Satureja hortensis* L. and *Smyrnium olusatrum* L. were not effective at 1.0 µL concentration, but produced inhibition zone ranging between 10 and 16 mm at 2.0 and 4.0 µL concentration. Carvacrol and thymol showed antifungal activity in the genus *Satureja*[22].

TABLE-2
ANTIFUNGAL ACTIVITY (MIC IN mg/mL)* OF THE ESSENTIAL OIL, CHLOROFORM, METHANOL AND HEXANE EXTRACT

Plant species	*Phytophthora capcici*			
	Essential oil (µg/mL)	Chloroform (mg/mL)	Methanol (mg/mL)	Hexane (mg/mL)
Pistacia terebinthus L.	1.75	NA	NA	4.750**
Querqus intectoria Oliv.	0.75	NA	7.750	NA
Pinus brutia TEN.	1.25	NA	NA	NA
Juniperus oxycedrus L.	1.00	8.00	4.500	1.250
Cedrus libani A.Rich.	2.50	8.25	NA	NA
Pinus nigra L.	0.75	NA	4.500	3.250
Abies cilicica Carr.	0.25	NA	NA	NA
Salvia aucheri L.	0.25	3.500	NA	3.125
Phlomis bourgei Boiss.	2.50	4.500	3.125	8.250
Parmelia furfuracea L.	2.50	4.750	4.500	3.125
Crocus chyranthus (Herb.) Herb.	2.50	4.500	NA	NA
Rumex scutatus L.	1,75	NA	NA	NA
Myrtus communis L.	1.00	NA	NA	NA
Eugenia caryophyllata Thumb.	1.75	NA	0.700	NA
Smyrnium olusatrum L.	0.25	3.250	NA	3.125
Ajuga orientalis L.	0.75	8.25	4.750	4.500
Astargalus schizopterus Boiss.	1,75	3.125	NA	4.500
Papaver hybridium L.	0,75	4.750	NA	NA
Salvia multicaulis Vahl.	1.75	3.125	NA	NA
Salvia viridis L.	0.25	NA	NA	NA
Satureja cilicia P.H.	0.25	3.250	4.750	3.125
Satureja hortensis L.	0.25	NA	NA	3.125

*Minimum inhibitory concentration,
**Not active at concentration range from 8.25 to 0.700 mg/mL, NA: Not available.

It was reported that *Salvia fruticosa, Laurus nohilis, Mentha pulegium, Inula viscosa, Pîmpmella anisum, Eucalyptus camaldulensis, Origanum minitiflorum* together with essential oils of *Thymbra spicata, Satureja thymbra*, have toxic effects against *Phytophthora capsici* and these effects were due to different phenolic fractions in essential oils[23].

The saponins obtained from seeds of *Ammi majus, Costus speciosus, Madhuca butyraceae, Madhuca indica, Mimusops elengi* and *Mimusops littoralis* were 86-100 % effective *in vitro* against *Phytophthora capsici* and they have no phytotoxic effect and were very effective when they were tested at field conditions during two years. These results are consistent with present results.

The MIC of the essential oil extracts fell in the range of 0.250 µg/mL for *Phytophthora capsici* (Table-2). *Abies cilicica, Salvia aucheri, Salvia viridis, Satureja cilicia, Satureja hortensis, Smyrnium olusatrum* extract showed the most potent inhibition for *Phytophthora capsici* (MIC 0.250 µg/mL).

The MIC of the chloroform extract fell in the range of 0.800 to 4.750 mg/mL for *Phytophthora capsici* (Table-2). *Juniperus oxycedrus, Cedrus libani, Ajuga orientalis* chloroform extract also showed the most potent inhibition for *Phytophthora capsici* (MIC 0.800 to 0.825 mg/mL). Table-2 also shows that the chloroform extracts of the *Papaver hybridum, Parmelia furfuraceae* showed at the very least inhibition effect of *Phytophthora capsici* (MIC 4.750 mg/mL). *Eugenia caryophyllata* methanol extract also showed the most potent inhibition for fungi *Phytophthora capsici* (MIC 0.700) mg/mL) and the *Juniperus oxycedrus* hexane extract showed the most potent inhibition for *Phytophthora capsici* (MIC 1.250 to 4.500 mg/mL).

Phytophthora capsici causes important economic loss in pepper agriculture and there is no success against it *via* chemical eradication. Cultural eradication methods are still utilizing. It is well known the pesticides, fertilizers and plant growth regulators that are used commonly on farmlands may accumulate in agricultural products and they may be harmful for consumers. On the other hand, determination of some substances obtained from various plants effective against pathogen fungi and harmless to environment may be pioneer for biological eradication methods on which researchers could work all around the world.

Turkey has a very rich plant flora and with *ca.* 10000 natural plant species, it is one of the most important floristic center in the world. It can be seen that those present plant potential may be useful for various industrial areas as shown by many investigations. The economic dilemmas in Turkey and in the other countries and the depletion of natural sources made mandatory to multipurpose use of natural products. Therefore, expected profits from these investigations may be considered as richness economically and obtained results may have practical value.

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