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Development of Pathogen *Ascochyta* Species of Wild Legumes in Different Media

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Abstract
Ascochyta spp., which are plant fungal pathogens in the order Ascomycota infect many legume species, causing Ascochyta blight disease and serious yield and quality loss in plants. The disease agent causes typical necrosis and cell death in the aboveground parts of the plant. Wild forms of many legumes, which are among the plants infected by *Ascochyta* spp., are important genetic resources in the domestication process and are very important in terms of genetic diversity. Genetic resources are affected and destroyed by abiotic effects such as erosion, flood, fire, etc., natural disasters, and by biotic effects from some important plant pathogens, such as *Ascochyta* spp. Since it also affects cultivated plants and causes serious yield losses, it is necessary to obtain more information about the development process and conidia formation ability of *Ascochyta* spp., which evolved together with the wild form of the plant during the cultivation process. In this study, it was observed whether 107 isolates of *Ascochyta* spp. isolated from *Vicia* spp., *Pisum sativum*, *Lens* spp. and *Lathyrus* spp. developed hyphae and formed conidia in different media [potato dextrose agar (PDA), chickpea seed meal dextrose agar (CSMDA), potato dextrose broth (PDB)]. While 32, 10, 7 and 8 of the isolates of *Vicia* spp., *Pisum sativum*, *Lens* spp. and *Lathyrus* spp., inoculated in PDA medium, exhibited hyphal growth, and 18, 5, 10 and 6 formed conidia as well as hyphal growth. All of the conidia-forming and non conidia-forming isolates in PDA were also inoculated in CSMDA medium, and 24, 5, 10, and 10 of the isolates formed hyphal growth, respectively, while 26, 10, 7, and 4 formed conidia as well as hyphal growth. It was observed that 11, 3, 5 and 2 isolates formed conidia in both media (PDA and CSMDA). Of the 54 isolates of *Vicia* spp. (25), *Pisum sativum* (9), *Lens* spp. (13) and *Lathyrus* spp. (7) grown in PDB medium, 14, 5, 8, and 5 exhibited only hyphal growth, and 11, 4, 5, and 2 showed only conidial growth, respectively. It is thought that the data obtained may form a basis for studies in the field of plant diseases.

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1.Introduction

Plant food sources have been the basis of human nutrition since ancient times (Obuseng et al., 2022). In addition to the experiencing global climate change in today's world, the rapid increase in population and the emergence of food starvations also increase the production of legumes with high plant-based-nutrient contents (Ray et al., 2015). Legumes are one of the first choices in terms of environmentally friendly sustainable food sources and nutritionally healthy diets due to their contribution to fossil energy conservation, carbon sequestration and reduction of greenhouse gas emissions thanks to the fixation of nitrogen in the soil in terms of agriculture (Bessada et al., 2019). However, today's food safety and security problems are a global problem of great importance. Interest in pulses is increasing daily due to their contribution to reducing global problems and ensuring the food supply (Oguiba et al., 2023). For this purpose, the cooperation of agriculture and food sustainability strategies with technology allows to be obtained much more efficient products (Rehman et al., 2022) and helps to solve problems and reduce worries (Kahraman, 2023).

Turkey, located in the Fertile Crescent lands, which is the homeland of many legumes, holds a significant position for ancestral species. While Turkey has achieved a prominent global ranking in some product groups (such as lentils and chickpeas) (FAOSTAT, 2021), it struggles with some challenges in the production and yields of these crops (Aydın, 2016; Keçeli, 2023). Some of the main reasons for these challenges are attributed to certain abiotic and biotic factors (Polatbilek et al., 2017; Kızılok et al., 2019). Fungal factors, one of the biotic stress factors, are at the top of the priority problems of plant diseases and are considered a major concern (Akveç et al., 2018; Altınok et al., 2023). Additionally, plant diseases, which play a crucial role in plant pathology, also constitute an important part of plant protection and breeding (Foresto et al., 2023).

Learning and understanding the genetic processes of plant populations are a source of fundamental information that guides plant pathogen evolution, which is very important for plant pathologists and evolutionary biologists. There is not enough information in the literature on the genetic makeup of populations of pathogens that cause wild plant infections in the natural ecosystem, including the studies carried out in this context (Bradley et al., 2008; Treindl et al., 2023). In the natural ecosystem of plant communities, many plants coexist and are exposed to diseases together. Differences in disease prognosis lead to different results in the infection of plant species with a convergent history with similar disease agents. Because host–pathogen interactions are phylogenetically conserved, traditionally the first considerations have been that similar results will be obtained (Gilbert and Webb, 2007). The distributions of host–pathogen genetic diversity within and between their populations can be explained by genetic diversity and genetic differentiation. These terms give us an idea about their evolutionary history, geographic distribution and disease dynamics, as well as playing a major role in determining the evolutionary potential of host–pathogen species that interact with each other (Treindl et al., 2023).

Many agricultural crops worldwide are highly susceptible to fungal plant pathogens. Ascochyta blight disease caused by *Ascochyta* spp., is seen as one of the most important biotic factors globally, which prevents production in many agricultural products (pea, chickpea, lentil, and *Vicia faba*) and causes yield losses at different rates depending on the products (Chasti et al., 2022; Singh et al., 2022; Foresto et al.,

2023; Oguiba et al., 2023; Talapov et al., 2023). Ascochyta blight, which is a devastating seed-borne disease, causes symptomatic spotting and drying of all aboveground parts of the host plants, and its progressive stages result in the death of the plants. As a source of inoculum (infected plant residue, mycelium, conidia, ascospores, etc.), *Ascochyta* spp., which experience harsh winter conditions in the agricultural field, can make the planted crops sick again under suitable conditions or remain in the soil until the appropriate host and condition.

Climate has many effects, from pathogens to hosts, host–pathogen interactions, and even host-infection stages. It is well known that the spread of *Ascochyta* spp. is much faster, especially in agricultural areas with humid and cool climate conditions (Pande et al., 2005; Vail and Banniza, 2008; Manjunatha et al., 2021). Thus, when *Ascochyta* spp. encounters suitable hosts in suitable conditions and climates, they make plants diseased and act as sources of yield loss (Nalcaci et al., 2021). Due to various factors such as these, *Ascochyta* spp. as a global threat can also infect ancestral wild plants similar to cultivars. Some genetic resources of ancestral plants, which are of great importance for genetic diversity, during the first cultivation stage are affected by many abiotic and biotic factors and are reduced or lost (Rajpal et al., 2023; Salgotra and Chauhan, 2023). Since it is thought that *Ascochyta* spp. has coevolved with the wild form of the plant, more information is needed about the growth process of *Ascochyta* spp., and its ability to form conidia at the stages of obtaining cultivated plants. The fact that the disease is associated with many host plants, the increase in yield losses and the decrease in the resources required for adequate nutrition every day lead human beings to an unavoidable concern. In addition, the variable nature of *Ascochyta* spp. (existence of sexual and asexual forms) is highly effective on virulence and genetic diversity, especially pathogenicity (Rhaiem et al., 2008; Atik et al., 2011). Such factors are some of the factors that complicate the management of Ascochyta blight diseases (Manjunatha et al., 2018).

