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Determination of Fungal and Bacterial Disease Agents on Significant Brassicaceous Vegetable Species Grown in Hatay Province

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ABSTRACT

The *Brassicaceae* family, commonly known as the *Cruciferae* or mustard family, encompasses plant species of global economic significance, including oilseed crops, vegetables, as well as condiment purposes. Hatay is one of the most important provinces in Turkey where *Brassicaceous* vegetable crops are grown. This study aimed to identify the causative disease agents affecting *Brassicaceous* vegetables including cabbage (red and white varieties), broccoli, cauliflower, garden cress, rocket, and radish in the districts of Hatay Province where vegetable cultivation took place during the 2020-2021 growing seasons. Isolations from suspicious cabbage, broccoli, cauliflower, and radish plants exhibiting disease symptoms in the surveyed areas revealed the presence of various fungal disease agents, including *Rhizoctonia solani, Sclerotinia sclerotiorum, Fusarium solani, Fusarium oxysporum, Fusarium exquisite, Alternaria alternata, Botrytis cinerea*, and *Stemphylum* sp. Furthermore, obligate oomycete pathogens such as *Albugo lepidii, Albugo candida*, and *Perofascia lepidii* were detected with varying prevalence and incidence rates on leaves and stems of water cress, rocket, and radish plants. In addition to fungal pathogens, primary bacterial pathogens, including *Xanthomonas campestris* pv. *campestris*, *Pectobacterium carotovorum* subsp. *carotovorum*, *Pectobacterium parmentieri, Pseudomonas corrugata*, and *Pseudomonas victoria*, were identified through morphological, biochemical, and pathogenicity tests, and MALDI-TOF analyses. *Pantoea agglomerans, Pseudomonas marginalis, Pseudomonas fluorescens, Enterobacter cloacae,* and *Bacillus pumilus* were also identified as opportunistic soft rot bacterial pathogens. To the best of our knowledge, this is the first report of *F. solani, F. oxysporum, F. exquisite, A. alternata, B. cinerea, Stemphylum sp.,* as fungal disease agents; *P. parmentieri, P. corrugata,* and *P. victoria* as primary soft rot bacterial disease agents; *P. agglomerans, P. marginalis, P. fluorescens, E. cloacae,* and *B. pumilus* as opportunistic secondary soft rot bacterial disease agents affecting different minor vegetables belong to *Brassica* spp, such as cabbage (red and white varieties), broccoli, cauliflower, radish, rocket, and garden cress, grown in Turkey.

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Hatay İlinde Yetiştirilen Önemli Brassicaceous Sebze Türlerinde Fungal ve Bakteriyel Hastalık Etmenlerinin Belirlenmesi

ÖZET

Genellikle lahanagiller, *Cruciferae* veya hardal ailesi olarak bilinen *Brassicaceae* familyası, dünya çapında ekonomik açıdan tarımsal öneme sahip yağlı tohum, sebze ve çeşni amaçlı kullanılan bitki türleri içerir. Hatay, Türkiye'nin *Brassicaceous* sebze türlerinin yetiştiriciliğinin yapıldığı en önemli illerinden biridir. Bu çalışmada 2020-2021 yetiştirme sezonlarında Hatay ilinin sebze yetiştiriciliğinin yapıldığı ilçelerde *Brassicaceous* sebzelerden lahana (kırmızı ve beyaz baş), brokoli,

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karnabahar, tere, roka ve turp bitkilerinde sorun olan fungal, oomycet ve bakteriyel hastalık etmenlerinin tanılanması ve yaygınlık durumlarının belirlenmesini amaçlanmıştır. Sürvey yapılan alanlarda hastalık belirtisi gösteren şüpheli lahana, brokoli, karnabahar ve turp bitkilerin kök, gövde ve yapraklarından yapılan izolasyonlarda *Rhizoctonia solani, Sclerotinia sclerotiorum, Fusarium solani, Fusarium oxysporum, Fusarium equiseti, Alternaria alternata, Botrytis cinerea* ve *Stemphylum* sp., ait fungal hastalık etmenlerin yanısıra yanısıra *Albugo lepidii, Albugo candida ve Perofascia lepidii* gibi obligat parazit oomycet patojenler tere, roka ve turp bitkilerinin yaprak ve gövdelerinde değişen yaygınlık ve rastlama sıklıklarında tespit edilmiştir. Fungal etmenlerin yanısıra yapılan morfolojik, biyokimyasal, patojenisite testler ve MALDI-TOF analizleri sonucunda *Xanthomonas campestris* pv. *campestris*, *Pectobacterium carotovorum* subsp. *carotovorum*, *Pectobacterium parmentieri*, *Pseudomonas corrugata* ve *Pseudomonas cichorii* primer bakteriyel hastalık etmenleri olarak belirlenmiştir. *Pantoea agglomerans, Pseudomonas marginalis, Pseudomonas fluorescens, Enterobacter cloacae* ve *Bacillus pumilus* ise fırsatçı sekonder bakteriyel yumuşak çürüklük etmenler olarak tanılanmışlardır.Bilindiği kadarıyla *F. solani, F. oxysporum, F. equiseti, A. alternata, B. cinerea*, *Stemphylum* sp., fungal hastalık etmenleri; *P. parmentieri*, *P. corrugata* ve *P. cichorii* primer bakteriyel yumuşak çürüklük hastalık etmenleri; *P. agglomerans, P. marginalis, P. fluorescens, E. cloacae* ve *B. pumilus* ise sekonder fırsatçı bakteriyel yumuşak çürüklük hastalık etmenleri olarak Türkiye'de yetiştirilen farklı *Brassica* spp ait lahana (kırmızı ve beyaz baş), brokoli, karnabahar, tere, roka ve turp gibi minör sebzelerde hastalıklara neden olduğu ilk kez bu çalışma ile tespit edilmiştir.

Anahtar Kelimeler
Cruciferae
Brassica
Fungal hastalık
Bakteriyel hastalık
Oomycete

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INTRODUCTION

The *Brassicaceae* family is commonly known as the Cruciferae or mustard family. This family encompasses a diverse array of plant species with significant global economic importance (Raza et al., 2020). Within the *Brassicaceae* family, there are 338 genera and more than 3700 plant species, each serving various purposes (Shankar et al., 2019). The *Brassica* genus is widely recognized as the most significant genus within the *Brassicaceae* family, encompassing several vital crops. This genus includes oilseeds like canola and mustard, as well as a variety of vegetables suitable for consumption in raw, cooked, or salad form, such as cabbage, Brussels sprouts, broccoli, cauliflower, radish, rocket, and Chinese cabbage. Additionally, it comprises plants used for seasoning purposes, including mustard, wasabi, and wild radish (Rakow, 2004; Chen et al., 2013; Gupta, 2016). Among the most extensively cultivated and utilized *Brassicaceous* vegetables, virtually all are edible, *Brassica oleracea* and *B. rapa* are prominent examples, while the seeds of *B. nigra, B. carinata*, and *B. juncea* find application as seasonings in kitchens. According to data from the Food and Agriculture Organization (FAO), *B. napus, B. rapa, B. juncea*, and *B. carinata*, which also serve as leaf and root vegetables, collectively contribute to 12% of the world's supply of edible vegetable oils (Anonymous, 2021a).

In recent decades, there has been a notable 33.8% increase in the global production of *Brassicaceous* vegetable species. As of 2020, China, India, and Russia emerged as the top producers (Anonymous, 2021b). Within Hatay Province, located in Turkey, the cultivation of minor *Brassicaceous* vegetables is widespread across various districts, including Antakya, Kırıkhan, Hassa, Reyhanlı, Kumlu, Altınözü, Samandağı, Arsuz, İskenderun, Erzin, and Dörtyol. These minor vegetables include white and red cabbage, cauliflower, radish, and rocket. In Hatay Province, white cabbage was cultivated on 727 da, resulting in a total production of 1,315 tons, while red cabbage covered 130 da, yielding 260 tons. Additionally, radish, rocket, and watercress were cultivated on 255, 530, and 814 days, respectively (Anonymous, 2020).

