Effects of broomrape and drought stress on antimicrobial activity of a drought-sensitive lentil variety

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Abstract. Lentil (*Lens culinaris*) is moderately resistant to drought and high temperatures but sensitive to weeds such as broomrape (*Orobanche crenata*). The current study was aimed at the evaluation of the antimicrobial properties of *L. culinaris* 'Çiftçi' under drought and broomrape infection stresses. Antimicrobial activity of the ethanolic extract of 'Çiftçi' was was assayed against some test bacteria and yeast culture by the agar disc diffusion method and micro broth dilution method. The results suggested that the ethanol extracts from the different studied treatments showed antimicrobial activities, with the diameters of the inhibition zone ranging from 7.0 to 13.0 mm and 5.0 to 20.0 µg/mL, respectively. The most intensive antimicrobial activity against *Proteus vulgaris* ATCC 13315 was demonstrated by the extract of 'Çiftçi' on the 7th day grown under drought. However, only the combined stress (drought and broomrape infection) treatment has promoted antagonistic effects of the extract against *Staphylococcus aureus* ATCC 25923 bacteria, as compared to the control on the 7th day.

Key words: Antimicrobial activity, broomrape, drought stress, lentil, *Lens culinaris* 'Çiftçi'

Introduction

Stress limits growth and development in plants and stress factors are divided into two groups: abiotic stress (drought, salinity, cold, etc.) and biotic stress (fungi, bacteria, insects, etc.). Drought is an important risk factor for agricultural yields and crop production. When plants lack sufficient amounts of water during their developmental stages, drought stress occurs causing damage. Abiotic stress conditions can induce the synthesis of pathogen-associated proteins (PRs) in plants (Van Loon & Rep 2006; Sehgal & Mohamad 2018; Acar & Hacioğlu Doğru 2019). Similarly, under severe drought conditions, PRs such as chitinase and glucanase have been reported in some wheat crops (Gregorova & al. 2015). On the other hand, antimicrobial activity has been reported to increase due to oxidative stress caused by environmental stress (Sharma & al. 2018; Schmidt & al. 2019). Furthermore, relations between plant-based antimicrobial activity and PRs, such as phytoalexins, have been observed under environmental stress (Li & al. 2011; Acar & Hacioğlu Doğru 2019; Schmidt & al. 2019).

Lentil (*Lens culinaris* Medik.) is moderately resistant to drought and high temperatures but sensitive to weeds, such as broomrape (*Orobanche crenata* Forssk.). Broomrapes, which obtain water, nutrients, and minerals from the host by clinging to the root of the plant, cause biotic stress that limit yields in lentil (Parker 2003; Rubiales & al. 2009). On the other hand, drought stress is one of the most important abiotic stress factors limiting yields by disrupting water balance in plant tissues (Farooq & al. 2009). The development of lentil is negatively affected by broomrape infection and drought stress.

Plants contain antimicrobial properties that are important for the drug design against diseases (Das & al. 2010; Bhattacharjee & Islam 2015). However, the resistance to antibiotics has become a global concern (Westh & al. 2004) and clinical efficacy of many existing antibiotics is threatened by the emergence of multidrug-resistant pathogens. Therefore, it is urgent to develop new antimicrobial compounds that are more active against new and emerging infectious diseases (Rojas & al. 1992). In this context, medicinal plants (antimicrobial and antioxidant, etc.) that produce and accumulate bioactive substances such as flavonoids, phenolic acid and anthocyanin are another important natural resource for the production of new bioactive compounds (Karakaş & Türker 2013). This extended study was conducted to evaluate the antimicrobial activities of the ethanolic extracts of *Lens culinaris* 'Çiftçi' exposed to two stress factors for seven days.

Material and method

Plant material: The drought-sensitive *Lens culinaris* 'Çiftçi' was used for this study and obtained from the Field Crops Central Research Institute (Ankara/Turkey). *Orobanche crenata* seeds were obtained from the Adana Plant Protection Research Institute (Adana/Turkey). The seeds of 'Çiftçi' were sterilized by washing with sodium hypochlorite solution (20 %, for 5 min) and with sterile distilled water for 7.5 min. Sterile seeds were germinated in sterile petri dishes and transferred to pots containing perlite. All seedlings were watered with Hoagland nutrient solution (Steward 1983). Fourteen-day-old seedlings were grown in water culture (25 ± 2 °C, 16/8 photoperiod).