Detection of the pathogen causing the disease is very important in terms of the effectiveness of disease management in plant protection and breeding. *In*–vitro applications help facilitate hyphal and conidial growth as well as direct detection, in an optimum area, due to the determination of all requirements of pathogens (Kosiada, 2012). Thus, the disease will be detected, and results that will form the basis for *in*–vivo applications can be obtained. Thanks to the support of all the data obtained and the combination of expert opinions, it helps to develop disease-causing conditions, disease prognoses, disease prevention and disease management strategies. However, the difficulties in determining the effect of environmental properties on pathogen-related properties with *in*–vivo applications highlight the importance of much more research for the standardization of *in*–vitro applications about the growth of disease caused by *Ascochyta* spp. (Endes, 2021).

In–vitro culturing of fungi, preserving their biodiversity, microscopic examinations and biochemical-physiological characterizations, etc., require special media to carry out studies. Additionally, the use of two or more media instead of one in the standardization of applications is an important factor in obtaining meaningful results in the morphological characterization of fungal disease diagnoses to examine many colony-related features (Sharma and Pandey, 2010). In studies carried out *in*–vitro under experimental conditions, the differentiation of media and the use of these different media are very important in terms of

phytopathology studies. These media, which are used for the morphological determination of diseases obtained from different plant species, can also form developmental differentiations in different media due to the differentiations within the species. The content of the media, light/dark environment conditions and temperature changes in addition to pH cause differences in the detection of fungal diseases. Especially within the scope of many studies on the detection of the disease within the species, it has been determined that the temperature has great effects. On the other hand, the necessity of light in conidial development for most fungi is emphasized, and it is known that it is also widely used. However, it has been determined within the scope of the studies that it encourages the growth of pycnidia in dark conditions as well as bright conditions. In addition, it is stated that there is only pycnidium growth under certain developmental conditions and that pycnidium growth is affected by these differences and plays a decisive role in the morphological determinations of the disease. In this manner, it reveals the necessity of determining the environmental factors that play a dependent and effective role in the growth of isolates for the diagnosis of diseases (Kosiada, 2012).

On the other hand, detecting the pathogen causing the disease with a fast, accurate and inexpensive method is a critical first step with the purpose of realizing effective disease management. Concentric necrotic rings formed by dark brown pycnidia known as raised spots are one of the most prominent symptoms in the detection of infected plants (leaf, stem, capsule and even seed surface, etc.) (Markell et al., 2008). However, during the diagnosis of diseases, morphological (colonial and conidial growth, pycnidia and pseudothecia examinations, etc.) and physiological evaluation of the pathogen is not always a method that can discriminate (Manjunatha et al., 2021). Diagnosing diseases with such traditional methods is one of the most frequently used methods in the field of plant pathology, although it is time consuming, challenging and requires expertise (Ahmed et al., 2015). In contrast, molecular techniques used in the field of plant pathology are the focus of attention today and in the future due to their potentially rapid, reliable and reproducible properties (Demirel et al., 2022; Bulat et al., 2023). A good understanding of the host range of *Ascochyta* spp. is the one of the great importance in the management of Ascochyta blight in places where the disease is endemic or where the primary infection is due to ascospores. Therefore, the management of Ascochyta blight relies on part on the host plants diversity, and also appropriate identification of the basis for variation in cross-pathogenicity (Barilli et al., 2016; Foresto et al., 2023).

In today's world, the diagnosis of plant diseases and the development of management strategies are extremely important issues to meet the increasing food demand and protect plant diversity from diseases. In addition, the importance of collecting, conserving and sustainably using of plant genetic resources, together with their discovery, to overcome current and future global problems is undeniable. Every study and information to be done on this subject is very necessary and has great value. Moreover, contrary to the increasing studies on wild chickpea genotypes, it is thought that this study will be considered to be the basis for the studies carried out both for the purpose of eliminating the deficiencies in the literature for the diagnosis, detection, identification (morphological, pathogenic, and genetic) and management of *Ascochyta* spp. as the cause of disease and yield losses in wild legumes (*Vicia* spp., *Pisum* spp., *Lens* spp. and *Lathyrus* spp.), and the data obtained from the morphological growth examinations of *Ascochyta* spp. for applications in the field of plant diseases.

2. Materials and Methods

2.1.Preparation for morphological examination of *Ascochyta* spp.

2.1.1. Fungal material

Within the study, a total of 107 *Ascochyta* spp. isolates obtained from wild legumes and found in the stocks of the Gaziantep University Biology Department, given in Table 1, were used.

2.1.2. Media

To observe the conidial and hyphal growth of *Ascochyta* spp. isolates, ½ PDA (potato dextrose agar), CSMDA (chickpea seed meal dextrose agar) and PDB (potato dextrose broth) after the media was prepared, it was sterilized (121 °C 15') and used.

2.1.2.1. PDA medium

½ PDA medium (g/L) was prepared by adding 24 g Agar to 19,5 g Potato Dextrose Agar and dissolving in ~100 mL sdH_2O to make up to 1 L.

2.1.2.2. CSMDA Medium

CSMDA medium (g/L) was prepared by adding 20 g Agar to 20 g Chickpea Seed Meal and 20 g Glukoz and dissolving in ~100 mL sdH_2O to make up to 1 L.

2.1.2.3. PDB Medium

PDB medium (g/L) was prepared by dissolving 24 g Potato Dextrose Broth in ~100 mL sdH_2O to make up to 1 L.

2.2. Growth of *Ascochyta* spp. in different media

2.2.1. PDA

Prepared sterile ½ PDA medium was poured into ~10 mL Petri dishes (sterile, 90 x 17 mm) and exposed to UV after freezing. The isolates in Table 1 were transferred to the medium and incubated at 22 ±2 °C in a dark condition incubator (Nüve, Turkey). After each of the growing isolates (~20 days) was examined in terms of hyphal growth, it was also examined whether each isolate showed conidial growth in the medium using a binocular light microscope (Zeiss PrimoStar, Germany). Isolates that did not grow in PDA medium were not examined.