Brassicaceous vegetable plants, encompassing vital vegetable species such as white and red cabbage, cauliflower, broccoli, radish, garden cress, and rocket, are susceptible to a range of disease agents, including bacteria and fungi, which can adversely affect both yield and quality (Smith et al., 1988; Koike et al., 2007; Srivastava et al., 2011). Among the factors that can limit yield and quality in *Brassicaceous* vegetables, soil and leaf-borne fungal, oomycete, and bacterial diseases play a prominent role. These disease agents have the potential to significantly influence crop yield and quality, manifesting at various stages of growth, from the seedling stage to later developmental phases (Bruehl, 1987). Fungal and oomycete diseases pose significant threats to *Brassicaceous* vegetables, including Alternaria leaf spot and blight (*Alternaria* spp.), anthracnose (*Colletotrichum* spp.), downy mildew (*Hyaloperonospora parasitica* [Syn. *Peronospora parasitica*], *Perofascia lepidic*), powdery mildew (*Erysiphe cruciferous*), Sclerotinia stem and head rot (*Sclerotinia sclerotiorum*), white rust (*Albugo candida, Albugo lepidic*), wilt (*Fusarium* spp.), leaf spot (*Leptosphaerulina brassicas*, *Cercospora chianti*), damping-off (*Pythium* spp.), clubroot (*Plasmodiophora brassica*e), and blackleg (*Leptosphaeria maculans*) (Koike, 2007; Srivastava et al., 2011; Kumar et al., 2017; Al-Lami et al., 2019; Shaw et al., 2021; Kiran et al., 2022; Greer et al., 2023; Mourou et al., 2023).

In addition to fungal and oomycete pathogens, bacterial diseases are significant biotic factors that can detrimentally affect the production and yield of vegetable species within this genus. Primary bacterial diseases include soft rot, caused by *Pectobacterium carotovorum subsp. carotovorum* (=*Erwinia carotovora* subsp. *carotovora*), *Pseudomonas viridiflava, Pseudomonas corrugata*, leaf spot disease caused by *Pseudomonas syringae* pv. *maculicola*, and black rot, caused by *Xanthomonas campestris* pv. *campestris*. Furthermore, species such as *Pseudomonas marginalis* and *Pseudomonas fluorescens* are known as opportunistic bacterial diseases (Koike et al., 2007; Rimmer et al. 2007).

While studies examining fungal and bacterial disease agents affecting key *Brassicaceous* vegetable species are conducted in regions around the world (Mourou et al., 2023), research in Turkey on these disease agents within such plants remains notably scarce. Several disease agents have been identified for the first time in Turkey, specifically within *Brassicaceous* vegetable species, including cabbage, cauliflower, broccoli, and Brussels sprouts, grown in various regions. Notably, *X. campestris* pv. *campestris*, the causative agent of bacterial black spot disease, along with soft rot agents *P. carotovorum* subsp. *carotovorum* and *P. viridiflava*, have been reported as the first disease records for *Brassicaceous* vegetable species (Mirik et al., 2008; Aksoy et al., 2017a, b; Aksoy & Öztürk, 2018; Öztürk et al., 2019; Öztürk & Soylu, 2022; Meral et al., 2022). Similarly, *R. solani*, responsible for root and crown rot, has been reported in red and white cabbage plants cultivated in various regions of Turkey (Saygi et al., 2020; Benli & Türkkan, 2020; Erper et al., 2021). In the case of cabbage, head rot disease caused by *S. sclerotiorum* has also been previously reported (Tozlu et al., 2016). Furthermore, oomycete disease agents such as *P. lepidii* were found to cause downy mildew, while *A. lepidii* was associated with white rust disease in garden cress (*Lepidium sativum*) plants growing in Hatay province (Soylu et al., 2017; Soylu et al., 2019).

In recent years, farmers in the districts of Hatay Province have been confronted with significant challenges related to economic losses stemming from fungal and bacterial diseases that afflict *Brassicaceous* vegetables. While some information is available regarding diseases caused by *P. lepidii* and *A. lepidii* in garden cress plants, there remains a dearth of knowledge regarding disease agents responsible for other ailments and their prevalence in production areas. This study is aimed at addressing this gap by identifying and assessing the prevalence of fungal, oomycete, and bacterial disease agents on significant *Brassicaceous* vegetables, including white and red cabbage, cauliflower, broccoli, radish, garden cress, and rocket growing in districts of Hatay Province.

MATERIAL and METHODS

Determination of the Prevalence of Fungal and Bacterial Disease Agents.

Disease survey investigations were conducted during the 2020-2021 cultivation season, taking into account data provided by the Hatay Provincial Agriculture Directorate for the year 2019. These surveys encompassed approximately 10% of the total cultivated area in Hatay Province, covering various districts including Antakya, Kırıkhan, Hassa, Reyhanlı, Kumlu, Altınözü, Samandağı, Arsuz, İskenderun, Erzin, and Dörtyol. These districts are known for the cultivation of different minor vegetable crops belonging to *Brassica* species, spanning a total area of 6181 da.

The survey studies were conducted at different growth stages of the plant, including the seedling (September-October 2020), before and after heading stages (November 2020-April 2021). In the surveyed fields, a systematic zigzag pattern was followed to inspect the entire area, and any observed symptoms were recorded (Bora & Karaca, 1970). Suspicious plant samples were assigned unique codes and subsequently transported to the laboratory for isolation and identification. The prevalence of disease agents was determined by calculating the number of fields in which the disease was observed out of the total surveyed fields. Additionally, the incidence rate for each field was calculated by relating the number of diseased samples to every 100 plants within that field.

Isolation of Fungal Disease Agents

Fungal pathogens were isolated from various parts of the host plants displaying characteristic disease symptoms. General and selective nutrient media, including Potato Sucrose Agar (PSA; Merck, Darmstadt, Germany), Czapek Dox Agar (CPA; Merck, Darmstadt, Germany), and Carnation Leaf Piece Agar (CLA) were used for the isolation process. Plant tissues, such as roots, stems, heads, and leaves from affected plants, were first washed under tap water. Subsequently, necrotic tissues resulting from the infection were cut into 5 mm pieces using a sterile scalpel. Tissue pieces were surface disinfected in 75% ethanol for 1 minute, followed by a 5-minute exposure to 3% hypochlorite. After disinfection, the tissue pieces were rinsed three times in sterile distilled water and left to air dry for 1-2 hours on sterile blotting papers. Once they were completely dried, the tissue pieces were transferred to 90 mm diameter Petri dishes containing general and selective culture media, supplemented with 50 µg ml^{-1} streptomycin sulfate. Four pieces were placed in each petri dish, which was then incubated at 25°C for 5-7 days. Following incubation, mycelial disks were taken from actively growing colonies of each isolate and transferred to 60 mm diameter PDA Petri dishes containing the 50 µg ml^{-1} streptomycin sulfate, and incubated at 25°C for 5 days. Subsequently, single spore cultures were prepared for all fungal isolates and preserved at -20°C in envelopes on filter paper for further studies.

