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Rhizoctonia species and anastomosis groups isolated from *Lavandula* species in Isparta, Türkiye

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ABSTRACT: The genus *Rhizoctonia* includes a wide host of various kinds of more than 200 species of plants, and the genus includes different groups that are destructive plant pathogens and nonpathogenic to plants. *Lavandula* species are aromatic and medicinal plants in the family Lamiaceae. In this study, *Rhizoctonia* species were isolated from diseased *Lavandula angustifolia* and *Lavandula x intermedia* plants exhibiting symptoms related to crown and root rot to determine the anastomosis groups (AGs) of these isolates through hyphal anastomosis utilizing the test strains and the sequence analysis of the ribosomal DNA internal transcribed spacer (ITS) region. The obtained data confirmed the species identity of 29 isolates out of 44 as *R. solani* AG 4 HG-II and the remaining 15 isolates as binucleate *Rhizoctonia* (*Ceratobasidium* sp.) AG-K. Pathogenicity tests showed that AG 4 HG-II isolates were highly virulent on lavender plants, but AG-K isolates were not pathogenic. To our knowledge, this is the first report of the occurrence of *R. solani* AG 4 HG-II and binucleate *Rhizoctonia* (*Ceratobasidium* sp.) AG-K on *L. angustifolia* and *L. x intermedia* in Türkiye and around the world.

Keywords: Anastomosis group; *Lavandula* spp.; Pathogenicity; *Rhizoctonia* spp.; Lavender.

1. INTRODUCTION

The genus *Lavandula* L. is a member of the family Lamiaceae, and greater than 30 varieties are known. Species of the *Lavandula* are fundamentally grown for their volatile oils that are employed in cosmetics, perfumes, aromatherapy products, and food processing. They have also been used medicinally for a long time, such as to treat parasitic infections, pain, burns, muscle spasms, insect bites, and cramps [1-3].

In the world, there are three major species of lavender with significant commercial value that are cultivated: lavandin (*Lavandula x intermedia* Emeric ex Loisel., a hybrid of *L. angustifolia* and *L. latifolia*), spike lavender (*Lavandula latifolia* Medik.), and lavender (*Lavandula angustifolia* Mill.) [4]. *Lavandula* species is cultivated in many areas that border the Mediterranean Sea, from southern Europe through north and east Africa, southeast India, southwest Asia, and the Middle East [5]. Lavender has adapted to the ecological conditions of Türkiye, especially in drought- and slope-like fields [6]. The lavender cultivation area in Türkiye has been increased from 50 to 5200 hectares since 2012 until 2023 [7].

Lavender production could be limited by several biotic stressors, with diseases being the main limiting factors. Several diseases caused by fungi and fungus-like organisms have been reported to affect lavender around the world, including *Botrytis cinerea*, *Dothiorella sarmentorum*, *Fusarium solani*, *F. sporotrichioides*,

Peronospora belbahrii, *Phoma lavandulae*, *Phoma multirostrata*, *Phytophthora cactorum*, *P. palmivora*, *P. citrophthora*, *P. cinnamomi*, *P. cryptogea*, *P. nicotianae*, *P. drechsleri*, *Rhizoctonia solani*, *Rosellinia necatrix*, *Sclerotinia sclerotiorum*, and *Septoria lavandulae* [8-10].

Rhizoctonia spp. are categorized as multinucleate *Rhizoctonia* (teleomorphs: *Thanatephorus* and *Waitea*), binucleate *Rhizoctonia* (teleomorphs: *Ceratobasidium* and *Tulasnella*), and uninucleate *Rhizoctonia* (teleomorph: *Ceratobasidium*), according to the number of nuclei for each hyphal cell [11]. Each group consists of distinct anastomosis groups (AGs) depending on the hyphal anastomosis reaction. Until today, 13 AGs designated as AG 1–AG 13 and AG BI have been assigned within multinucleate *Rhizoctonia*, which are also divided into subgroups, while 19 AGs of binucleate *Rhizoctonia* (*Ceratobasidium* sp.), referred to as AG A–AG W, have been designated [11-14]. For so many decades, *Rhizoctonia* AGs were determined by pairing hyphae with test isolates. Nevertheless, the sequence analysis of the internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) is presently most of the correct method for the classifying of *Rhizoctonia* isolates into AGs and subgroups [11, 15-19].

The soilborne plant pathogen *R. solani* is a basidiomycota that comes globally and leads to economically significant diseases to an extensive variety of field and vegetable crops, turf grasses, forest trees, fruit, and ornamentals [20]. *R. solani* causes crown rot, stem rot, root rot, seed and fruit decay, foliar blight, damping-off, and stem canker in various types of plants [21]. Nowadays, cultivation of lavender is faced with a significant problem with *Rhizoctonia* fungal infections, which result in the most cases of crop destruction [22].