2.2.2. CSMDA

Sterile ½ PDA medium was poured into ~10 mL Petri dishes (sterile, 90 x 17 mm) and exposed to UV after freezing. The isolates in Table 1 were transferred to the medium and incubated at 22 ±2 °C in an incubator. After incubation (~20 days), the growth of each isolate was examined hyphally and conidially. One isolate from each genus showing conidial growth [21 ERG KSTŞ VA2 (*Vicia* spp.), PS3 (*Pisum sativum*), YB90-27 LA 03 (*Lathyrus* spp.), 02 BSN 02/5 (*Lens* spp.)] was examined under a light microscope (Leica, Germany), and their conidial dimensions were determined.

2.2.3. PDB

The PDB medium, which was used as liquid medium, was sterilized and then exposed to UV after adding ~50 mL of medium into sterile sample containers (100 mL). After reculturing the isolates in Table 1 in PDA, 3-4 explants were taken and left in the medium, and sterile sample containers were incubated for ~10 days in an orbital shaker (Gerhardt, Germany) at room temperature. Each isolate was examined to determine its hyphal and conidial growth following incubation. Thirty–two isolates showing hyphal growth in PDB medium were not examined for conidial growth.

Table 1. Isolates used in examinations of the morphological growth of *Ascochyta* spp.

PLANT SPECIES	LOCATIONS	ISOLATE NAME
Pisum sativum	Kayatepe/Savur/Diyarbakır	21 KYTP SVR PS
	Hatunköy/Sivrice/Elazığ	23 HTKY SVRC PS1
	Hatunköy/Sivrice/Elazığ	23 HTKY SVRC PS2
	Hatunköy/Sivrice/Elazığ	23 HTKY SVRC PS3
	Hatunköy/Sivrice/Elazığ	23 HTKY SVRC PS4
	Çermik/Diyarbakır	21 ÇRMK/KK-1 PS
	Gaziantep	YB55-27 PS 01
	Gaziantep	YB56-27 PS 02
	Gaziantep University Stocks	PS1
		PS2
		PS3
		PS4
		PS5
		PS6
		PS7
Vicia sativa	Oyalı/Besni/Adıyaman	02 BSN OYL VS1
	Oyalı/Besni/Adıyaman	02 BSN OYL VS2
	Kesentaş/Ergani/Diyarbakır	21 ERG KSTŞ VS
	Dargeçit/Barıştepe/Mardin	47 DRGT-BRTP VS1
	Ekindüzü/Pervari/Siirt	56 EKDZ PRV VS
	Gaziantep	YB1-27 VS 01
	Gaziantep	YB2-27 VS 02
	Gaziantep	YB3-27 VS 03
	Gaziantep	YB4-27 VS 04
	Gaziantep	YB8-27 VS 05
	Gaziantep	YB9-27 VS 06
	Gaziantep	YB10-27 VS 07
	Gaziantep	YB11-27 VS 08
	Gaziantep	YB12-27 VS 09
	Gaziantep	YB13-27 VS 10
	Gaziantep	YB14-27 VS 11
	Gaziantep	YB15-27 VS 12
	Gaziantep	YB16-27 VS 13
	Gaziantep	YB17-27 VS 14
	Gaziantep	YB18-27 VS 15
Vicia anatolica	Köklüce/Gerger/Adıyaman	02 KKLC GG VA
	Karadut Köyü/Adıyaman	02 KRDT KHT VA1
	Karadut Köyü/Adıyaman	02 KRDT KHT VA2
	Kesentaş/Ergani/Diyarbakır	21 ERG KSTŞ VA
	Kesentaş/Ergani/Diyarbakır	21 ERG KSTŞ VA2
	Kayatepe/Savur/Diyarbakır	21 SVR KYTP VA
	Ekindüzü/Pervari/Siirt	56 EKDZ PRV VA
Vicia cassiae	Yeşilova/Burdur	15 YŞV VC
	Hatunköy/Sivrice/Elazığ	21 HTKY SVRC VC
	Hazar Gölü/Elazığ	23 HZRG VC
	Sarıkaya/Midyat/Mardin	47 MDYT SRKY VC1
	Sarıkaya/Midyat/Mardin	47 MDYT SRKY VC2-1
	Sarıkaya/Midyat/Mardin	47 MDYT SRKY VC2-2
	Sarıkaya/Midyat/Mardin	47 MDYT SRKY VC2-3
	Sarıkaya/Midyat/Mardin	47 MDYT SRKY VC3
	Gaziantep	YB45-27 VC 01
	Gaziantep	YB46-27 VC 02
Vicia narbonensis	Sarıkaya/Midyat/Mardin	47 MDYT SRKY VN
	Ömerli/Anıttepe/Mardin	47 ÖMRL ANTP VN1
	Ömerli/Anıttepe/Mardin	47 ÖMRL ANTP VN1/1
	Ömerli/Anıttepe/Mardin	47 ÖMRL ANTP VN1/2
	Ömerli/Anıttepe/Mardin	47 ÖMRL ANTP VN1/3
	Ömerli/Anıttepe/Mardin	47 ÖMRL ANTP VN2
	Ömerli/Anıttepe/Mardin	47 ÖMRL ANTP VN2-2
	Ömerli/Anıttepe/Mardin	47 ÖMRL ANTP VN2/1