Morphological Identification of Fungal Disease Agents

All single-spored cultures of fungal isolates were evaluated for their colony morphologies, pigment formations, presence of overwintering structures (sclerotic), mycelium, conidiophores, and conidial developments on general (V8 Juice Agar) and selective media (CLA) kept at 25°C with 12-hour alternating fluorescent/UV light and darkness. To measure the dimensions of the conidia produced by each isolate, conidial suspensions were prepared from 7-10-day-old cultures grown on PDA using sterile distilled water, and the shape and size measurements of their conidia were determined using a trinocular light microscope (Olympus BX51, Japan). Identification was performed using diagnostic keys based on morphocultural characteristics published by Ellis (1971), Sneh et al. (1991), Leslie & Summerell (2006), and Simmons (2007). The identification of oomycete pathogens was made based on the conidia and conidiophore structures taken directly from the diseased tissues, as previously described (Constantinescu & Fatehi, 2002; Choi et al., 2007).

Pathogenicity Tests of Fungal and Oomycete Pathogens

The pathogenicity of the fungal and oomycete isolates was confirmed on different parts (leaves, root collar, etc.) of 8-week-old seedlings of host plants, including white head cabbage (cv. Lades F1), red cabbage (cv. Karmen F1), cauliflower (cv. Bahara F1), broccoli (cv. Vole), garden cress (cv. Arzuman), rocket (cv. Arzuman), and radish (cv. Kadirli). For leaf pathogens such as *A. lepidii*, *A. candida*, and *P. lepidii*, conidial suspensions were prepared at a concentration of 10^6 conidia ml^{-1} in sterile water and sprayed onto the leaf surface (Soylu et al., 2019). Isolates of *A. alternata*, *B. cinerea*, and *Stemphylum* sp. were inoculated by applying 20 µl of conidial suspensions, at a concentration of 10^6 conidia ml^{-1} in sterile water, onto wounds made on the leaf surface using a sterile syringe needle (Blagojević et al., 2020). Isolates of *R. solani*, *S. sclerotiorum*, *F. solani*, *F. oxysporum*, and *F. equiseti* were inoculated by placing mycelial pieces obtained from 5-day-old mycelial cultures into wounds at the base of stems of 8-week-old healthy cabbage seedlings. In the pathogenicity tests, ten seedlings for each representative isolate were used. Following the onset of disease symptoms at the inoculation points, the fungal pathogens were re-isolated and compared with the original isolates.

Molecular Identification of Fungal Disease Agents

To confirm the morphological identification of the fungal pathogens from infected plants, molecular identification studies were conducted. Amplification of internal transcribed spacer (ITS) rDNA locus was performed using universal primer pairs ITS1 (5'-TCCGTAGGTGAACCTGCG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990). Fungal genomic DNA was extracted from 5-day-old aerial mycelia of pure cultures of representative isolates grown on PDA. Fungal mycelia were homogenized in 2 ml Eppendorf tubes, and their DNA was extracted by using the genomic DNA isolation kit (DNeasy Plant Mini Kit, Qiagen Inc., Valencia, CA) following the manufacturer's recommendations. The quantity and quality of the extracted genomic DNA were assessed using the Qubit 3.0 fluorometer. The PCR procedure was carried out in a thermal cycler (Applied Biosystems, Singapore), with each mixture consisting of 1x PCR buffer, 0.2 µM dNTP, 0.5 µM of each primer, 1 U Taq polymerase (Invitrogen), and 2 µl of genomic DNA. PCR condition was set to initiate denaturation at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 60 seconds, concluding with a final extension step at 72°C for 10 minutes (White et al., 1990). The quality of PCR products was visualized by capillary electrophoresis (QIAxcel Advanced, Qiagen, Germany) as previously described (Uysal et al., 2022). Molecular identification of fungal isolates, relying on sequencing results, involved using the BLAST program at the

National Center for Biotechnology Information (NCBI) to compare the sequences with known species.

Isolation and Pathogenicity of Bacterial Disease Agents

Bacterial pathogens were isolated directly from infection sites and cultured on general (Tryptic Soy Agar, TSA) and selective media (King B Agar, KB). The plates were then incubated at 25°C for 24-48 hours in incubators. Bacterial isolates with varying morphological structures that grew on the plates were subcultured from single colonies onto TSA following the procedures outlined by Lelliot & Stead (1987). Each bacterial colony isolated from a different plant was treated as a separate isolate and preserved at -80°C in a 40% glycerol solution for use in future studies. Biochemical tests, such as colony color, colony form, cell morphology, gram reaction, oxidase and catalase production, as well, and levan formation, were employed to make a preliminary selection of isolates by the methods outlined by Lelliott & Stead (1987). All isolates originating from single colonies were initially tested for Hypersensitive Reaction (HR) on tobacco leaves and subsequently, rotting tests on various parts of host plants, including leaves, stems, flowers, and potato slices. Pathogenicity tests of representative isolates were conducted on their original host plants. A bacterial suspensions of *Xanthomonas campestris pv. campestris* at 10^6 cfu ml^{-1} concentration was injected into the leaves of 8-week-old white cabbage seedlings (cv. Yalova). Pathogenicity tests of soft rot bacterial disease agents were conducted on cabbage leaves, broccoli, cauliflower, and radish. Two-day-old bacterial colonies were taken with a sterile toothpick and directly inoculated on the stem of their host plants. The inoculation site was covered with parafilm. The inoculated plant parts were placed into a transparent storage box and incubated at 25 ^{C} for 3 days and the formations of soft rot lesions at the inoculation site were examined (Öztürk & Soylu, 2022).

Identification of Bacterial and Fungal Disease Agents by MALDI-TOF

Morphological and biochemical identification of fungal and bacterial isolates were also confirmed by using the MALDI-TOF (Bruker Daltonics GmbH, Bremen, Germany) Biotyper identification system, (Pavlovic et al., 2012). Bacterial isolates on TSA and fungal isolates in Potato Dextrose Broth were cultivated for 24-48 hours. The ethanol-formic acid method was utilized for protein extraction (Soylu et al., 2022). Specific protein spectra were obtained through the MALDI-TOF Biotyper. Obtained spectra were then compared with the protein spectra of reference bacterial isolates available in the device's microbial library, employing the Maldi Biotyper Real-Time Classification (RTC) software (Biotyper 3.0; Microflex LT; Bruker Daltonics GmbH, Bremen, Germany) for species-level identification, as detailed by Soylu et al. (2023).

RESULTS and DISCUSSIONS

The initial disease survey was conducted in cultivation areas of cabbage (white and red), cauliflower, broccoli, rocket, and radish in September 2020, coinciding with the emergence of the first seedlings. No signs of disease were detected on the seedlings when they were transplanted into the fields. Based on communication with local farmers, sporadic reports mentioned a slight drying of some seedlings approximately one week after planting. Since farmers promptly replaced the affected seedlings with healthy ones, the surveys did not reveal any notable instances of such drying.

Subsequent 3 surveys were carried out in October and November 2020, aligning with the growth of the seedlings and the commencement of the heading stage (for white and red cabbage and cauliflower). These surveys collectively covered a total of 77 fields located in the districts of Antakya, Kırıkhan, Kumlu, Reyhanlı, Hassa, and Arsuz. The presence of root rot was identified at the root and crown of certain red cabbage seedlings (Figure 1).

In cabbage fields, only one field exhibited signs of mild wilting, affecting a minimal percentage of plants, approximately 2-3% (see Figure 2A-E). In the third and fourth surveys conducted in October and November 2020, the prevalence of pathogens exhibited considerable variation across the surveyed fields. A cross-section of the stem of the plant displaying such symptoms revealed darkening in the vascular bundles, reminiscent of the thinning observed at the root collar in red cabbage seedlings. Subsequent isolations from symptomatic plants collected during all surveys yielded 76 different fungal isolates. Based on morphological characteristics of the fungal structures of each isolate, 6 different fungal species tentatively identified as *R. solani* (Sneh et al., 1991), *S. sclerotiorum* (Kohn, 1979), *Fusarium* spp. (Leslie & Summerell, 2006), *Alternaria* spp (Simmons, 2007) and *Stemphylum* spp. (Woudenberg et al., 2017).