The main objectives of this study were to be able to identify anastomosis groups of *Rhizoctonia* isolates obtained from *Lavandula* species in Isparta, Türkiye, and to determine their pathogenicity.

2. MATERIALS AND METHODS

2.1. Sample collection and isolation of *Rhizoctonia* spp.

During November 2021, diseased *L. angustifolia* and *L. x intermedia* plants with symptoms of root and crown rot (Fig. 1) were collected from the Isparta province of Türkiye.



Figure 1. Field symptoms of lavender (*Lavandula angustifolia*) crown and root rot.

Plant roots were cleaned in flowing tap water to remove soil particles. Isolations were made from sections of the roots and crowns of symptomatic lavender plants. Small sections (0.5 cm long) from the edge of symptomatic tissues were surface disinfected using 2% sodium hypochlorite (NaClO) for 2 min, rinsed once in

distilled water that is sterile, dried between filter papers, and placed on 1.5% water agar (WA) containing 50 mg/l streptomycin sulfate [23]. Plates were incubated at 25 °C for 3 days in the dark. Growing colonies were examined under a microscope, and typical *Rhizoctonia*-like fungi were transferred to potato dextrose agar (PDA) and incubated for one week at 25 °C in the dark. The *Rhizoctonia* pure cultures were transferred to a slant PDA and stored at 4 °C.

2.2. Identification of *Rhizoctonia* spp.

Rhizoctonia spp. isolates obtained in this manner were identified on the basis of their vegetative hyphae [24], nuclear condition [25], and hyphal anastomosis characteristics with known tester isolates of *R. solani* and binucleate *Rhizoctonia* spp. by using standardized techniques for anastomosis group determination [26].

The anastomosis results of two representative isolates from *L. angustifolia* (CEL-2 and CEL-6) and one representative isolate from *L. x intermedia* (CEL-10) were confirmed by analyzing their ribosomal DNA-ITS regions using the following procedure: Fungal mycelia were developed on PDA for 7 days at 25 °C, after which total DNA was isolated from fresh mycelium using a GeneMATRIX Plant & Fungi DNA Purification Kit (EURx Ltd., Poland) in accordance with the manufacturer's instructions. Following DNA isolation, a spectrophotometric measurement was taken with a Thermo-Fisher Scientific Nanodrop 2000 to determine the quantity and purity of the DNA. Polymerase chain reaction (PCR) analyses were performed to amplify the internal transcribed spacers (ITSs) using primers ITS-1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS-4 (5'-TCCTCCGCTTATTGATATGC-3') [27]. The following were the conditions for amplification: 5 min initial denaturation at 95 °C, followed by 40 cycles of 45 s denaturation at 95 °C, 45 s annealing at 57 °C, 1 min extension at 72 °C, and a final extension of 5 min at 72 °C. Sanger sequencing and purification of the PCR products were performed by BM Labosis, Ankara, Türkiye. In order to generate a consensus sequence, the primer readings were contig-formed. The process was carried out using the CAP contig assembly algorithm in BioEdit 7.2.3 software [28]. Moreover, sequences of ITS were uploaded to the National Center for Biotechnological Information (NCBI) database, and an accession number was provided. Nucleotide sequences of additional isolates of *Rhizoctonia* were retrieved from GenBank and shown in Table 1.

Table 1. The isolates and accession numbers of *Rhizoctonia* anastomosis groups used in the analysis of the ITS region.

Isolate code	Anastomosis Groups	Country of origin	GenBank accession number
CEL-2	AG 4 HG-II	Türkiye	PP986394.1
CEL-10	AG 4 HG-II	Türkiye	PP986949.1
NM-7	AG 4 HG-II	China	MH172669.1
HLJ-50	AG 4 HG-II	China	MH172659.1
DB105	AG 4 HG-II	USA	OM045297.1
C213	AG 4 HG-II	USA	MZ379602.1
RhBnES-143	AG 4 HG-II	Sweden	MW999149.2
RhCaES-119	AG 4 HG-II	Sweden	MW999173.2
CEL-6	AG-K	Türkiye	PP986897.1
HB-12	AG-K	China	MH172636.1
N9	AG-K	China	MH172612.1
C191	AG-K	USA	MZ396079.1
DB224	AG-K	USA	OM046570.1
WUF-ST-Rhwhf26	AG-K	Australia	JQ859868.1
WUF-ST-RhT4-14	AG-K	Australia	JQ859875.1

A phylogenetic tree was generated using MEGA 6 software utilizing the Maximum Likelihood technique with 1000 replications for each bootstrap value [29]. *Helicobasidium purpureum* (MH854843.1) was used as an outgroup.