Orobanche crenata seeds were washed with 70 % ethyl alcohol for 2 min, then sterilized by sodium hypochlorite (5 %, 10 min) and washed with water. Sterilized seeds were incubated for 1 week in a plant-growth cabinet at 22 °C. *Orobanche crenata* seeds were induced with 1ppm GR24 and the roots of 21-day-old lentil seedlings were infected. Drought stress was created by applying with polyethylene glycol 6000 (10 % PEG 6000, –1.5 MPa) (Gökçay 2012). Antimicrobial properties were determined in leaf samples taken on days 1st and 7th. Lentil seedlings (21-day-old) were divided into four groups for the 1st and 7th day samples: (1) control (1st day); (2) broomrape infection (1st day); (3) drought stress (1st day); (4) drought stress + broomrape infection (1st day); (5) control (7th day); (6) broomrape infection (7th day); (7) drought stress (7th day); (8) drought stress + broomrape infection (7th day).

Preparation of extracts for antimicrobial activity: Air-dried leaf samples of 'Çiftçi' were ground into fine powder in a grinding mill. Pulverized plant samples (1 g) were extracted with 10 mL of 80 % ethanol (1:10 w/v) by an orbital shaker for 8 h at room temperature. The extract was separated from the solids by filtration with Whatman No 1 filter paper. The remaining solids were extracted twice with the same solvent and extracts combined. Extracts were stored in a refrigerator (4 °C) until analyzed (Sultana & al. 2007).

Test for microorganisms: Gram-negative bacteria (*Escherichia coli* NRRLB 3704, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 13315, *Acinetobacter baumanii* ATCC 19606), Gram-positive bacteria (*Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538P, *Staphylococcus haemolyticus* ATCC 43252) and yeast (*Candida albicans* ATCC 10231) were used for determining the antimicrobial activities of 'Çiftçi'.

Screening for antimicrobial activities: Empty sterilized antibiotic discs with a diameter of 6 mm (Schleicher and Schull No. 2668, Dassel, Germany) were impregnated each with 50 µL of extract (10 mg/disc). All above-mentioned bacteria were incubated at 35 ± 0.1 °C for 24 h by inoculation into Nutrient Broth (Difco Laboratories, MI, USA) and the yeast culture was incubated in Malt Extract Broth (Difco Laboratories, MI, USA) at 25 ± 0.1 °C for 48 h. An inoculum containing 106 bacterial cells or 10^8 yeast cells/mL was spread on Mueller Hinton Agar (MHA) (Oxoid Ltd., Hampshire, UK) plates (1 mL inoculum/plate). The discs injected with extracts were placed on the inoculated agar by pressing slightly. Petri dishes were placed at 4 °C for 2 h, plaques injected with the yeast culture was incubated at 25 ± 0.1 °C and bacteria were incubated at 35 ± 0.1 °C for 24 h (CLSI 2006). At the end of the period, the inhibition zones formed in the medium were evaluated in millimeters. Studies were performed in triplicate. Treatments with Penicillin (P10), and Nystatin (NYS30) served as positive controls, and treatments with ethanol, without bacterial or fungal materials, served as negative controls.

Minimum inhibitory concentration assay: Minimum Inhibitory Concentration (MIC) was checked as recommended by the instruction of the Clinical and Laboratory Standards Institute (CLSI 2006). The lowest concentration of extracts inhibiting visible growth of each test microorganism was taken as MIC. The medium, 0.1 % (w/v) Streptomycin (ST), Nystatin (NYS100) and 10 % DMSO, were used as non-treated, positive and negative controls, respectively (Teanpaisan & al. 2017).

Results

Table 1 indicates the antimicrobial activity of 'Çiftçi' plant extracts and the inhibition zones formed by standard antibiotic disks and MIC ratios. The ethanol extracts from the different treatments showed antimicrobial activities, with diameters of the inhibition zone ranging from 7 mm to 13 mm and from 5.0 to 20 μg/mL, respectively. 'Çiftçi' has shown the highest antimicrobial activity against *Proteus vulgaris* ATCC 13315 under drought conditions on the 1^{st} day (C1 in Table 1). However, the antagonistic effects of the extract against *Staphylococcus aureus* ATCC 25923 bacteria were promoted only by combined treatment, as compared to the controls on the 7^{th} day (C8 in Table 1).