The last 3 surveys, which occurred just before harvest, were conducted in March and April 2021, encompassing 130 fields of cabbage (white and red), garden cress, rocket, radish, and cauliflower plants that were either harvested or at a stage of delayed harvest (Figure 3). These fields were spread across the districts of Antakya, Arsuz, Payas, İskenderun, Dörtyol, Erzin, Hassa, Kırıkhan, Reyhanlı, Kumlu, and Altınözü. Subsequent isolations from symptomatic plants collected during these surveys yielded 38 different fungal isolates White-headed cabbage plants exhibited symptoms of head rot disease, caused by *S. sclerotiorum* (with a prevalence ranging from 6% to 11%), leaf spot disease caused by *Alternaria spp.* (with a prevalence of 2% to 7%), and gray mold diseases associated with *B. cinerea* (with a prevalence of 1% to 5%).

During all surveys, a total of 114 fungal isolates were obtained, 32 isolates were identified as *Fusarium* spp., 25 isolates as *R. solani*, 21 isolates as *S. sclerotiorum,* 19 isolates as *Alternaria* spp., 9 isolates as *B. cinerea*, and 8 isolates as *Stemphylum* spp.. According to observations and identification results, prevalence rates range from 3% to 11%, 1% to 8%, 1% to 7%, 1% to 4%, 2% to 7%, 1% to 3%, 1% to 2%, and <1% were recorded for *R. solani, S. sclerotiorum, F. solani, F. oxysporum, F. exquisite, Alternaria* spp., *B. cinerea*, and *Stemphylum* spp., respectively. Morphological identification of these isolates was confirmed by MALDI-TOF analysis results.

Figure 1. General wilting symptoms (arrows) associated with root and root collar rot on some seedlings, indicating the presence of fungal disease agents in *Brassica* planting areas in Hatay Province during the October 2020 surveys.

Şekil 1. Hatay ili önemli Brassica ekim alanlarında Ekim 2020 tarihinde yapılan sörveylerde bazı fidelerin köklerinde kök ve kökboğazı çürüklüğüne (ok) bağlı genel solgunluk belirtileri.

The isolates, initially characterized based on morphological features and MALDI-TOF, were conclusively identified using molecular methods employing universal primer pairs ITS1-4. Molecular analyses demonstrated that the morphologically identified isolates exhibited a remarkable similarity of 99.83-100% with sequences available in the NCBI database. Notably, among the obtained isolates, the following fungal species were molecularly identified and their sequence data deposited in GenBank: *Alternaria alternata* LAa21 (OM854806, 100% matched with the sequence of KU360613), *Sclerotinia sclerotiorum* LRs11 (OM867578, 100% matched with the sequence of MG516658), *Fusarium exquisite* LFe33 (OM883923, 100% matched with the sequence of MT601958), *Rhizoctonia solani* LRs5 (OM883982, 99.83% matched with the sequence of HF912172), *Fusarium solani* KFs9 (OM883985, 100% matched

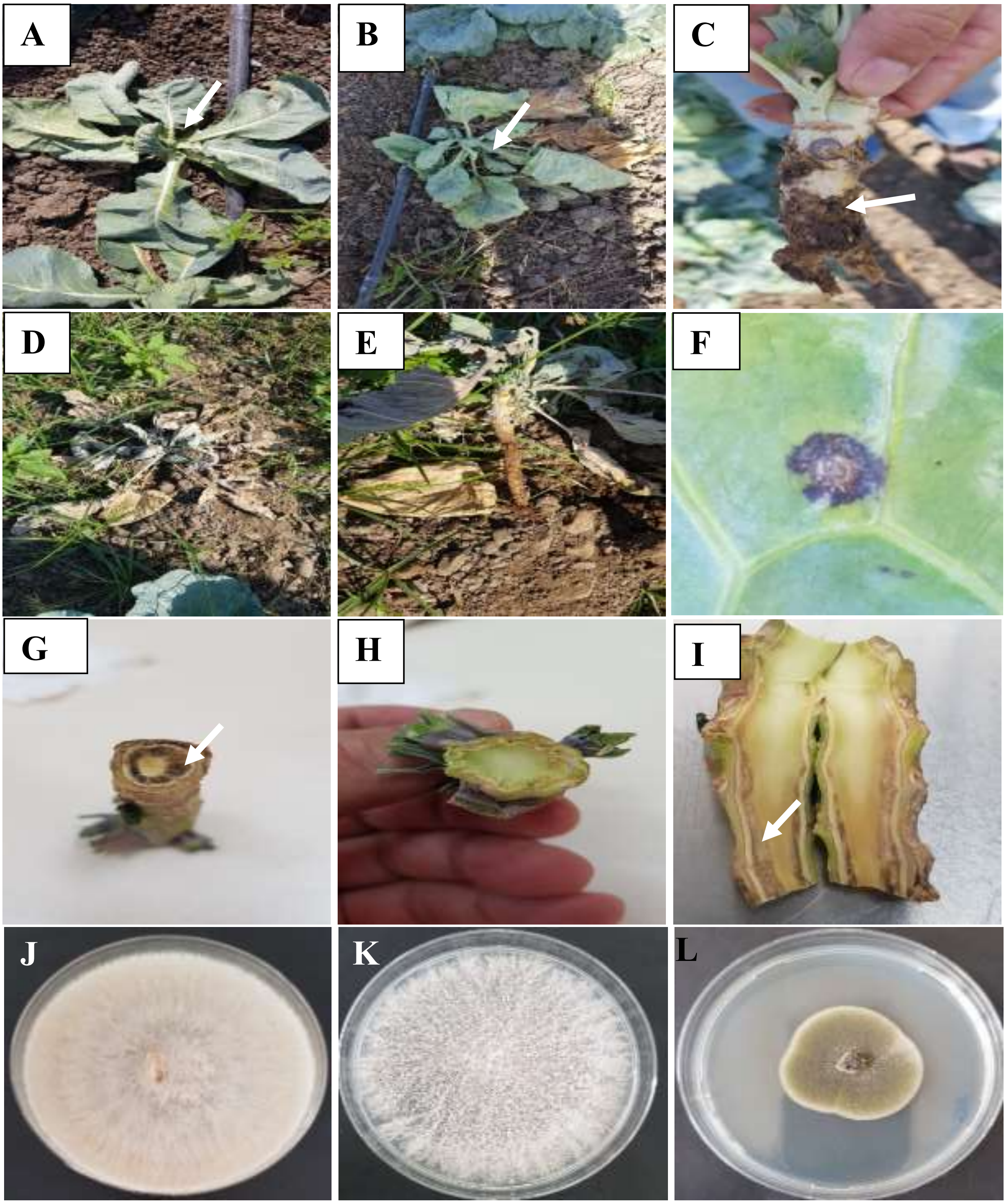

Figure 2. (A-E) Typical wilting symptoms associated with root thinning and root rot (indicated by arrows) were observed during surveys in significant *Brassica* cultivation areas in Hatay Province in November 2020. (F) Notable necrotic lesion symptoms were observed on leaves. (G-I) Darkening of the vascular bundles in stem cross-sections of plants displaying wilting symptoms (indicated by arrows). Petri dish images of isolates of *Rhizoctonia solani* (J), *Fusarium solani* (K), and *Alternaria alternata* (L) were obtained from tissues exhibiting disease symptoms.