	Ömerli/Anıttepe/Mardin	47 ÖMRL ANTP VN2/2
Vicia narbonensis L. var.	Gaziantep	YB25-27 VN 01
Vicia palaestina	Ömerli/Anıttepe/Mardin	47 ÖMRL ANTP VP
Vicia palaestina Boiss.	Gaziantep	YB23-27 VP 01
	Gaziantep	YB24-27 VP 02
Vicia villosa	Pervari/Uzunca Dağı/Siirt	56 PRV UZDĞ VV
Vicia galilaea	Oyalı/Besni/Adıyaman	02 BSN OYL VG
Vicia galilaea Plitmann & Zohary	Gaziantep	YB47-27 VG 01
	Gaziantep	YB48-27 VG 02
	Gaziantep	YB49-27 VG 03
	Gaziantep	YB50-27 VG 04
	Gaziantep	YB51-27 VG 05
	Gaziantep	YB52-27 VG 06
	Gaziantep	YB53-27 VG 07
Vicia hybrida L.	Gaziantep	YB7-27 VH 01
Lens spp.	Besni/Adıyaman	02 BSN 01/4
	Besni/Adıyaman	02 BSN 02/5
	Besni/Adıyaman	02 BSN 03/1
	Besni/Adıyaman	02 BSN 04/2
	Besni/Adıyaman	02 BSN 05/4
	Besni/Adıyaman	02 BSN 06/9
Lens culinaris	Midyat/Gercüş/Mardin	47 MDYT GRCŞ LeCu
Lens culinaris subsp. *orientalis*	Gaziantep	YB66-27 LeCu 01
	Gaziantep	YB67-27 LeCu 02
	Gaziantep	YB68-27 LeCu 03
	Gaziantep	YB69-27 LeCu 04
	Gaziantep	YB70-27 LeCu 05
	Gaziantep	YB71-27 LeCu 06
	Gaziantep	YB72-27 LeCu 07
	Gaziantep	YB73-27 LeCu 08
	Gaziantep	YB74-27 LeCu 09
	Gaziantep	YB75-27 LeCu 10
Lens orientalis	Sarıkaya/Midyat/Mardin	47 MDYT SRKY LeOr
Lathyrus gorgoni var.*gorgoni*	Oyalı/Besni/Adıyaman	02 BSN OYL LG1
	Oyalı/Besni/Adıyaman	02 BSN OYL LG2
	Oyalı/Besni/Adıyaman	02 BSN OYL LG3
Lathyrus aphaca L.	Kesentaş/Ergani/Diyarbakır	21 ERG KSTŞ LA-1
	Kesentaş/Ergani/Diyarbakır	21 ERG KSTŞ LA-2
	Gaziantep	YB88-27 LA 01
	Gaziantep	YB89-27 LA 02
	Gaziantep	YB90-27 LA 03
Lathyrus cicera L.	Adıyaman	02 KRDT3 LC
	Gaziantep	YB19/A-27 LC 01
	Gaziantep	YB19/B-27 LC 02
	Gaziantep	YB20-27 LC 03
	Gaziantep	YB21-27 LC 04
	Gaziantep	YB26-27 LC 05

3. Results and Discussion

Ascochyta blight, a universal fungal plant disease, is very difficult, costly and complex to manage. Currently, it is recommended to use disease-resistant varieties, fungicides and disease-free seeds, as well as rotational planting within the scope of integrated disease management (Stoddard et al., 2010; Ahmed et al., 2016). Although it is known that the best management is through the use of disease-resistant varieties, the complexity of the disease and the problems in identifying

resistant lines make them limited in the management of *Ascochyta* spp. (Rubiales and Khazaei, 2022). In addition, alternative, eco-friendly, cost-free and sustainable innovative methods are needed due to the negative effects of the chemical control methods used, such as economy-environment-resistance to disease (Gikas et al., 2022; Güneş et al., 2022). Accordingly, a good understanding of disease-related characteristics (morphological, pathogenic and genetic) and the identification of new disease-resistant varieties, taking into account their genetic diversity, form the basis of management. In this context, 107 *Ascochyta* spp. isolates (Table 1) collected from different provinces of Southeastern Anatolia, Eastern Anatolia and Mediterranean regions and stocked from pure culture were used together with different media (PDA, CSMDA and PDB) to examine their growth morphologies. Data obtained using different media for morphological observations of isolates known to cause Ascochyta blight disease in *Vicia* spp., *Pisum sativum (P. sativum)*, *Lens* spp. and *Lathyrus* spp. wild legumes are listed in Table 2.

Table 2. Growth differences in different media within the scope of morphological examinations

ISOLATES	PDA		CSMDA		PDB	
	Only Hyphae	**Hyphae + Conidia**	**Only Hyphae**	**Hyphae + Conidia**	**Hyphae**	**Only Conidia**
21 KYTP SVR PS	+	-	+	-	Not selected	Not selected
23 HTKY SVRC PS1	+	-	+	-	Not selected	Not selected
23 HTKY SVRC PS2	+	-	-	+	+	X
23 HTKY SVRC PS3	+	-	-	+	+	X
23 HTKY SVRC PS4	+	-	-	+	Not selected	Not selected
21 ÇRMK/KK-1 PS	-	+	-	+	+	X
YB55-27 PS 01	+	-	+	-	Not selected	Not selected
YB56-27 PS 02	+	-	-	+	Not selected	Not selected
PS1	+	-	-	+	+	X
PS2	-	+	+	-	-	+
PS3	-	+	-	+	-	+
PS4	+	-	-	+	Not selected	Not selected
PS5	-	+	-	+	-	+
PS6	+	-	-	+	+	X
PS7	-	+	+	-	-	+
02 BSN OYL VS1	-	+	+	-	-	+
02 BSN OYL VS2	+	-	+	-	Not selected	Not selected
21 ERG KSTŞ VS	-	+	+	-	-	+
47 DRGT-BRTP VS1	-	-	-	-	-	-
56 EKDZ PRV VS	+	-	+	-	Not selected	Not selected
YB1-27 VS 01	-	+	+	-	+	X
YB2-27 VS 02	+	-	+	-	Not selected	Not selected
YB3-27 VS 03	+	-	+	-	Not selected	Not selected
YB4-27 VS 04	-	+	+	-	-	+
YB8-27 VS 05	+	-	+	-	Not selected	Not selected
YB9-27 VS 06	+	-	+	-	Not selected	Not selected
YB10-27 VS 07	-	-	-	-	-	-
YB11-27 VS 08	+	-	+	-	Not selected	Not selected
YB12-27 VS 09	+	-	+	-	Not selected	Not selected
YB13-27 VS 10	+	-	+	-	Not selected	Not selected
YB14-27 VS 11	+	-	+	-	Not selected	Not selected

