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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Determination of fungal and bacterial microbiota of broomrape species found in their major host plants grown in Hatay province of Türkiye

Türkiye'nin Hatay ilinde yetiştirilen önemli tarımsal ürünlerde karşılaşılan canavar otu türlerinin fungal ve bakteriyel mikrobiyotasının belirlenmesi

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ABSTRACT

The aim of this study was to determine the fungal and bacterial microbiomes of broomrape species (*Orobanche ramosa* and *O. crenata*) encountered in their major host crops in different districts of Hatay province of Türkiye. Fungal isolates obtained from plants showing disease symptoms were identified as *Rhizoctonia solani, Fusarium incarnatum, Sclerotinia sclerotiorum, Alternaria alternata, Epicoccum nigrum, Aspergillus niger* and *Rhizopus oryzae* as a result of morphological, MALDI-TOF MS and molecular identification studies. Antagonist/plant growth promoting bacterial species such as *Bacillus cereus, Bacillus megaterium, Bacillus mycoides, Bacillus weihenstephanensis, Bacillus pumilus, Bacillus putida, Bacillus simplex, Enterobacter cloacae, Glutamicibacter mysorens, Lysinibacillus sphaericus, Pseudomonas cedrina* ssp. *cedrina, Pseudomonas chlororaphis, Pseudomonas pseudomycoides, Pseudomonas trivialis, Pseudomonas thivervalensis, Pseudomonas umsongensis, Rhizobium radiobacter, Solibacillus silvestris, Stenotrophomonas maltophilia* and *Variovorax paradoxus* were isolated from healthy broomrape plants and pathogenic bacterial species such as *Pseudomonas cichorii, Pseudomonas corrugata, Pseudomonas marginalis* and *Pseudomonas mediterranea* were isolated from plant samples of broomrape plants showing symptoms of disease and identified by MALDI-TOF MS analysis.

ÖZET

Bu çalışmada, Türkiye'nin Hatay ilinin farklı ilçelerinde yetiştiriciliği yapılan önemli tarımsal öneme sahip kültür bitkilerinde karşılaşılan canavar otu türlerinin (*Orobanche ramosa* ve *O. crenata*) fungal ve bakteriyel mikrobiyomlarının belirlenmesi amaçlanmıştır. Hastalık belirtisi gösteren bitkilerden yapılan izolasyonlarda elde edilen fungal izolatlar morfolojik, MALDI-TOF MS ve moleküler tanılama çalışmaları sonucunda *Rhizoctonia solani, Fusarium incarnatum, Sclerotinia sclerotiorum, Alternaria alternata, Epicoccum nigrum, Aspergillus niger* ve *Rhizopus oryzae* olarak teşhis edilmiştir. Sağlıklı canavar otu bitki örneklerinden *Bacillus cereus, Bacillus megaterium, Bacillus mycoides, Bacillus weihenstephanensis, Bacillus pumilus, Bacillus putida, Bacillus simplex, Enterobacter cloacae, Glutamicibacter mysorens, Lysinibacillus sphaericus, Pseudomonas cedrina* ssp. *cedrina, Pseudomonas chlororaphis, Pseudomonas pseudomycoides, Pseudomonas trivialis, Pseudomonas thivervalensis, Pseudomonas umsongensis, Rhizobium radiobacter, Solibacillus silvestris, Stenotrophomonas maltophilia* ve *Variovorax paradoxus* gibi antagonist/bitki gelişimini teşvik eden bakteri türleri, hastalık belirtileri gösteren canavar otu bitki örneklerinden ise *Pseudomonas cichorii, Pseudomonas corrugata, Pseudomonas marginalis* ve *Pseudomonas mediterranea* gibi patojen karakterli bakteri türleri izole edilerek MALDI-TOF MS ile tanılanmışlardır.