2.3. Pathogenicity tests

The pathogenicity of two representative isolates each from *L. angustifolia* (CEL-2 and CEL-6) and *L. x intermedia* (CEL-7 and CEL-10) was determined using *L. angustifolia* cv. Sevtopolis plants according to Koch's postulates. The inoculum of *Rhizoctonia* isolates was prepared using infected wheat grains following the method described by Eken et al. [19]. Each isolate's inoculum was prepared on moistened wheat grains in glass Petri dishes (20 mL of distilled water and 10 g of wheat grain per dish), which were then autoclaved twice at 24-hour intervals and inoculated with plugs of mycelium from cultures grown on PDA. After inoculation, Petri dishes were incubated at 25 °C for 15 days in the dark until the mycelium colonized the substrate. Five colonized wheat grains were inserted into the soil 10 mm deep beside the root of each two-year-old lavender plant. Control plants were inoculated with similar amounts of sterile wheat grains. The lavender plants were incubated in a greenhouse, which was maintained at 25 °C to 27 °C with a 12-hour photoperiod, and watered while the soil appeared dry on the surface. Six weeks later, the incidence of the disease was determined visually as the percentage of affected roots. Disease assessments were made utilizing a scale designed by Yang et al. [30], with minor modifications: 0 = no disease, 1 = less than 10%, 2 = 11–50%, 3 = 51–100%, and 4 = plants dead. To verify Koch's postulates, reisolutions were made by plating 5-mm pieces of tissue removed from the symptomatic root and crown tissues on the WA. The experiment was repeated, and each experiment was carried out utilizing a randomized complete block design with four block replicates for the each treatment.

3. RESULTS

Rhizoctonia spp. was initially identified as having right-angle branching, a septum nearby the branch, and a tiny narrowing at the branch base, based on hyphal characteristics. As a total, 44 *Rhizoctonia* isolates were recovered successfully from the diseased *Lavandula* species roots with the symptoms of crown and root rot gathered from Isparta province. On the basis of culture morphology and hyphal anastomosis by pairing with tester isolates, 29 of the *R. solani* isolates were identified as AG 4 HG-II. Fifteen isolates of binucleate *Rhizoctonia* (*Ceratobasidium* sp.) were identified as AG-K. Considerably lower numbers of isolates of *Rhizoctonia* were recovered from *L. x intermedia* compared to *L. angustifolia* (Table 2).

Table 2. *Rhizoctonia* isolates obtained from *Lavandula* species in Isparta province, Türkiye.

<i>Lavandula</i> species	Species / Anastomosis Groups (AGs)	
	<i>Rhizoctonia solani</i> / AG 4 HG-II	Binucleate <i>Rhizoctonia</i> (<i>Ceratobasidium</i> sp.) / AG-K
<i>L. angustifolia</i>	22	12
<i>L. x intermedia</i>	7	5
Total	29	15

PCR amplification of the DNA of *R. solani* and binucleate *Rhizoctonia* (*Ceratobasidium* sp.) isolates retrieved from *Lavandula* species in Isparta province, Türkiye, using universal primers ITS-1 and ITS-4 generated fragments ranging in length from 671 to 717 bp. BLASTn analysis verified the AG identity determined by hyphal anastomosis.

A maximum likelihood phylogenetic tree with 1000 bootstrap values was created using the alignment of 12 representative isolates from the NCBI GenBank nucleotide database and our three isolates (Fig. 2). Two

distinct clades were mainly divided in the phylogenetic tree (Fig. 2); one clade was composed of the binucleate *Rhizoctonia* (*Ceratobasidium* sp.) isolates belonging to AG-K, and the other clade consisted of the *R. solani* isolates belonging to AG 4 HG-II.