YB15-27 VS 12	+	-	+	-	Not selected	Not selected
YB16-27 VS 13	+	-	+	-	Not selected	Not selected
YB17-27 VS 14	+	-	+	-	Not selected	Not selected
YB18-27 VS 15	+	-	+	-	Not selected	Not selected
02 KKLC GG VA	-	+	+	-	+	X
02 KRDT KHT VA1	-	-	-	-	-	-
02 KRDT KHT VA2	+	-	+	-	Not selected	Not selected
21 ERG KSTŞ VA	+	-	-	+	Not selected	Not selected
21 ERG KSTŞ VA2	-	+	-	+	Not selected	Not selected
21 SVR KYTP VA	+	-	+	-	Not selected	Not selected
56 EKDZ PRV VA	-	+	-	+	Not selected	Not selected
15 YŞV VC	-	+	-	+	-	+
21 HTKY SVRC VC	-	-	-	-	-	-
23 HZRG VC	-	+	+	-	+	X
47 MDYT SRKY	-	+	-	+	+	X
47 MDYT SRKY	-	+	-	+	+	X
47 MDYT SRKY	-	+	+	-	-	+
47 MDYT SRKY	+	-	-	+	-	+
47 MDYT SRKY	-	+	-	+	+	X
YB45-27 VC 01	+	-	-	+	Not selected	Not selected
YB46-27 VC 02	+	-	-	+	+	X
47 MDYT SRKY VN	-	+	-	+	-	+
47 ÖMRL ANTP	+	-	-	+	-	+
47 ÖMRL ANTP	-	-	-	-	-	-
47 ÖMRL ANTP	-	-	-	-	-	-
47 ÖMRL ANTP	+	-	-	+	+	X
47 ÖMRL ANTP	+	-	-	+	Not selected	Not selected
47 ÖMRL ANTP	-	+	-	+	+	X
47 ÖMRL ANTP	-	-	-	-	-	-
47 ÖMRL ANTP	-	-	-	-	-	-
YB25-27 VN 01	+	-	-	+	-	+
47 ÖMRL ANTP VP	-	+	-	+	+	X
YB23-27 VP 01	+	-	+	-	Not selected	Not selected
YB24-27 VP 02	+	-	-	+	Not selected	Not selected
56 PRV UZDĞ VV	-	+	-	+	+	X
02 BSN OYL VG	-	-	-	-	-	-
YB47-27 VG 01	-	+	-	+	+	X
YB48-27 VG 02	+	-	-	+	-	+
YB49-27 VG 03	+	-	-	+	Not selected	Not selected
YB50-27 VG 04	+	-	-	+	-	+
YB51-27 VG 05	+	-	-	+	Not selected	Not selected
YB52-27 VG 06	+	-	-	+	+	X
YB53-27 VG 07	+	-	-	+	+	X
YB7-27 VH 01	+	-	+	-	Not selected	Not selected
02 BSN 01/4	-	+	+	-	+	X
02 BSN 02/5	-	+	-	+	+	X
02 BSN 03/1	+	-	-	+	-	+
02 BSN 04/2	+	-	-	+	-	+
02 BSN 05/4	-	+	-	+	+	X
02 BSN 06/9	-	+	-	+	+	X
47 MDYT GRCŞ	-	+	-	+	-	+
YB66-27 LeCu 01	-	+	+	-	+	X
YB67-27 LeCu 02	-	+	-	+	-	+
YB68-27 LeCu 03	-	+	+	-	-	+
YB69-27 LeCu 04	-	+	+	-	+	X

YB70-27 LeCu 05	+	-	+	-	Not selected	Not selected
YB71-27 LeCu 06	+	-	+	-	Not selected	Not selected
YB72-27 LeCu 07	-	+	+	-	+	X
YB73-27 LeCu 08	+	-	+	-	+	X
YB74-27 LeCu 09	+	-	+	-	Not selected	Not selected
YB75-27 LeCu 10	+	-	+	-	Not selected	Not selected
47 MDYT SRKY	-	-	-	-	-	-
02 BSN OYL LG1	+	-	-	+	+	X
02 BSN OYL LG2	+	-	+	-	Not selected	Not selected
02 BSN OYL LG3	-	+	-	+	+	X
21 ERG KSTŞ LA-1	-	+	+	-	-	+
21 ERG KSTŞ LA-2	-	+	+	-	-	+
YB88-27 LA 01	+	-	+	-	Not selected	Not selected
YB89-27 LA 02	+	-	-	+	+	X
YB90-27 LA 03	-	+	-	+	+	X
02 KRDT3 LC	-	+	+	-	+	X
YB19/A-27 LC 01	+	-	+	-	Not selected	Not selected
YB19/B-27 LC 02	-	+	+	-	Not selected	Not selected
YB20-27 LC 03	+	-	+	-	Not selected	Not selected
YB21-27 LC 04	+	-	+	-	Not selected	Not selected
YB26-27 LC 05	+	-	+	-	Not selected	Not selected

(+: There is growth/ -: No growth/ X: Out of the scope of examination

To observe morphological growth in PDA medium, which is the first step of the study, the isolates were recultured in PDA medium and incubated at 22 ±2 °C. According to the study conducted by Nalcaci et al. (2021), for the purpose of performing pathogenic characterization and mating type analysis of 237 *Didymella rabiei* isolates isolated from 106 chickpea cultivation areas in 44 provinces of Turkey, the isolates were incubated in a 22 ±2 °C or in the dark at room temperature in cabinets (~10 days), and fungal colony growth in the medium was examined. As a result of microscopic examination of the isolates whose growth was examined, the isolates were pure-cultured in PDA medium, and morphological differences were revealed similar to Nene (1982). Thus, information about the growth morphology of *D. rabiei* isolates was obtained, and differences were determined. Accordingly, they stated that *D. rabiei* morphologically showed annular and pycnidial growth on the plant, while microscopically, it showed special conidial growth in terms of form and structure. It was determined that the performed study and the findings obtained support each other, and the representative images of 1 isolate from each genera, whose growth morphologies of *Ascochyta* spp. were determined in PDA medium, are given in Figure 1, and the observational data of the grown-up state of the ~20-day-old isolates are as given in the explanation of Figure 1. Within the examinations carried out with 107 *Ascochyta* spp. isolates from 12 species of 4 genera of wild legumes carried out in PDA medium, it was determined that a total of 58 only hyphal growth, additionally 39 hyphal growth and conodial growth were observed, and there was no growth in 10 isolates (Table 2).