Şekil 2. (A-E) Hatay ili önemli Brassica ekim alanlarında Kasım 2020 tarihlerinde yapılan sörveylerde gözlenen bitkilerin köklerinde incelme ve kök çürüklüğüne bağlı genel solgunluk belirtileri (ok); F, Yapraklarda gözlenen nekrotik leke belirtileri. ***G-I****, solgunluk belirtisi gösteren bitkilerin gövde kesitlerinde iletim demetlerindeki kararma belirtileri (ok). Hastalık belirtileri gösteren dokulardan elde edilen Rhizoctonia solani (J), Fusarium solani (K) ve Alternaria alternata (L) izolatlarına ait petri görüntüleri.*

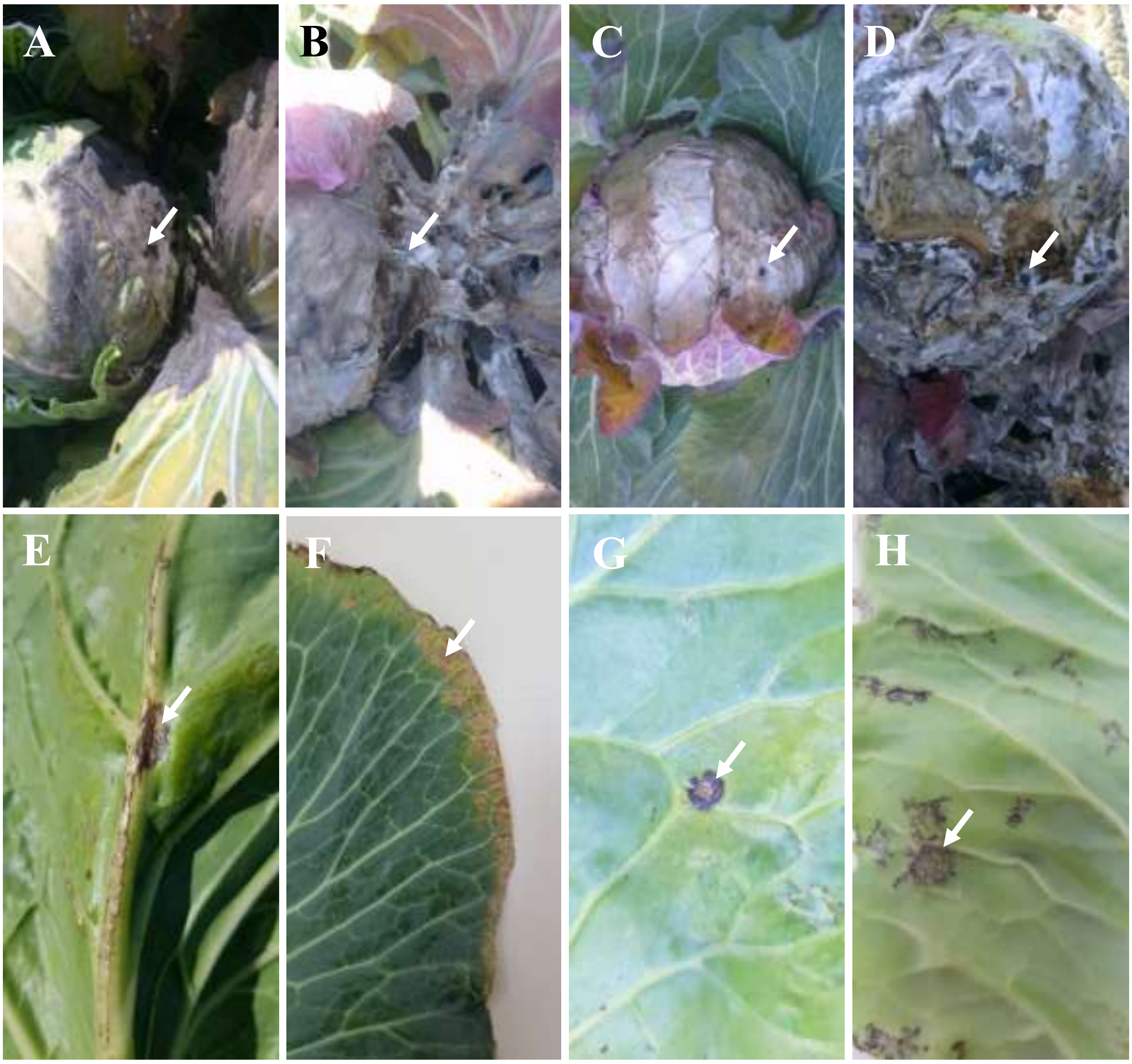

Figure 3. Diseases caused by different fungal pathogens identified in *Brassica* spp. cultivation areas of Hatay Province. (A) Abundant sporulation of gray mold disease caused by *Botrytis cinerea* on main heads and leaves (indicated by arrows). (B-D) Formation of sclerotia due to severe infection on cabbage heads and leaves, resulting from head rot disease caused by *Sclerotinia sclerotiorum* (indicated by arrows). (E-H) Disease symptoms induced by fungal pathogens, including Stemphylim sp. (E and F), *Alternaria alternata* (G), and *Rhizoctonia solani* (H), were observed on cabbage leaves (indicated by arrows).

Şekil 3. Hatay ili önemli Brassica ekim alanlarında yapılan sörveylerde tespit edilen hastalıklar. (A) Botrytis cinerea tarafından neden olunan gri küf hastalığının ana baş ve yapraklardaki yoğun sporulasyonu (ok). (B-D) Sclerotinia sclerotiorum tarafından neden olunan beyaz çürüklük hastalığının lahana baş ve yapraklardaki yoğun enfeksiyon sonucu oluşturduğu sklerotlar (ok). (E-H) Lahana yapraklarında Stemphylim sp., (E ve F) Alternaria alternata (G) ve Rhizoctonia solani (H) gibi fungal hastalık etmenleri tarafından neden olunan hastalık belirtileri (ok).

with the sequence of MG991246), *Fusarium oxysporum* KFo27 (OM883986, 100% matched with the sequence of KU528846), and *Stemphylum* sp. LSt33 (OM884463, 100% matched with the sequence of OK560128). The sequence of the *Stemphylum* sp. LSt33 isolate exhibited a 100% match with the sequence of *Stemphylium eturmiunum*, which is the causal agent of black spot disease in apples (OK560128), as confirmed by BLAST analysis.

During the last surveys conducted from March to April 2021, a distinct pattern emerged, highlighting variations in the prevalence of diseases caused by oomycete pathogens in leafy vegetables, such as rocket and garden cress, in comparison to the fungal and bacterial diseases observed in cabbage and cauliflower, affecting their leaves, shoots, and stems (Figure 4). The prevalence of white rust disease, caused by *A. lepidii*, was notably higher in garden cress plants, ranging from 14% to 55% (see Figure 4A). In contrast, downy mildew disease, caused by *P. lepidii*, exhibited relatively lower prevalence rates, ranging from 4% to

11% (see Figure 4C). This trend was also observed in rocket and radish plants, which share similar small planting areas with garden cress (Figure 4B and Figure 5). In rocket and radish plant cultivated areas, white rust disease, caused by *A. candida*, was common (Figure 4B and Figure 5). The prevalence rate of *A. candida* ranged from 25% to 70%, whereas downy mildew disease, caused by *P. lepidii* agent, exhibited a relatively lower prevalence, ranging from 9% to 18% in garden cress plants. Importantly, powdery mildew disease caused by *Erysiphe cruciferarum* was not detected during any of the surveys.