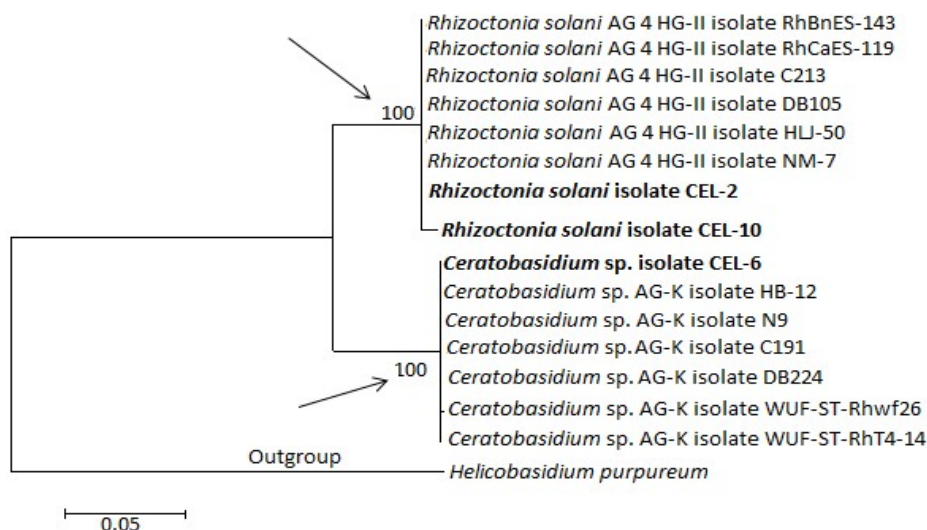


Figure 2. Phylogenetic tree constructed with the Maximum Likelihood technique from the sequences of the ITS region of rDNA of three representative *Rhizoctonia* isolates in the present study (bold) and 12 reference *Rhizoctonia* isolates retrieved from NCBI GenBank. Indicated as a percentage of 1000 replications are the bootstrap values. The sequence of rDNA-ITS of *Helicobasidium purpureum* (GenBank accession number MH854843.1) was used as the outgroup.

As a result of pathogenicity tests, two AG 4 HG-II isolates were found to be pathogenic. The disease incidence of the two AG 4 HG-II isolates, CEL-2 and CEL-10, was determined to be 75% and 76%, respectively. No significant differences in disease index were detected among the isolates representing the two AG 4 HG-II isolates. Binucleate *Rhizoctonia* (*Ceratobasidium* sp.) isolates CEL-6 and CEL-7 were found to be nonpathogenic on *L. angustifolia* cv. Sevtopolis plants. No symptoms were observed on the control plants. *Rhizoctonia* spp. was re-isolated from affected lavender plants, fulfilling Koch's postulates.

4. DISCUSSION

Rhizoctonia solani, which was the soilborne plant pathogen, is a basidiomycota that is revealed all around the world and reasons that are economically significant diseases to a wide range of field and vegetable crops, ornamentals, turf grasses, forest trees, and fruit, including the family Lamiaceae [10, 20, 31, 32]. This species has already been reported on lavender. Garibaldi et al. [33] first reported *R. solani* AG 1-1B as the cause of the web blight of lavender in Italy, and Aktaruzzaman et al. [34] also recorded it in Korea. In the present study, 44 *Rhizoctonia* isolates associated with *Lavandula* spp. root and crown rot were identified and belonged to two AGs or subgroups, including AG 4-HGII, and AG-K. Phylogenetic analysis confirmed the results obtained by the morphological method for the determination of anastomosis groups. AGs can be distinguished by recent molecular approaches such as internal transcribed spacer (ITS) region-based analysis to support the separation of *R. solani* and binucleate *Rhizoctonia* (*Ceratobasidium* sp.) into genetically different groupings. In the most research, isolates of *Rhizoctonia* have been characterized only based on the rDNA-ITS sequences [11, 14, 17, 19, 35]. To our knowledge, this is the first report of *R. solani* AG 4 HG-II and binucleate *Rhizoctonia*

(*Ceratobasidium* sp.) AG-K causing root and crown rot on *L. angustifolia* and *L. x intermedia* in Türkiye and around the world.

R. solani AG 4 was the primary *Rhizoctonia*, which causes root rot, seed rot, and post-emergence damping-off in many important crop plants, including the Lamiaceae family [32]. The conclusions of the present research verified that *R. solani* AG 4 was also the predominant AG that causes lavender crown and root rot.

Furthermore, in our study, binucleate *Rhizoctonia* (*Ceratobasidium* sp.) isolates (AG-K) were not pathogenic to lavender plants. Some isolates of binucleate *Rhizoctonia* (*Ceratobasidium* sp.) have been described as avirulent, weakly pathogenic, or pathogenic on cultivated plants [19, 36-38]. Additionally, several studies reported the presence of these isolates that colonize plant roots and could be successfully controlled against *R. solani*, *Fusarium*, and *Pythium*-caused diseases [39-48] or considered mycorrhizal species [49].

5. CONCLUSIONS

For this research, samples of *L. angustifolia* and *L. x intermedia* plants were collected from the Isparta province of Türkiye, which display symptoms of crown and root rot. In the present research, *Rhizoctonia* isolates were identified using both traditional morphological classification and rDNA-ITS sequence analysis. This is the first report of the occurrence of *R. solani* AG 4 HG-II and binucleate *Rhizoctonia* (*Ceratobasidium* sp.) AG-K on *L. angustifolia* and *L. x intermedia* in Türkiye and around the world, and it may contribute towards an efficient control strategy for lavender diseases caused by *Rhizoctonia* species.

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