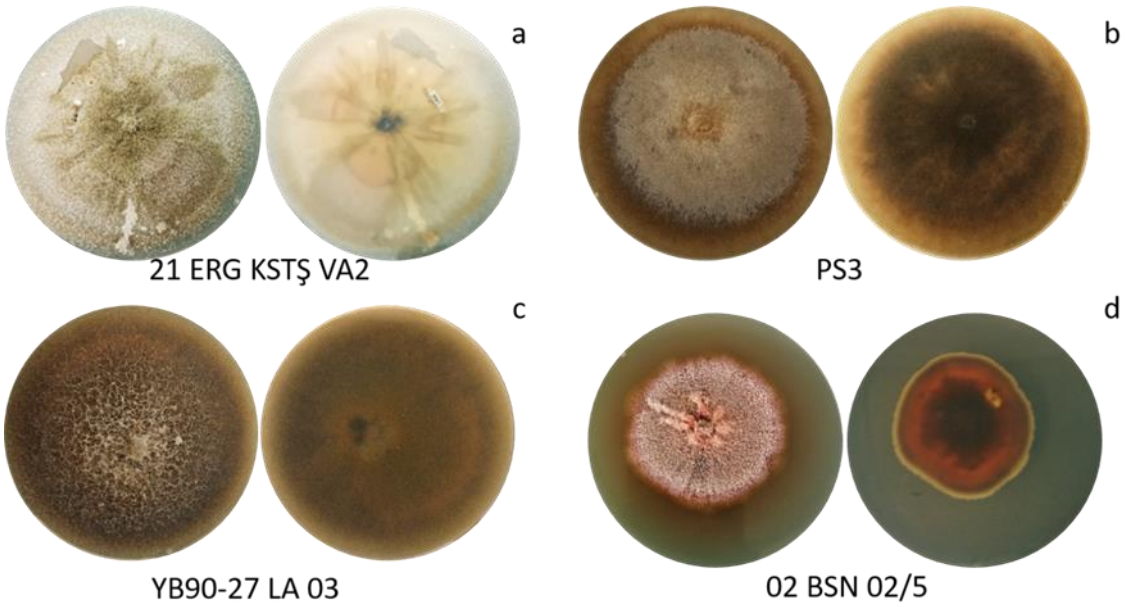

Figure 1. Growth morphology of *Ascochyta* spp. in PDA medium
(Growth morphology of Ascochyta spp. isolated from **a:** *Vicia* spp., **b:** *P. sativum*, **c:** *Lathyrus* spp., **d:** *Lens* spp. wild legumes in Petri dishes (front-back) containing PDA medium)

a.The isolate covers the whole of the Petri dish, and its edges are whitish-gray in color, darkening towards the middle of the Petri dish and taking on a reed green color similar to khaki. Colony edges have a partite appearance and structure with varying color tones.

b.The isolate covers most of the Petri dish and shows a circular structure with dark brown edges and a grayish color in the middle. The colony has a dispersed form with circular development and intense hyphal development on its edges.

c.The isolate shows regular circular growth in camel color covering the entire Petri dish. Colony edges are smooth, and the middle part has a white netted structure.

d.The isolate is slow growing, covering a small part of the Petri dish, and shows hyphal growth in the middle with wild rose colored growth on the obverse. On the back of the Petri dish, scattered growth, which is not white annular, on the edges and darker colourations are observed in the middle parts.

In the second part, the growth morphology of *Ascochyta* spp. isolates was investigated using CSMDA, also known as a special medium for *Ascochyta* studies. All isolates (97 isolates) that grew in PDA medium were cultured in CSMDA medium and incubated at 22 ±2 °C. Within the literature review, Mahiout et al., in 2015, isolated 16 isolates of the pathogen from diseased plants in 6 chickpea growing regions [Mascara (called C), Mostaganem (called M), Ain Temouchet (called A), Ain Defla (called Z), Sidi Belabbes (called B) and Relizane (called R)] in the northwestern Algeria, similar to the study of Benzohra et al. (2013), on CSMDA medium (CSMDA; 40 g chickpea seed meal; 20 g dextrose; 20 g agar and 1 L dsH_2O) at 20 ±2 °C. They performed symptomatic, morphological, pathogenicity and mating type analyses of the pathogen isolates they isolated. It has been determined that the study is similar to the results of Mahiout et al. (2015) within the scope of the findings we have obtained. In addition, the CSMDA medium prepared in the study was used by modification (20 g chickpea flour), and it was observed that it did not affect the growth of the isolates. This result supports the opinion of Gowen

(1986), who stated that *Ascochyta* spp. is the most suitable environment for conidial development (Maden, 2007).

In the continuation of the study using CSMDA, 97 *Ascochyta* spp. isolates grown in PDA medium and diagnosed within microscopic examinations were cultured in CSMDA medium and incubated at 22 ± 2 °C, and information about the effect on morphological growth was obtained. Moreover, after the detection of hyphal growth, whether the isolates showed conidial growth was also examined using binocular light microscopy. To achieve information about morphological examinations of 16 *Ascochyta rabiei* isolates, Mahiout et al. (2015) incubated at 20 ±2 °C by placing discs with a diameter of 5 mm, cut from each of the pure cultures of the isolates, in the center of Petri dishes (90 mm) containing CSMDA. In their 6-replication study, they observed the morphological characteristics (colony color, diameter (mm), quantitative and physiological conidial growth and size of conidia, etc.) of the isolates after incubation. They stated that the findings they obtained within the scope of the examinations were clearly observed to differ between the isolates, especially in terms of three criteria (colony colouration, conidia size and colony growth diameter). They observed that most of the isolates formed colonies with olive green or mouse gray color, while the remaining formed distinctive gray and white colouration in the center. They also determined that only the colony of the R2 isolate was colored with a dark brown center. They showed that the conidial dimensions of the isolates also differed in length and width (the length of the conidia was 5.2 - 17.2 µm, and the width of the conidia was 2.4 - 6.7 µm). They explained that the conidial difference can be compared with the data obtained by Udupa and Weigand (1997) and Basandrai et al. (2005). To determine the lowest and highest colony diameters of 16 isolates, they examined all isolates inoculated (~10 days) in CSMDA medium and found that it varied between 30.75 mm for the B2 isolate and 54.5 mm for C1.

They also explained that the findings of their study showed resemblance in accordance with other studies in which the colony diameters of 16 *Ascochyta rabiei* isolates developed in CSMDA medium were determined to be related to the variability of colony growth of the isolates (Basandrai et al., 2005; Ozkilinc et al., 2010). When the information in the literature was examined (Kaiser, 1973; Grewal, 1984), they stated that similar morphological differences were obtained and emphasized that this had never been based on geographical or pathogenic diversity sources before (Pande et al., 2005). According to the data obtained from the study in which CSMDA medium was used, hyphae and conidial growth were detected in 47 isolates, while 50 isolates showed only hyphal growth. In addition, it was found that 4 isolates obtained from 4 wild legumes and had conidial growth in CSMDA medium had similar characteristics according to conidial size examinations. The conidial dimension image of the "02 BSN 02/5" isolate, which was representatively isolated from *Lens* spp., is given in Figure 2. Accordingly, it was observed that the conidial diameter was ~19.68 x 5.66 µm in length and width. Moreover, in terms of colony development, the growth images of *Ascochyta* spp. in the Petri dish obtained in the CSMDA medium are shown in Figure 3, similar to the representative images of one isolate from each genus whose growth morphology was determined for these four isolates in PDA medium. Thus, the observational data of the developed state of the ~20-day-old isolates were determined as indicated in the description of Figure 3.