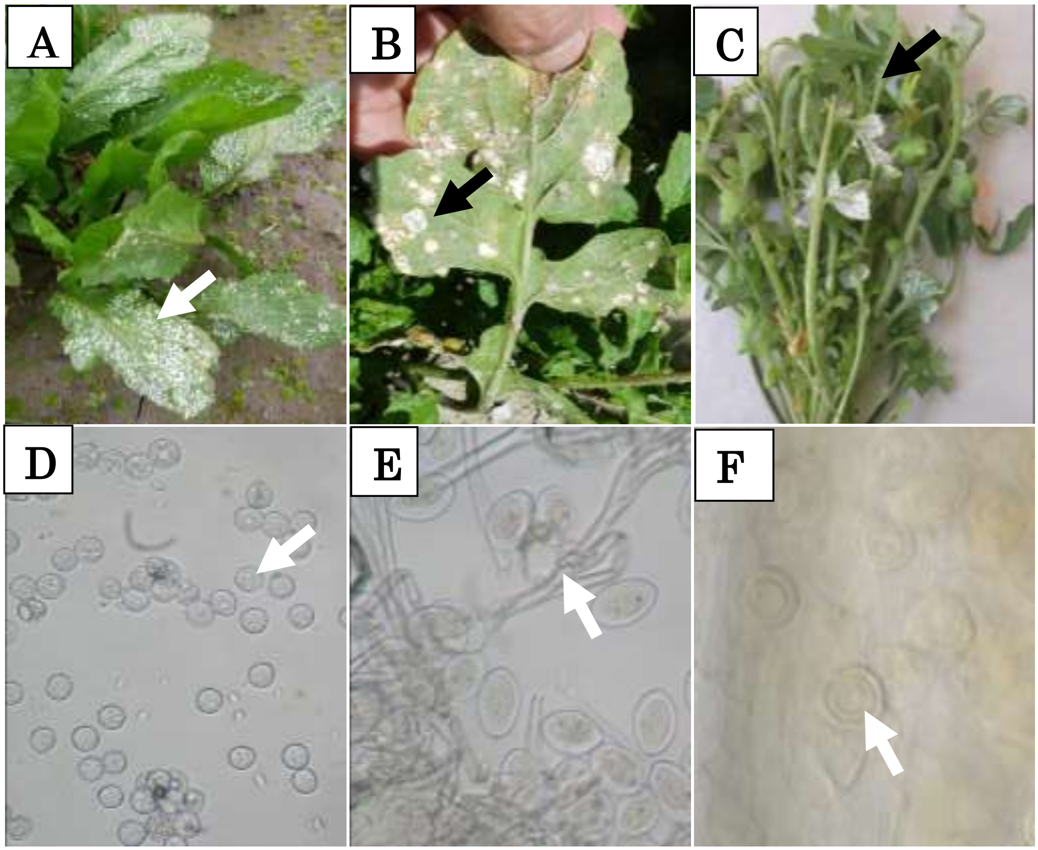

Figure 4. Typical disease symptoms (arrows) caused by the causal agents of white rust, *Albugo lepidii* (A), Albugo candida (B), and downy mildew, *Perofascia lepidii* (C), on leaves of garden cress (A, C) and rocket (B) plant surveyed in Hatay province. (D) Dense sporangia (arrow) of *Albugo*. (E) Conidiophores (arrow) and ellipsoidal-oval conidia are produced by the downy mildew pathogen, *Perofascia lepidii*. (F) Thick-walled oospores (arrows) produced by *Albugo lepidic* on leaf surfaces

Şekil 4. Hatay ilinde yapılan sörveylerde tere (A, C) ve roka (B) bitkilerinde beyaz pas hastalık etmeni Albugo lepidii (A), Albugo candida (B) ve (mildiyö hastalık etmeni Perofascia lepidii C) tarafından neden olunan tipik hastalık belirtileri (ok). (D) Albugo lepidii nin yoğun sporangiumları (ok). (E) Mildiyö hastalık etmeni Perofascia lepidii konidioforu (ok) ve elipsik-oval yapılı konidileri. (F) Albugo lepidii'nin yaprak üzerinde oluşturduğu kalın duvarlı oosporlar (ok)

During the surveys, unusual physiological disorders, characterized as abiotic stress-induced edema (Oedema), were also observed on cabbage leaves in only three fields in the Arsuz and Kırıkhan districts (Figure 6). Edema represents a physiological anomaly that arises in conditions where air temperatures are cooler than the soil, and there is a high relative humidity discrepancy between the soil and the atmosphere. This phenomenon heightens cell turgor by promoting water uptake, diminishes transpiration rates, leads to the rupture of epidermal cells, facilitates the expansion of underlying cells, and ultimately culminates in the formation of wart-like outgrowths. These outgrowths, although mimicking symptoms induced by parasitic organisms, ultimately result in cellular necrosis and discoloration.

In the surveyed fields, beyond the presence of fungal diseases, symptoms indicative of black rot, caused by *X. campestris* pv. *campestris* (with a prevalence ranging from 1% to 7%), as well as soft rot disease caused by diverse bacterial species (ranging from 1% to 13%), were observed on the leaves, stems, heads, and tubers of cabbage, broccoli, cauliflower, and radish plants (Figure 7 and Figure 8). Black rot symptoms were typically characterized by V-shaped wilting along the margins of the cabbage leaves, encircled by a yellow discoloration around the affected area, and the presence of darkened veins (Figure 7A, B).

Subsequently, 17 isolates were obtained from infected plant leaves of white-headed cabbage and identified using MALDI-TOF.

Figure 5. (A) Black necrotic lesions (arrow), caused by *Stemphylium* sp., were observed on radish tubers during surveys conducted in the significant *Brassica* cultivation regions of Hatay Province. (B-C) Distinctive disease symptoms manifest on radish plant leaves caused by *Albugo candida* (arrow).

Şekil 5. (A) Hatay ili önemli Brassica ekim alanlarında yapılan sörveylerde turp yumrularında Stemphylium sp. tarafından neden olunan siyah nekrotik çöküntüler (ok). (B-C) Turp bitki yapraklarında beyaz pas hastalık etmeni Albugo candida tarafından neden olunan tipik hastalık belirtileri (ok)

Figure 6. Typical edema (Oedema) symptoms, caused by abiotic factors, in cabbage leaves grown in the *Brassica* cultivation regions of Hatay Province.

Şekil 6. Hatay ili önemli Brassica ekim alanlarında yapılan sörveylerde lahana yapraklarında abiyotik etkenlerden kaynaklanan ödem (Oedema) belirtileri (ok)

Using MALDI-TOF analysis, the identification of 56 pure colonies, isolated from the root collars, stems, and heads of white cabbage and cauliflower plants exhibiting soft rot symptoms, confirmed the prevalence of primary causative agents responsible for soft rot disease, including *P. caratovorum* subsp. *caratovorum* (15 isolates), *P. parmentieri* (8 isolates), *P. victoria* (7 isolates), and *P. corrugata* (6 isolates), which were recorded at a prevalence of 4% to 13%. Secondary opportunistic bacterial agents, such as *P. agglomerans* (6 isolates), *P. fluorescens* (4 isolates), *P. marginalis* (3 isolates), *E. cloacae* (4 isolates), and *B. pumilus* (3 isolates), were detected at a prevalence rate of less than 1%.

All bacterial isolates were subjected to pathogenicity tests on their respective host plants. *P. caratovorum, P. parmentieri, P. victoria,* and *P. corrugata* caused typical soft rot disease symptoms on artificially inoculated cabbage, broccoli, cauliflower, and radish plants (Figure 9). Opportunistic secondary soft rot disease agents *P. agglomerans, P. fluorescens, P. marginalis, E. cloacae,* and *B. pumilus*, however, did not cause typical soft rot disease symptoms at the inoculation sites (Figure 10).