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INTRODUCTION

Parasitic weed species of the genus *Orobanche*, commonly called broomrape, attack almost all vegetables, tobacco, muskmelon, and sunflower in Europe, the Middle East, North Africa and Asia (Parker, 2009). Tomatoes, parsley, potatoes and carrots are widely grown in greenhouse and in the open fields, while tobacco, peas and thyme plants are grown in relatively limited areas in the Hatay province of Türkiye. Broomrape has been reported to cause yield losses of 33% in tobacco (Emiroğlu et al., 1987), 50-100% in broad bean (Aksoy, 2003), 33% in sunflower (Mijatovic & Stojanovic, 1973), 24-88% in carrot (Wurgler, 1973; Üremiş et al., 2020; Üremiş et al., 2023), and 21-29% in tomato (Cordas, 1973; Aksoy & Uygur, 2008). There are currently no herbicides registered in Türkiye for use against broomrape, which is encountered in tomato, pepper, aubergine, parsley, carrot, tobacco, chickpea, lentil, broad bean and potato. Methods such as solarisation, crop rotation, use of trap crops, deep ploughing, appropriate fertilisation, resistant plant breeding, biological control and chemical control are used for control (Aksoy & Pekcan, 2014; Sokat, 2019; Sokat, 2020). However, some of these control methods are difficult to apply and some of them are not economical. In addition, very small broomrape seeds can spread rapidly over very large areas by means of wind, water, tools and equipment, etc. This situation causes the problem to grow rapidly and makes it difficult to control (Kadıoğlu, 2009). The lack of an effective and economical method of control in cultivated crops increases the importance of the control of broomrape species. In addition, the number of effective herbicides for broomrape is very limited and some of the effective chemicals cannot be used for chemical control because they are phytotoxic to their host crops. For this reason, attempts are being made to develop new alternative control methods for broomrape. Many phytophagous insects that feed on broomrape have been identified in studies in Türkiye and other countries. It has been reported that the larvae of *Phytomyza orobanchia* Kalt, which feed only on the seeds, stems and capsules of broomrape (*Orobanche* spp.) and cause 20-100% damage, can be used as a biological control agent (Trencheu, 1981; Giray & Nemli, 1983; Mihajlovic, 1986; Horvath, 1987; Linke et al., 1990; Linke et al., 1992; Civelek & Demirkan, 1997; Kroschel & Klein, 1999; Amsellem et al., 2001; Klein & Kroschel, 2002; Bayram & Çıkman, 2017; Piwowarczyk et al., 2018). In addition, toxins produced by some fungi of the genus Fusarium have been reported to inhibit germination of *O. ramosa* seeds and these fungi can be used to control of *O. ramosa*. Many phytopathogenic fungi have been isolated from *O. ramosa* in field studies and it is suggested that some of these fungi may be used as mycoherbicides in the biological control of broomrape (Abouzeid et al., 2004). In Nepal, more than 70% of the fungal strains on *O. aegyptiaca* Pers. were identified as *Fusarium* spp., while other fungi were *Acremonium fusidioides, Alternaria alternata, Cladosporium cladosporioides, Epicoccum nigrum, Moltierella alpina, Papulaspora* sp., *Phoma* spp., *Sordaria fimicola, Rhizoctonia* sp., *Trichoderma* spp. and *Trichothecium roseum* (Thomas et al., 1999). In surveys conducted in tomato greenhouses in the Western Mediterranean Region of Türkiye, diseased broomrape samples were collected from 50 tomato greenhouses and 99 fungi were obtained from the infected broomrape plants. Consequently, 69 of the isolated fungi were identified as *Fusarium* spp., and 30 as *Rhizoctonia* spp. (Başbağcı et al., 2023). With the area and density of broomrape increasing day by day, the lack of an effective control method makes this weed more important every day. In addition, the fact that broomrape is a root parasite and its appearance and attractiveness keep both growers and outsiders from worrying about the problem. However, in areas where broomrape is widespread, growers are anxiously awaiting an effective control method.

The aim of this study was to determine the fungal and bacterial microbiome of healthy and diseased *Orobanche* spp. in different crops grown in agricultural areas of Hatay province.

MATERIALS and METHODS

Isolation of fungal and bacterial microbiomes from healthy and diseased broomrape plants

Fields in different districts of Hatay province were inspected using a random sampling method. Fungal and bacterial microbiomes were isolated from healthy and diseased broomrape plant tissues. Fungal species were isolated from the surface sterilised tissues of plants showing disease symptoms on the common medium Potato Dextrose Agar (PDA) and identified according to morphological characteristics (Ellis, 1971; Sutton, 1980; Domsch et al., 1980). After isolation, petri dishes were kept in incubators at 22 °C for 5 days and purifications of fungal isolates with different morphological structures were carried out.

Bacterial isolations were carried out on bacterial selective [King B Agar (King B)] and semi-selective [Yeast Dextrose Chalk Agar (YDCA)] media as previously described (Lelliot & Stead, 1987). Bacterial pathogens were isolated by dividing plant tissues showing symptoms of disease with a sterile scalpel and isolating the internal tissues by direct contact with the media using the direct imprint technique (Aktan & Soylu, 2020). After isolation, the petri dishes were kept in incubators at 26°C for 2 days and purifications were made from a single colony with different morphological structures.

Fungal and bacterial cultures with different/similar morphological appearance isolated from the plants selected to represent the plant and district where the samples were collected were accepted as separate isolates to be used in pathogenicity testing.

Pathogenicity of fungal and bacterial isolates

Orobanche seeds were sown in 18 cm × 18 cm pots containing a sterilised mixture of peat:soil:sand:animal manure (1:1:1:1:1). Subsequently, surface-sterilised broomrape (*O. ramosa*) seeds (0.5 g) were scattered to a depth of 10 cm where the tomato seedlings were to be transplanted, and the soil was mixed. Four-week-old tomato seedlings (cv. M-82) were planted at the same height (10 cm) (Hershenhorn et al., 1996; Dor & Hershenhorn, 2009; Üremiş & Arslan, 2021). The pots were kept in a growth chamber at 25°C, 16 h day/8 h night and 77 µE m^{-2} s^{-1} light intensity. Broomrape plants appeared on tomato roots 50-55 days after sowing. Orobanche plants emerging from the bottom of the seedlings were suitable for inoculation after 10 days and were used for pathogenicity studies (Figure 1).

Figure 1. Cultivation of broomrape (*O. ramosa* L.) plants for use in pathogenicity tests

Şekil 1. Patojenite testlerinde kullanılmak üzere yetiştirilen canavar otu (O. ramosa L.) bitkisi

PDA discs (5 mm) containing mycelium/spores from 5-day-old fungal cultures were used in pathogenicity tests of fungal agents. Wounds were made with a sterile cork borer on the stems of healthy broomrape plants closest to the soil level and mycelial discs taken from 5-day-old fungal isolates were placed in the wound. Sterile wet cotton wool was placed on the wounds and the inoculation sites were thoroughly wrapped with parafilm (Vural & Soylu, 2012).