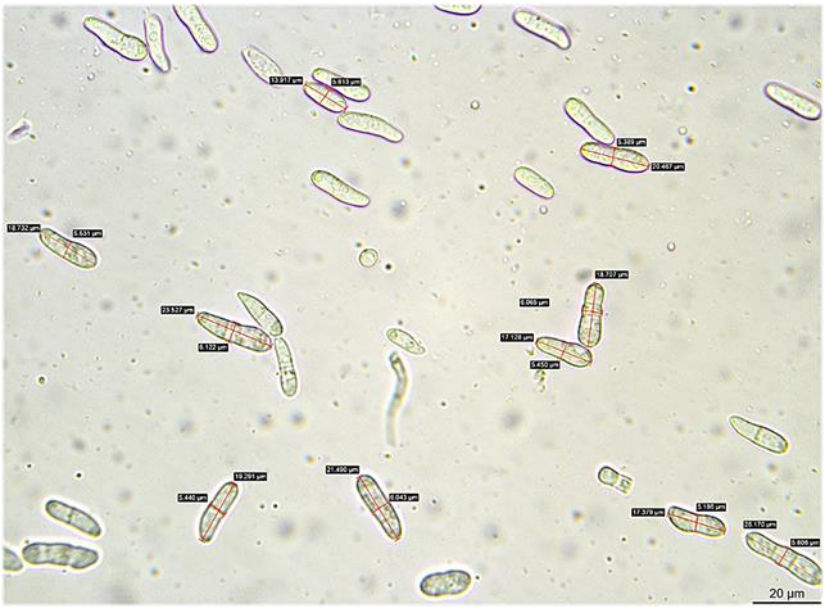

Figure 2. Quantitative conidial growth of *Ascochyta* spp.

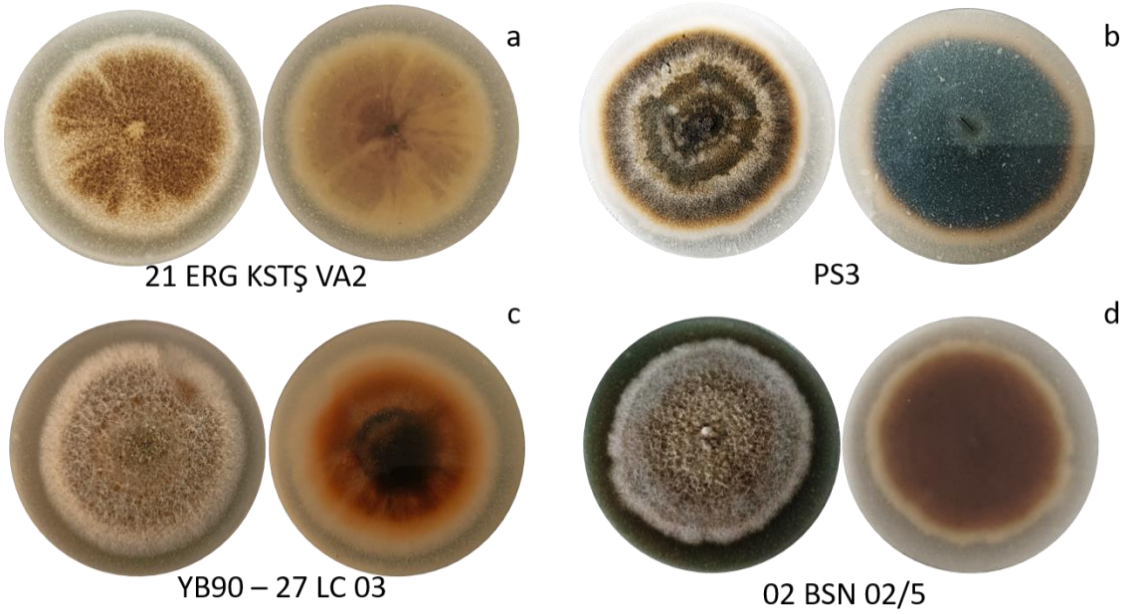

Figure 3. Growth morphology *Ascochyta* spp. in CSMDA medium

a.The isolate covers a large part of the Petri dish, has whitish and brownish colourations in places and has a circular development.

b.The isolate covers most of the Petri dish and forms nested annular structures. Intense hyphal development is observed towards the middle part. The inner annular structures of the colony show growth with white and brown colour and light brown colour in the outer annular structure.

c.The isolate has rapid growth and shows brown ring formation in the middle parts and white rings in the outer parts, almost covering the Petri dish. The upper surface of the Petri dish is covered with a white net-

like structure over time. Colony edges are in white ring structure and regular symmetry.

d.The isolate shows circular growth, and the colony has smooth symmetrical growth with white edges that turn gray and brown towards the middle.