According to literature records, there is limited research focusing on the identification of fungal diseases, primarily affecting *Brassicaceous* vegetables such as cabbage (white and red), cauliflower, and broccoli in Turkey. The results obtained in this study revealed the prevalence of various fungal pathogens in different districts of Hatay Province, where cabbage,

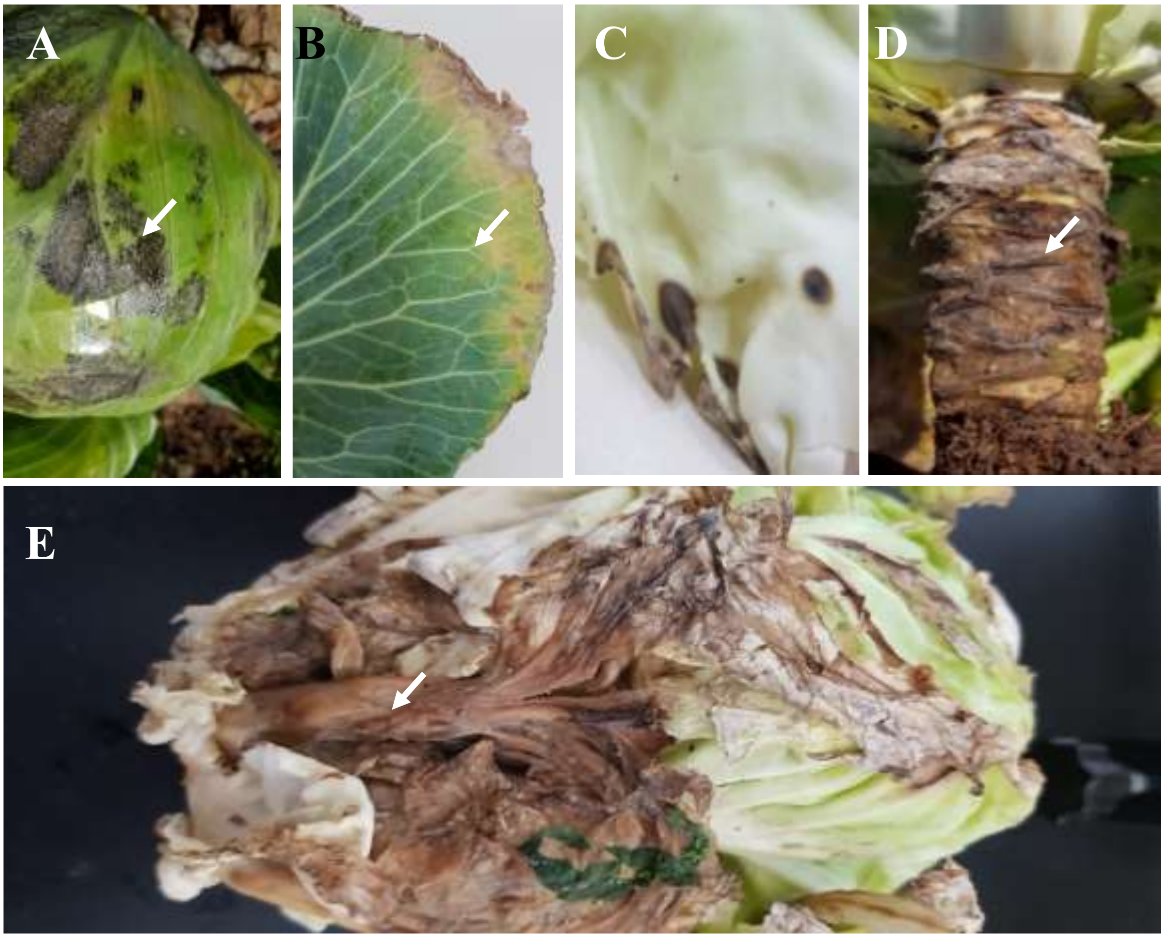

Figure 7. Disease Symptoms on cabbage leaves from surveyed *Brassica* cultivation areas in Hatay Province. (A-B) Symptoms caused by *Xanthomonas campestris* pv. *campestris*, (C) Symptoms associated with *Pantoaea agglomerans*. Typical symptoms of soft rot disease caused by *Pectobacterium* spp. and *Pseudomonas* spp. on cabbage stems (D) and heads (E) are indicated by arrows.

Şekil 7. Hatay ili önemli Brassica ekim alanlarında yapılan sörveylerde lahana yapraklarında (A-B) Xanthomonas campestris pv. campestris ve Pantoaea agglomerans (C) tarafından neden olunan hastalık belirtileri. Lahana kök boğazı (D) ve başlarda (E) farklı Pectobacterium spp., Pseudomonas spp., türleri tarafından neden olunan yumuşak çürüklük hastalık belirtileri (ok)

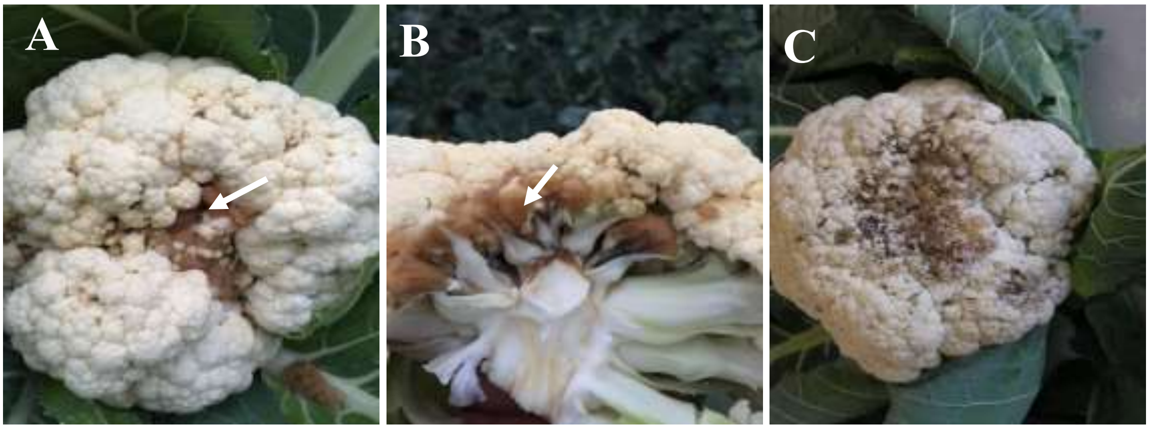

Figure 8. Soft rot disease symptoms (arrows) caused by different *Pectobacterium* spp. in cauliflower heads in the surveyed *Brassica* cultivation areas of Hatay Province.

Şekil 8. Hatay ili önemli Brassica ekim alanlarında yapılan sörveylerde Karnabahar başlarında farklı Pectobacterium spp. tarafından neden olunan yumuşak çürüklük hastalık belirtileri (ok)

Figure 9. Typical soft rot disease symptoms caused by different *Pectobacterium* spp. and *Pseudomonas* spp. on various *Brassica* spp. during pathogenicity tests.

Şekil 9. Hatay ili önemli Brassica ekim alanlarında yapılan sörveylerde farklı Pectobacterium spp. ve Pseudomonas spp. tarafından farklı Brassica türleri üzerinde yapılan patojenite testlerinde oluşturdukları tipik yumuşak çürüklük hastalık belirtileri (ok)

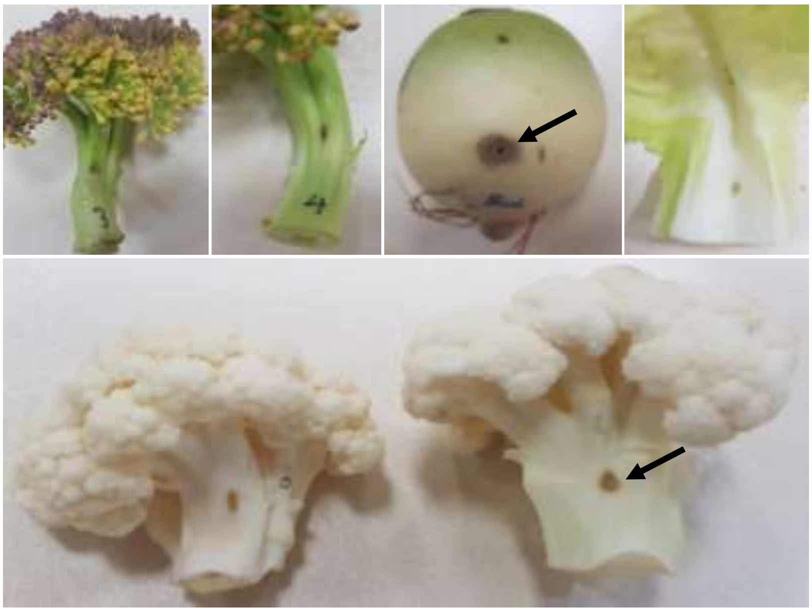

Figure 10. Atypical disease symptoms (arrow) caused by opportunistic bacterial isolates of *Pantoae agglomerans, Pseudomonas fluorescens, Pseudomonas marginalis, Enterobacter cloacae,* and *Bacillus pumilis* on various *Brassica* spp. during pathogenicity tests.