For pathogenicity tests of bacterial pathogens, bacterial cells taken from a 2-day bacterial culture were suspended in 10 ml of sterile 50 mM $MgCl_2$ buffer solution and their concentration was adjusted to 10^8 cfu/ml using a spectrophotometer. The prepared bacterial suspensions were injected with a sterile syringe into the stems of healthy Orobanche plants closest to the soil level and the inoculation sites were thoroughly wrapped with parafilm after covering the inoculation point with wet cotton wool.

Five replicates were used for each microorganism. PDA discs and sterile 50 mM $MgCl_2$ buffer solution were used to inoculate control plants. Observations were made on days 3, 6 and 9 after inoculation. Bacterial and fungal agents were re-isolated from the plants showing symptoms of disease.

Detection of fungal and bacterial isolates by Matrix Assisted Laser Desorption Ionisation-Time of Flight Mass Spectrometry (MALDI-TOF MS)

Morphological identification of the fungal isolates was confirmed by MALDI-TOF MS (Microflex LT; Bruker Daltonics GmbH, Bremen, Germany). Cultures were transferred to tubes containing 8 ml of potato dextrose broth (PDB) liquid medium. The cultures were allowed to grow in the rotator for 2-3 days. Formic acid-ethanol extraction procedures were then initiated for MALDI-TOF MS analysis (Kara & Soylu, 2022). Spectra were captured using Flex control software. These spectra were then compared using Maldi Biotyper V 2.0 software and identification was performed (Pavlovic et al., 2012). After the samples were loaded into the MALDI-TOF MS instrument, the protein spectra obtained were compared with the spectra of standard species in the microorganism library using the instrument's software (BIOTYPERTM 1.1 software, Bruker Daltonics GmbH, Bremen, Germany).

Molecular identification of fungal isolates

Fungal isolates selected as representative of the region/plant variety from the re-isolates causing typical disease symptoms were also identified using a universal primer pair (ITS1/ITS4 and ITS4/ITS5) specific for the ITS gene region (White et al., 1990). Genomic DNA was isolated from fungal cultures grown on PDA medium for 5-7 days. Small amounts of fungal hyphae were collected from the fungal culture grown on PDA medium by scraping with a scalpel into 2 ml eppendorf tubes. These hyphae were then homogenised using a homogeniser. DNA isolation was performed using QIAGEN DNeasy (250) Plant mini kit. DNA concentration was measured using a spectrophotometer (Nanodrop) and PCR was started on the samples (Kurt et al., 2020). For each reaction, 1× enzyme buffer, 0.2 µL dNTP, 0.5 µL primer, 1.5 µL $MgCl_2$ and 0.2 µL (ng) DNA were added to a total of 25 µL sterile distilled water. DNA amplification was performed in a thermal cycler PCR apparatus. PCR conditions were denaturation at 94 °C for 45 s, annealing temperature of 52 °C for ITS, 72 °C for 1 min and final step of 72 °C for 10 min. A capillary gel electrophoresis device was used to determine the quality of the PCR products. Depending on the quality of the bands, the PCR products were sequenced by commercial companies (ABI 3100, Applied Biosystems). The consensus sequences obtained for the ITS locus of the fungal isolates were deposited in the NCBI GenBank database (Boratyn et al., 2013).

RESULTS and DISCUSSIONS

Fungal and bacterial microbiomes isolated from healthy and diseased broomrape plants

The study was carried out between April and September 2021 at 25 different locations in tomato, carrot, tobacco, pepper potato, sunflower, pea, melon and thyme production areas in Altınözü, Antakya, Arsuz, Belen, Dörtyol, Erzin, Hassa, İskenderun, Kırıkhan, Kumlu, Reyhanlı, Payas, Samandağ and Yayladağı districts of Hatay province (Figure 2).

As a result of the disease screening studies carried out on 148 fields with different crops, broomrape weed species were found in 29 fields (Üremiş et al., 2023). In the surveyed fields, healthy broomrape plants and broomrape plants with different disease symptoms were collected. A total of 91 plant samples (31 plants showing disease symptoms and 60 healthy broomrape plants) were obtained as a result of random sampling. The fungal and bacterial species on the broomrape plants in the surveyed areas were collected together with the host plant (Figure 2), placed in a paper bag in a cold chain and taken to the laboratory for isolation on nutrient media.

Figure 2. Detection of broomrape species (arrow) in different host crops during surveys in Hatay province. (A-E) Typical *Orobanche ramosa* in parsley fields, (F-H) *O. crenata* in carrot fields, (İ-J) *O. ramosa* in tomato, (K-M) tobacco, (N) eggplant fields

Şekil 2. Hatay ilinde yapılan sürveylerde farklı konukçu bitkilerde canavar otu (ok) türlerinin tespiti. (A-E) Maydanoz tarlalarında tipik Orobanche ramosa, (F-H) havuç tarlalarında O. crenata, (İ-J) domates (K-M) tütün ve (N) patlıcan tarlalarında O. ramosa

Isolation and identification of fungal and bacterial species in broomrape plants

Isolations were made to identify fungal and bacterial species from the blackening of root bundles, crowns and stems near the root collar of healthy and diseased broomrape (*Orobanche ramosa* and *Orobanche crenata*) collected during the surveys (Figure 3).