Discussion

Lentils have high nutritional value, polyphenols and bioactive compounds (Ganesan & Xu 2017) and have been found to have strong antimicrobial activity against *S. aureus* (Nair & al. 2013). Similarly, antimicrobial activity against *S. aureus* was determined in the 'Çiftçi' variety as compared to the controls under drought stress. Thus, antimicrobial activity in lentils under drought stress has been recorded for the first time in this study. On the other hand, antimicrobial activity of lentil under normal conditions was found to be on a low level, as compared to the conventional antibiotics (Nair & al. 2013). Therefore, the activity of lentil under stress conditions can be compared to conventional antibiotics in the future.

In lentil plants, lectins (Nair & al. 2013) and defensins (Drikvand & al. 2019) induce antimicrobial activity, which indicates that, if those molecules increase under stress conditions, antimicrobial activity will also increase. Furthermore, it was found that the *O. crenata* leaf extract used for biotic stress manifests

Table 1. Disc diffusion and MIC ratios of the *L. culinaris* cv. 'Çiftçi' extracts.

Test microorganisms	Plant extracts																	
	*Disc diffusiona									MIC								
	cv. 'Çiftçi'								Control P10/ NY100	cv. 'Çiftçi'								Control ST/ NY100
	C1	C2	C3	C4	C5	C6	C7	C8		C1	C2	C3	C4	C5	C6	C7	C8	
E.coli **NRRLB 3704**	–	–	–	–	–	–	–	–	16.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	4.0
P. aeruginosa **ATCC 27853**	9.0	7.0	8.0	8.0	9.0	8.0	8.0	8.0	8.0	10.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	1.0
P. vulgaris **ATCC 13315**	13.0	7.0	8.0	9.0	8.0	8.0	11.0	10.0	13.0	5.0	20.0	20.0	5.0	20.0	20.0	10.0	10.0	4.0
A. baumanii **ATCC 19606**	11.0	8.0	9.0	–	–	8.0	8.0	–	12.0	10.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	2.0
B. subtilis **ATCC 6633**	7.0	8.0	8.0	9.0	8.0	8.0	8.0	8.0	14.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	4.0
S. aureus **ATCC 6538P**	10.0	7.0	8.0	–	–	–	–	10.0	15.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	10.0	4.0
S. haemolyticus **ATCC 43252**	10.0	9.0	–	–	9.0	9.0	9.0	9.0	14.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	5.0
C. albicans **ATCC 10231**	9.0	–	8.0	8.0	8.0	9.0	9.0	9.0	16.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	2.5

(C: *L. culinaris* cv. 'Çiftç'; C1: control (1^{st} day); C2: pre-treatment of seeds with broomrape (1^{st} day); C3: drought stress (1^{st} day); C4: drought stress + pre-treatment of seeds with broomrape (1^{st} day); C5: control (7^{th} day); C6: pre-treatment of seeds with broomrape; C7: drought stress (7^{th} day); C8: drought stress and pre-treatment of seeds with broomrape (7^{th} day)).
Inhibition zone (mm); a – diameter of disk (6 mm); P10 = Penicillin (10 ug/disc); ST: Streptomycin; NY100 Nystatin 100 ug/disc.

antimicrobial activity against Gram-positive strains (Genovese & al. 2019), *Salmonella enteritidis* (Abbes & al. 2014) and six different pathogens (Gatto & al. 2015).

Although there is a study on the bioactivities of lentil cultivars grown under different stress conditions, our results have shown that the use of plant extracts for antimicrobial activity is a potential source of anti-infective agents. Medicinal plants are a valuable and readily available resource for primary health care and complementary health systems. The results of this study provide evidence that the drought-sensitive 'Çiftçi', which is cultivated under different conditions, still is an important asset for health care in different societies. Moreover, this variety needs further pharmacological investigation. In the future, some species of *Fabaceae* may serve as new antimicrobial sources, especially if grown under different stress conditions.

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