Şekil 10. Hatay ili önemli Brassica ekim alanlarında yapılan sörveylerde yumuşak çürüklük belirtilerinden izole edilmiş Pantoae agglomerans, Pseudomonas fluorescens, Pseudomonas marginalis, Enterobacter cloaceae ve Bacillus pumilis izolatları tarafından farklı Brassica türleri üzerinde yapılan patojenite testlerinde oluşturdukları atipik hastalık belirtileri (ok)

brccoli, and cauliflower are cultivated. Morphological and molecular results confirmed that soil-borne fungal pathogens such as *R. solani*, *S. sclerotiorum*, *F. solani*, *F. oxysporum*, *F. equiseti*, and *A. alternata* were major causal disease agents on various plants. Notably, oomycete white rust disease agents *A. candida* and *A. lepidii*, along with the downy mildew pathogen *P. lepidii*, were identified as major pathogens affecting leafy *Brassicaceous* vegetables such as rocket, garden cress, and radish. Previous studies have reported the existence of different anastomosis groups of *R. solani* in cabbage cultivation areas in the Black Sea region

(Erper et al., 2017; Türkkan et al., 2020, Saygı et al., 2020; Benli et al., 2021; Erper et al., 2021). *S. sclerotiorum* has been identified as the causative agent of head rot in cabbage crops in Erzurum Province (Tozlu et al., 2016). Recent studies have also reported the presence of *A. candida* on rocket and garden cress in Ankara and Eskişehir (Canpolat & Tülek, 2019), *A. lepidii* and *P. lepidii* on garden cress plants in Hatay Province (Soylu et al., 2017; Soylu et al., 2019).

To the best of our knowledge, *A. alternata* LAa21 (OM854806), *F. equiseti* LFe33 (OM883923), *F. solani* KFs9 (OM883985), *F. oxysporum* KFo27 (OM883986), and *Stemphylium* sp. LSt33 (OM884463) have been identified as causal agents in different *Brassicaceous* vegetables cultivated in Turkey for the first time. The presence of *Fusarium equiseti* in cabbage plants grown in Korea (Afroz et al., 2021), *F. oxysporum* and *F. solani* in cabbage plants grown in Egypt (Khafagi et al., 2018) and China (Yan et al., 2018) and *S. sclerotiorum* in cabbage and canola crops in Sri Lanka and Iran (Yousefdoost & Ghosta, 2013; Mahalingam et al., 2017; Khan et al., 2022) has been previously reported during routine disease surveys.

In addition to fungal diseases, *Brassicaceous* plants are also susceptible to various bacterial pathogens. Among the bacterial pathogens known to affect *Brassicaceous* plants, *Pectobacterium* spp. and *Pseudomonas* spp. are associated with soft rot disease, *P. syringae* pv. *maculicola* causes leaf spot disease and *X. campestris* pv. *campestris* is responsible for black rot disease (Rimmer et al., 2007). The present study also investigated the presence of bacterial diseases in different *Brassicaceous* vegetable crops cultivated in Hatay Province, Turkey. The identification of these bacterial species was accomplished through a combination of biochemical analyses and MALDI-TOF analyses. *X. campestris* pv. *campestris* was isolated from the leaves of cabbage plants. This pathogen was more prevalent in fields with high groundwater levels, especially in late-harvested fields. Isolates of *Pectobacterium* spp., *Pseudomonas* spp., *P. agglomerans, E. cloacae,* and *B. pumulis* were identified from white cabbage, cauliflower, broccoli, and radish plants displaying symptoms of soft rot disease. To assess the pathogenicity of all bacterial isolates obtained from soft rot symptoms, experiments were conducted on both the plant species from which they were isolated and on potato slices. This comprehensive approach allowed us to determine the virulence of these isolates. Following the inoculation process and subsequent re-isolation from *Brassicaceous* vegetable crops and potato slices, pure colonies were examined and their species were reconfirmed. This rigorous methodology ensured the accuracy of our findings regarding the pathogenicity of these bacterial isolates.

Previous studies have documented the presence of *X. campestris pv. campestris* (Mirik et al., 2008; Aksoy et al., 2018; Öztürk et al., 2019; Öztürk & Soylu, 2021) and *P. caratovorum* (Aksoy et al., 2017; Öztürk & Soylu, 2022) on *Brassica* spp growing in various provinces of Turkey, including Adana, Samsun, and Yozgat. This study represents a significant contribution as it is the first report of primary soft rot pathogens such as *P. parmentieri, P. victoria*, and *P. corrugata*. Additionally, this is also the first report of opportunistic (secondary) soft rot pathogens, including *P. agglomerans, P. fluorescens, P. marginalis, E. cloacae,* and *B. pumilis* on *Brassica* spp growing in Turkey. *P. parmentieri, P. victoria,* and *P. corrugata* have been previously documented as primary soft rot disease agents in various vegetables in Turkey (Mirik et al., 2011; İmriz and Çınar, 2015; Öztürk et al., 2018; Soylu et al., 2022). Similarly, *P. agglomerans* has been reported to cause soft rot diseases in Napa cabbage in China (Guo et al., 2019). Furthermore, *P. fluorescens, P. marginalis,* and *E. cloacae* have been reported in various vegetables displaying soft rot symptoms worldwide, including Turkey (Godfrey & Marshall, 2002; Koike et al., 2007; Mikiciński et al., 2010; Hausdorf et al., 2011; Achbani et al., 2014; Dadaşoğlu & Kotan, 2017; García-González et al., 2018; Soylu et al., 2022).

CONCLUSION

This study conducted a comprehensive investigation into the identification, prevalence, and incidence of fungal, oomycete, and bacterial pathogens in various *Brassicaceous* plants, which hold significance as minor vegetables cultivated in the Hatay province of Turkey. Among the fungal pathogens examined, it is noteworthy that *A. alternata* LAa21 (OM854806), *F. equiseti* LFe33 (OM883923), *F. solani* KFs9 (OM883985), *F. oxysporum* KFo27 (OM883986), and *Stemphylum* sp. LSt33 (OM884463) were identified as the causal agents of diseases on *Brassicaceous* species grown in Turkey for the first time. This expands our knowledge of the diversity of pathogens affecting these crops. In addition to the fungal agents, the results of this study also revealed the presence of bacterial pathogens. Among the bacterial pathogens examined, *P. parmentieri, P. cichorii,* and *P. corrugata,* along with opportunistic (secondary) soft rot pathogens like *P. agglomerans, P. fluorescens, P. marginalis, E. cloacae,* and *B. pumilis*, were determined as causative bacterial disease agents on *Brassicaceous* vegetable plants for the first time in Turkey. The identification of these previously unreported disease agents is of significant importance. Neglecting to implement necessary precautions against these pathogens could potentially result in substantial yield and quality losses in the region in the forthcoming periods.

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Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

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