Figure 3. Disease symptoms detected on broomrape species are a problem in different areas of Hatay province where important agricultural products are grown. (A-D) Healthy broomrape plants. ((E-L) Typical blackening, fungal mycelia development, softening, and bacterial soft rot symptoms (arrow) on the stems of broomrape plants showing disease symptoms of different severities (arrow)

Şekil 3. Hatay ilinde önemli tarımsal ürünlerin yetiştirildiği farklı alanlarda sorun olan canavar otu bitki türlerinde tespit edilen hastalık belirtileri. (A-D) Sağlıklı canavar otu bitkileri. ((E-L) Farklı şiddetlerde hastalık belirtileri gösteren canavar otu bitkilerinin gövdelerinde tipik kararma, fungal misel gelişimi, yumuşama ve bakteriyel yumuşak çürüklük belirtileri (ok)

Fungal isolates with different morphological development (Figure 3A,B) were isolated from the tissues where disease symptoms were observed by surface sterilisation on PDA nutrient media. A total of 43 representative

fungal isolates were obtained from the isolations. Representative single spores and/or colonies of similar colonies were used for identification studies.

As a result of morphological characteristics of fungal isolates on PDA, sporulation and mycelial structures, 8 isolates of *Rhizoctonia solani* (Sneh et al., 1991) (Figure 4C) showed light brown mycelial development on PDA medium, hyphae were transparent, compartmentalised and showed 10 µm wide hyphal development in the form of right-angled side branches. While no sporulation was observed in the medium, light brown-black microsclerotia of 0.5-2.0 µm diameter (n=50) were observed in the long-term cultures.

Four *Fusarium incarnatum* (Leslie & Summerell, 2006) isolates showed pink-red mycelial growth on the PDA medium (Figure 4D) and formed dense micro- and macroconidia on day 5 of incubation. Microconidia were unicellular, transparent, oval, without compartments, 10-12.5 x 3-5 µm, macroconidia were 4-5 compartments, slightly curved, tapered at the apex and 25-32.5 x 3-5 µm (n=50).

Two isolates of *Sclerotinia sclerotiorum* (Mordue & Holliday, 1976) developed as cottony white mycelium on the petri and formed typical black sclerotia with a diameter of 2.5-7.5 mm (n=50) on the edges of the petri from the 5th-6th day of incubation (Figure 4E).

Nine *Alternaria alternata* isolates showed olive green-greyish black mycelial growth on the medium (Figure 4F). Microscopic observations made on the 4th day of incubation showed dark brown conidia characteristic of *Alternaria* type, especially with 1-2 transverse and 4-9 longitudinal divisions. The dimensions of the conidia were measured as 30-62.5x12.5-27.5 µm (n=50). Based on the observed cultural and morphological characteristics, the fungus was identified as *Alternaria alternata* (Simmons, 2007).

Four *Aspergillus niger* isolates showed dark brown-black mycelial growth on the PDA medium (Figure 4G). The vesicles of the fungus were spherical, and covered with irregular metulae and phialides. The conidia were generally dark brown to black, with rough cell walls, spherical, with a mean diameter of 3.0-3.5 µm (n = 50), while conidiophores were smooth-walled, hyaline, and melanised towards the vesicle. These characteristics of the fungal isolate were similar to those described for *Aspergillus niger* van Tiegh (Ahmed & Ravinder Reddy, 1993).

Two isolates of endophytic *Epicoccum nigrum*, produce coloured pigments (Figure 4H) after the 5th day of incubation on the PDA. Mycelial colonies developed as felted mycelial colonies giving reddish-orange diffusible pigment. Conidiophores were aggregated in sporodochia, densely compressed, and dark brown. The conidia were spherical or pyriform, dark, multicellular, 15.0-× 27.5 µm (n = 50) in diameter, and these morphological characteristics were found to be in complete agreement with previously reported morphological characteristics of the fungus (Colavolpe et al., 2018).

The most common fungal species isolated was identified as *Rhizopus oryzae* (syn. *Rhizopus arrhizus*) with 12 isolates. The fast-growing colonies of the fungus on PDA medium had a white cottony growth that turned brownish-grey to blackish-grey (Figure 4I), mycelia were unbranched, solitary, transparent to slightly dark sporangiophores 4.5-10.0 µm in diameter. Sporangia were round, dark, light brown oval spores, 40.0-200.0 µm in diameter, and rhizoids were located in the knot position adjacent to the sporangioform. The columella was globose to subglobose, pale brown and mostly 90–115 µm in diameter. These morphological characteristics were found to be in complete agreement with the previously reported morphological characteristics of the fungus (Kwon et al., 2012).

Representative isolates of fungal species with different morphological structures, representing each region/field in terms of disease severity, were used in the pathogenicity studies. Similar symptoms to those seen in the field were caused by A*. niger, R. solani, F. incarnatum, A. alternata* and *Sclerotinia sclerotiorum*, which are potential plant pathogens, at inoculation sites on broomrape plants, while no symptoms developed on control plants. The fungal isolates were re-isolated from the inoculated plants and were morphologically identical to the original isolates, thus fulfilling Koch's postulates. Isolates of *E. nigrum* and *R. oryzae* caused mild disease symptoms at the inoculation site.

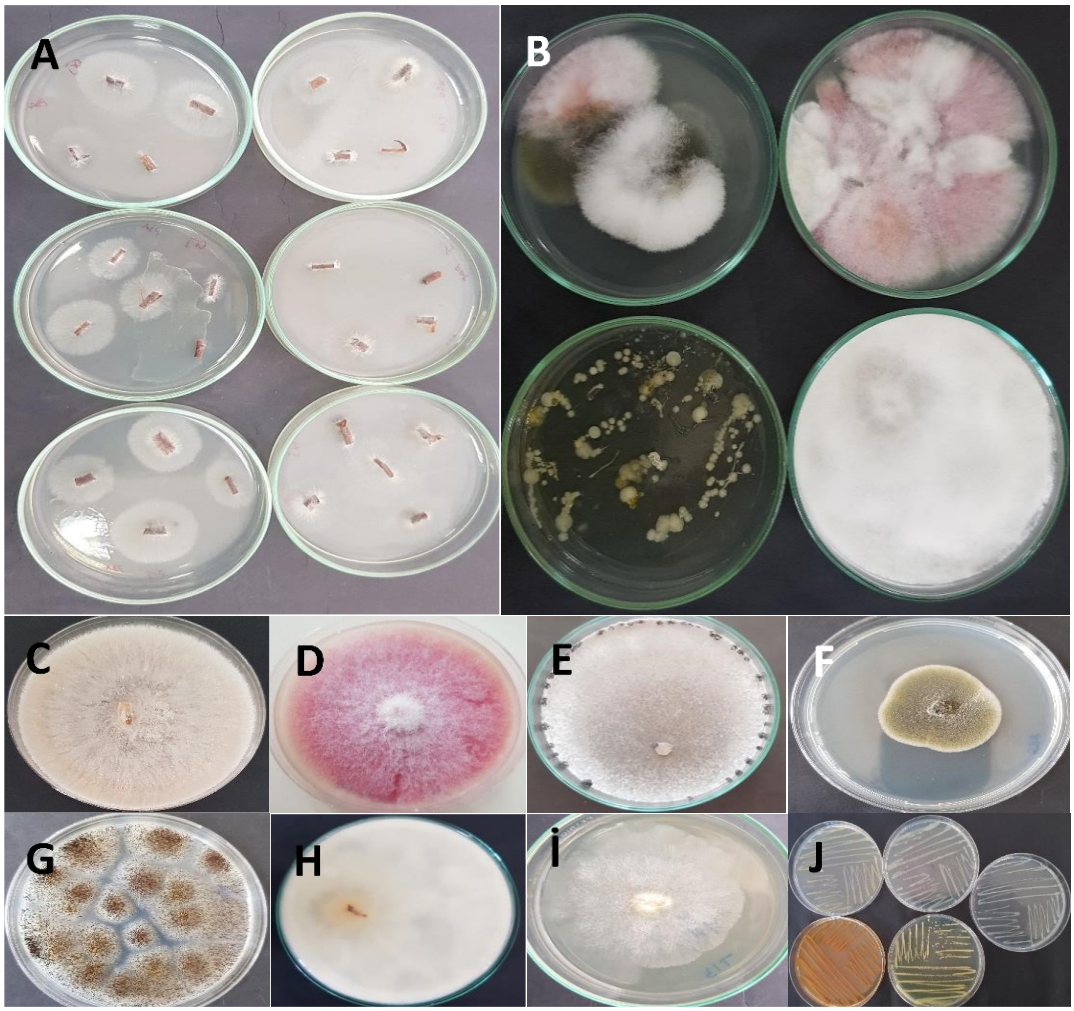

Figure 4. (A-B) Pure and mixed fungal and bacterial isolates of different species isolated from healthy and diseased broomrape plants. Typical mycelial growth of (C) *Rhizoctonia solani*, (D) *Fusarium incarnatum*, (E) *Sclerotinia sclerotiorum*, (F) *Alternaria alternata*, (G) *Aspergillus niger*, (H) *Epicoccum nigrum*, (I) *Rhizopus oryzae*, isolated from typical blackening, softening and root rot symptoms, on PDA medium. (J) Bacterial isolates with different morphological appearances developed from tissues showing soft rot symptoms

Şekil 4. (A-B) Sağlıklı ve hastalıklı canavar otu bitkileri üzerinden izole edilen farklı türlere ait saf ve karışık gelişen fungal ve bakteriyel izolatlar. Bitkilerin gövdelerindeki tipik kararma, yumuşama ve kök çürüklük belirtilerinden izole edilen (C) Rhizoctonia solani, (D) Fusarium incarnatum, (E) Sclerotinia sclerotiorum, (F) Alternaria alternata, (G) Aspergillus niger, (H) Epicoccum nigrum, (İ) Rhizopus oryzae türlerinin PDA besi yeri üzerindeki tipik misel gelişimleri. (J) Yumuşak çürüklük belirtileri gösteren dokulardan gelişen farklı morfolojik görünüşlü bakteri izolatları

Morphological identifications of fungal isolates were also confirmed by MALDI-TOF analysis. As protein profiles of reference isolates of all morphologically diagnosed fungal species were not available in the MALDI-TOF instrument, only *A. niger, A. alternata, F. incarnatum, E. nigrum, R. solani* and *R. oryzae* present in the microbial library of the instrument were identified by MALDI-TOF analysis (Figure 5).

Analyte ID: Or21-1
Analyte Creation Date/Time: 2021-06-09T06:35:04.289
Applied MSP Library(ies): Filamentous Fungi
Applied Taxonomy Tree:

Rank (Quality)	Matched Pattern	Score Value	NCBI Identifier
1 (+++)	Alternaria alternata DSM 62006 DSM	2.343	5599
2 (+++)	Alternaria alternata DSM 12633 DSM	2.332	5599
3 (++)	Alternaria alternata DSM 62010 DSM	2.198	5599

Analyte ID: Or2-2
Analyte Creation Date/Time: 2021-06-09T06:35:02.592
Applied MSP Library(ies):
Applied Taxonomy Tree: Projects, Bruker Taxonomy, Taxonomy

Rank (Quality)	Matched Pattern	Score Value	NCBI Identifier
1 (++)	Aspergillus niger M10 RLH	2.2	126754417
2 (++)	Aspergillus niger M16 RLH	2.133	126754417
3 (++)	Aspergillus niger M14 RLH	2.114	126754417

Analyte ID: Or9-7
Analyte Creation Date/Time: 2021-06-09T06:35:04.847
Applied MSP Library(ies):
Applied Taxonomy Tree: Projects, Bruker Taxonomy, Taxonomy

Rank (Quality)	Matched Pattern	Score Value	NCBI Identifier
1 (+)	Fusarium incarnatum DSM 62403 DSM	1.979	298378
2 (-)	Fusarium equiseti DSM 62203 DSM	1.609	61235
3 (-)	Fusarium sp DSM 63310 DSM mod	1.548	5506

Analyte ID: Or4-2
Analyte Creation Date/Time: 2021-06-09T06:35:30.718
Applied MSP Library(ies):
Applied Taxonomy Tree: Projects, Bruker Taxonomy, Taxonomy

Rank (Quality)	Matched Pattern	Score Value	NCBI Identifier
1 (++)	AGK R-32_Rhizoctonia solani-20170220	2.006	100134258
2 (-)	Pseudomonas rhodesiae DSM 14020T HAM	1.333	76760
3 (-)	Chryseobacterium oranimense 901900074 LBK	1.307	59732

Figure 5. MALDI-TOF MS diagnostic results of fungal species of different species isolated from diseased broomrape species found in different host crops growing in Hatay province

Şekil 5. Hatay ilinde yetişen farklı tarımsal ürünlerde hastalık belirtileri gösteren canavar otu bitkilerinden izole edilen farklı türlere ait fungusların MALDI-TOF MS tanı sonuçları

All representative fungal isolates were also used in molecular identification studies. The diagnosis of all fungal isolates was confirmed by the sequences of the Internal Transcribed Spacer (ITS) rDNA gene regions using the ITS1/4 or ITS4/5 primer pairs (White et al., 1990). The ITS locus sequences of the fungal isolates were compared with those of various fungal isolates from different host plants available in the NCBI GenBank database using the BLAST tool (Table 1). The PCR products were purified and sequenced. The resulting consensus sequences of all fungal isolates were deposited in the NCBI GenBank (Table 1).

Morphological and MALDI-TOF identification of *Rhizoctonia solani* Or4-2 (accession number: PP989821), *Fusarium incarnatum* Or9-7 (accession number: PP989825), *Sclerotinia sclerotiorum* Or14-5 (accession number: PP989826), *Alternaria alternata* Or21-1 (accession number: PP989827), *Epicoccum nigrum* Or5-3 (accession number: PP989828), *Aspergillus niger* Or2-2 (accession number: PP989829) and *Rhizopus oryzae* Or7-3 (accession number: PP989830) were also confirmed by molecular methods. In a recent study conducted by Başbağcı et al. (2023), isolates belonging to *Fusarium* spp. and *Rhizoctonia* spp. were identified at the genus level based on morphological characteristics of the fungal isolates. This study, however, clearly demonstrated for the first time that *Rhizoctonia solani, Fusarium incarnatum, Sclerotinia sclerotiorum, Alternaria alternata, Epicoccum nigrum, Aspergillus niger* and *Rhizopus oryzae* cause disease on *Orobanche* spp. grown in Türkiye. The fact that the fungal species that cause disease on *Orobanche* spp. also cause disease on cultivated plants makes it impossible to use these species in biological control studies. In previously published studies, *Alternaria alternata* and *Rhizoctonia solani* were found to be relatively low pathogenic to *Orobanche aegyptiaca* in Israel (Dor & Hershenhorn, 2009), *Sclerotinia sclerotiorum* was found to be pathogenic to *Orobanche cumana* in China (Ding et al., 2012), *Epicoccum nigrum* was reported to be a potential biological control agent of *Orobanche aegyptiaca* in Nepal (Amsellem et al., 2001). *Rhizopus oryzae* has also been reported as a potential pathogen of *Orobanche aegyptiaca* in China (Zhang et al., 2013). Zhang et al. (2018) also reported the stem rot of sunflower broomrape (*O. cumana*) caused by *Sclerotinia minor* Jagger in Inner Mongolia, China. Cignitas et al. (2024) recently reported *Fusarium fujikuroi* as a potential biocontrol agent of the parasitic weed *Phelipanche aegyptiaca* in tomato growing in Türkiye and

Hemmati and Gholizadeh (2019) reported *Talaromyces trachyspermus* on branched broom rape (Orobanche ramosa) in tomato growing in Iran. Impact of *Fusarium oxysporum* on the holoparasitic weed *Phelipanche ramosa* was also studied and its biocontrol efficacy under field-grown conditions were clearly revealed by Kohlschmid et al. (2009).

The isolation of the bacterial pathogen(s) from the soft rot symptoms observed on the stem and the swellings where they are attached to the root collar (Figure 3L) was carried out on KB media. Following isolation procedures, 72 bacterial isolates, both saprophytic (Figure 6A-B) and plant pathogenic (Figure 4C-D), were obtained and identified by MALDI-TOF analysis (Figure 4J). Pathogenicity tests of the bacterial species obtained were performed on healthy broomrape stems.

Table 1. Identification of fungal isolates isolated from broomrape plants displaying disease symptoms based on sequence analysis of the ITS gene region

Çizelge 1. Hastalık belirtisi gösteren canavar otu bitkilerinden izole edilen fungal izolatların ITS gen bölgesine ait dizi analizlerine bağlı tanılama sonuçları

Fungal species	**GenBank Accession number**	**Matched accession number (identification %) and host plant**
Rhizoctonia solani Or4-2	PP989821	MT380171 (100%) Strawberry
Fusarium incarnatum Or9-7	PP989825	PP087967 (100%) Muskmelon
Sclerotinia sclerotiorum Or14-5	PP989826	MN105884 (100%) Carrot
Alternaria alternata Or21-1	PP989827	MF167293 (100%) Radish
Epicoccum nigrum Or5-3	PP989828	MT000383 (100%) Common reed
Aspergillus niger Or2-2	PP989829	KY357318 (100%) Groundnut
Rhizopus oryzae Or7-3	PP989830	KT899481 (100%) Papaya

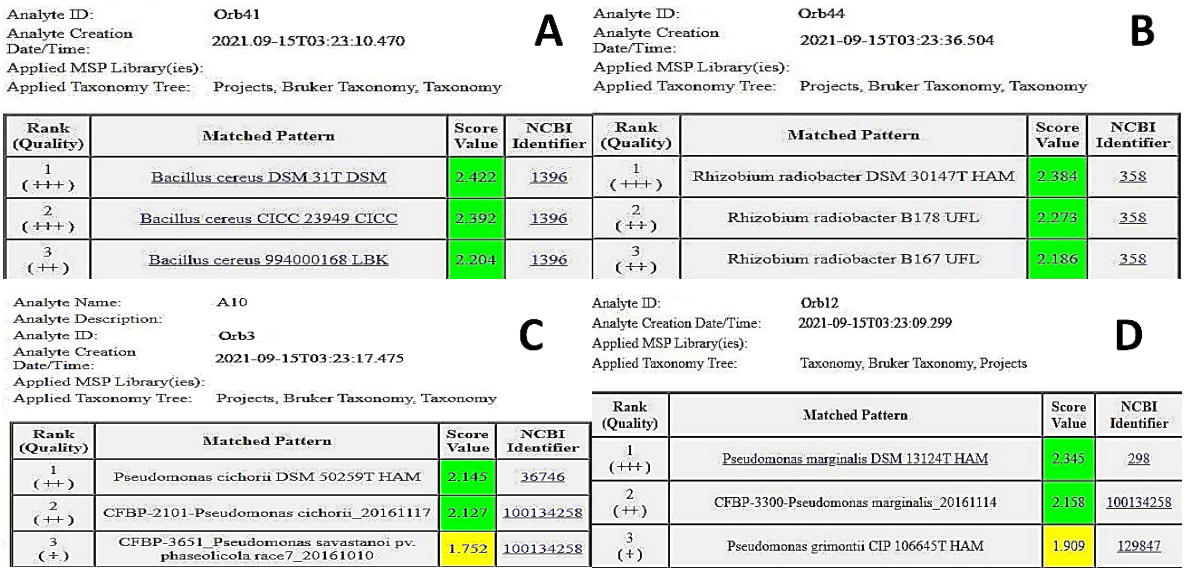

Figure 6. (A-B) MALDI-TOF MS diagnostic results of antagonist/PGPB species detected on healthy broomrape plants. (C-D) MALDI-TOF MS diagnostic results of bacterial species obtained from broomrape plants displaying soft rot disease symptoms

Şekil 6. (A-B) Sağlıklı canavar otu türleri üzerinde tespit edilen antagonist/PGPB izolatların MALDI-TOF tanı sonuçları. (C-D) Yumuşak çürüklük hastalık belirtilerinden elde edilen hastalık etmeni bakteriyel türlerin MALDI-TOF MS teşhis sonuçları

As a result of MALDI-TOF analysis, 53 bacterial isolates were obtained from healthy broomrape plant samples such as *Bacillus cereus* (3 isolates), *Bacillus megaterium* (5 isolates), *Bacillus mycoides* (2 isolates), *Bacillus weihenstephanensis* (2 isolates), *Bacillus putida* (4 isolates), *Bacillus simplex* (4 isolates), *Glutamicibacter mysorens* (2 isolates), *Lysinibacillus sphaericus* (4 isolates), *Pseudomonas cedrina* ssp. *cedrina* (3 isolates), *Pseudomonas chlororaphis* (4 isolates), *Pseudomonas pseudomycoides* (3 isolates), *Pseudomonas trivialis* (3 isolates), *Pseudomonas thivervalensis* (4 isolates), *Pseudomonas umsongensis* (1 isolate), *Rhizobium radiobacter* (4 isolates), *Solibacillus silvestris* (1 isolate), *Stenotrophomonas* sp. (Figure 5A,B). None of these isolates caused disease symptoms on the healthy broomrape stems into which they were inoculated. These isolates also did not cause soft rot symptoms at inoculation sites on potato tubers. The literature review reported that these bacterial species are well-known as potential antagonists and plant growth promoting bacteria (PGPB) species, which are commonly isolated from healthy weeds and cultivated plants and used in biological control of plant pathogens (Shaikh & Sayyed, 2015; Saxena et al., 2019).

A total of 19 bacterial isolates belonging to *Pseudomonas cichorii* (2 isolates), *Pseudomonas corrugata* (3 isolates), *Pseudomonas marginalis* (2 isolates), *Pseudomonas mediterranea* (3 isolates), *Bacillus pumilus* (4 isolates), and *Enterobacter cloacae* (5 isolates) were obtained from 12 different plants showing soft rot symptoms in the surveyed field and identified by MALDI-TOF analysis (Figure 5C,D). These bacterial isolates caused soft rot symptoms in the healthy broomrape plants and potato tubers 3 days after inoculation.

In the literature review, bacterial species such as *Pseudomonas corrugata* (Xu et al., 2013), *Pseudomonas marginalis* (Li et al., 2018), *Pseudomonas mediterranea* (Alippi & Lopez, 2010), *Pseudomonas cichorii* (Ruan et al., 2018), *Enterobacter cloacae* (Li et al., 2022), *Bacillus pumilus* (Peng et al., 2013) species cause "soft rot" disease symptoms in the roots, tubers, root collar, stem, and fleshy leaves of different cultivated plants, while *Rhizobium radiobacter* (Syn. *Agrobacterium tumefaciens*) species have been reported to cause the disease known as "root and crown gall and crown gall" (Lippincott et al., 1981). In the surveys conducted in Türkiye, *Pseudomonas corrugata, Pseudomonas mediterranea*, and *Pseudomonas cichorii* isolates caused soft rot disease symptoms in the tubers, stems, and fruits of different host plants (Saygılı et al., 2008; Aysan & Horuz, 2016), while *Rhizobium radiobacter* species generally cause the disease known as “knot or crown gall” in the fruit trees (Yüzbaşıoğlu & Aysan, 2021; Bozkurt & Soylu, 2019). The presence of these bacterial pathogens as primary or opportunistic-secondary soft rot disease agents of carrots and different Brassica species grown in the Hatay province of Türkiye has been recently reported (Soylu et al., 2022; Soylu et al., 2024).

Recently, surveys were carried out to identify broomrape species in tomatoes, carrots, tobacco, peppers and potatoes, sunflowers, peas, clover, melons and thyme in the Altınözü, Antakya, Arsuz, Belen, Dörtyol, Erzin, Hassa, İskenderun, Kırıkhan, Kumlu, Reyhanlı, Payas, Samandağ and Yayladağ districts of Hatay province (Üremiş et al., 2023). In this study, a large number of healthy and diseased broomrape plant samples were collected during these survey to determine the diversity of fungal and bacterial microbiota in broomrape plants. The fungal and bacterial isolates obtained were identified by morphological, MALDI-TOF and molecular studies. Based on the morphological characteristics of the fungal isolates on PDA, they were identified as *Rhizoctonia solani, Fusarium incarnatum, Sclerotinia sclerotiorum, Alternaria alternata, Epicoccum nigrum, Aspergillus niger* and *Rhizopus stolonifera*. The identification of the fungal isolates was also confirmed by MALDI-TOF and molecular identification studies. Morphological, MALDI-TOF and molecular identification studies have identified fungal isolates as plant pathogenic species on agriculturally important crops on which broomrape grows. This is the main issue limiting the possibility of using these fungal isolates for biological control of broomrape. Bacterial species such as *Bacillus cereus, Bacillus megaterium, Bacillus mycoides, Bacillus weihenstephanensis, Bacillus pumilus, Bacillus putida, Bacillus simplex, Enterobacter cloacae, Glutamicibacter mysorens, Lysinibacillus sphaericus, Pseudomonas cedrina* ssp. *cedrina, Pseudomonas chlororaphis, Pseudomonas cichorii, Pseudomonas corrugata, Pseudomonas marginalis, Pseudomonas mediterranea, Pseudomonas pseudomycoides, Pseudomonas trivialis,*

Pseudomonas thivervalensis, Pseudomonas umsongensis, Rhizobium radiobacter, Solibacillus silvestris, Stenotrophomonas sp., *Stenotrophomonas maltophilia,* and *Variovorax paradoxus* have been isolated from healthy broomrape plants and identified. All bacterial isolates were determined to be antagonistic/plant growth promoting bacteria (PGPB) species and did not cause disease symptoms in soft rot of potato slices or in pathogenicity tests on broomrape stems. In addition to these isolates, bacterial pathogens causing soft rot disease in cultivated plants such as *Pseudomonas corrugata, Pseudomonas marginalis, Pseudomonas mediterranea, Pseudomonas cichorii, Enterobacter cloacae* and *Bacillus pumilus* were also obtained from broomrape samples showing symptoms of soft rot. The potential plant pathogenicity of these species was considered as the most important issue limiting their use in the biological control of broomrape species. The results of this study, revealling the fungal and bacterial microbiota of healthy and diseased broomrape, will provide information for various studies to be carried out on this plant in the future.

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STATEMENT OF CONFLICT OF INTEREST

The authors declare that there is no conflict of interest between them.

AUTHOR'S CONTRIBUTIONS

All author contributed equally for analyses, writing and interpretation of the article. The authors read and approved the final version of the manuscript.

STATEMENT OF ETHICS CONSENT

This article does not require ethical approval as there are no experiments with human or animal subjects.

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