In the study in which *Ascochyta* spp. isolated from *Vicia* spp., *P. sativum*, *Lens* spp. and *Lathyrus* spp. wild legumes were used, the isolates grown in PDA and CSMDA media were examined using binocular light microscopy, and quantitative and morphological observations of conidia were carried out. Mahiout et al. (2015), emphasizing that the biggest significant difference between isolates in terms of literature research is observed in conidial growth in terms of quantity, stated that this difference belongs to the diversity of colonies. They state that diversity is highly effective on differentiation and that these differences cause a change in the number of conidia. They explained that the conidial number is between 0,13 x 10^7 sp mL^{-1} (for C2 and C3 isolates isolated from Mascara) and 2.52 x 107 sp mL^{-1} (for R3 isolate isolated from Relizane). Moreover, they determined that the investigations they made on the basis of the area where the isolates were collected also contributed to this. They found that the conidial numbers of the isolates they isolated from Mascara and Mostaganem (0,242 x 10^7 sp mL^{-1} and 0,279 x 10^7 sp mL^{-1}), respectively, were much lower than those isolated from Ain Temouchet, Ain Defla, Sidi Belabbes and Relizane (0,947 x 10^7 sp mL^{-1}, 1,309 x 10^7 sp mL^{-1}, 1.616 x 10^7 sp mL-1 and 2.049 x 10^7 sp mL^{-1}), and as a result of statistical analysis, in terms of the collecting area effect of infected plants was divided into 4 different groups. In addition, they also emphasized that colony growth rate and conidial number were inversely related in CSMDA. In particular, they pointed out that the isolates isolated from C1, C2 or B1 had rapid colony growth but low quantitative conidial growth and associated this with the results of the pathogenicity test. They argued that the findings were consistent with Grewal's (1984) findings, and as Schmit (2002) stated, the inverse relationship could be associated with the changes between vegetative development and reproduction. Similarly, Kaur (1995) reported that *Ascochyta rabiei* isolates with rapid colony growth showed less conidial growth than other isolates, and their pathogenicity was determined at a much lower level. For this reason, they stated that, by examining the literature studies, the results of the pathogenicity tests have not been related so deeply in any previous study and that the results have no effect on each other (Ali et al., 2009). In addition, the studies of Baite and Singh (2016) and Manjunatha et al. in 2021 and the detection of pycnidia nests and conidia (pycnidiospores), known as the secondary inoculum source of *Ascochyta* spp., were obtained from the microscopic examination of isolates grown in PDA medium. Moreover, blunt-tipped rod-shaped conidia, which were observed to form as single or bicelled conidia during quantitative conidial growth, were examined under a binocular light microscope (Figure 4).

On the other hand, in the study of Al-Maaroof and Salih (2022) to determine the physiological and morphological growth and to identify genetic variations in *Ascochyta rabiei* isolates obtained from infected plants collected from chickpea growing areas in Iraq, they reported that CSMDA medium was the most suitable medium for hyphal growth. In contrast to from other studies, *A. rabiei* isolates were divided into 3 groups according to pycnidium color; in addition, they determined the diameters of pycnidia nests. Thus, they determined that the mean conidia and pycnidia sizes ranged from 20.0 x 7.5 µm and 70.8 x 47.9 µm to 21.8 x 9.0 µm and 140.7 x 93.6 µm. Moreover, in this

study, which also examined the effect of temperature on the growth of isolates, they stated that while the maximum growth was achieved at 25 °C, it was now terminated at 35 °C. In the study in which the morphological growth of *Ascochyta* spp. obtained from 3 regions of Turkey was examined, and the isolates in which *Ascochyta* conidia with their unique forms were detected within the microscopic observations of the isolates in PDA medium are listed in Table 1. Pycnidia nests of *Ascochyta* spp., defined as small pear- or spheroidal (ostiole)–shaped structures, from which asexual fungal conidia form and are expelled from a pore or opening, have been observed under a binocular light microscope (Figure 4).

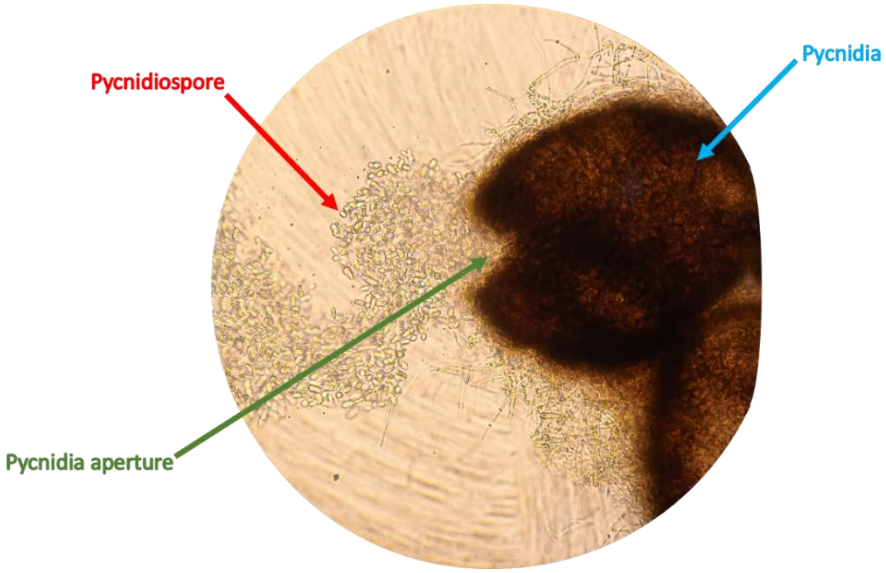

Figure 4. Morphological conidial growth of *Ascochyta* spp. (pycnidia and conidial growth in PDA)

PDB medium, also known as liquid medium, was used as the last step to determine the growth morphology of *Ascochyta* spp. For this purpose, 50 mL of PDB medium was added to 100 mL sterile sample containers after UV exposure, and 3-4 pieces of fungal explant were placed in the container and incubated at room temperature in the dark for ~10 days. Conidial and hyphal growth was observed following incubation. Mahiout et al. (2015) obtained discs from pure culture colonies on CSMDA medium and added the discs to 250 mL bottles containing 200 mL potato dextrose broth medium to prepare for mating type analysis. They stated that they showed mycelial growth after incubating all broth suspensions at 20 ±2 °C for 7 days. Similarly, Nalcaci et al. (2021) stated that they obtained hyphal and conidial growth from *A. rabiei* isolates incubated (~15 days) using PDB medium and stored in ultradeep freezers at -80 °C after cleaning from residues with sdH_2O for use in future studies. Within the scope of the study carried out with *Ascochyta* spp. causing Ascochyta blight in wild legumes, applications in liquid media were carried out similarly to those of Mahiout et al. (2015) and Nalcaci et al. (2021). In the study, where 54 isolates from 107 *Ascochta* spp. isolates were selected and used, it was determined that 32 isolates formed hyphal growth and 22 isolates only conidial growth. According to the literature, some fungi form conidial growth in different morphologies and structures to protect themselves under the minimum nutrient concentration. It is also reported that the amount of nutrients in the environment supports hyphal growth or spore formation (Morkunas and Ratajczak, 2014; Çapa, 2022). In this study, hyphal growth was also not observed in any of the isolates, and the presence of direct conidial growth was detected. Thirty–two isolates showing hyphal growth in PDB medium were not examined in terms of conidial growth, and it was emphasized that the

medium supported conidial growth due to the insufficient amount of nutrients for these isolates (Figure 5).

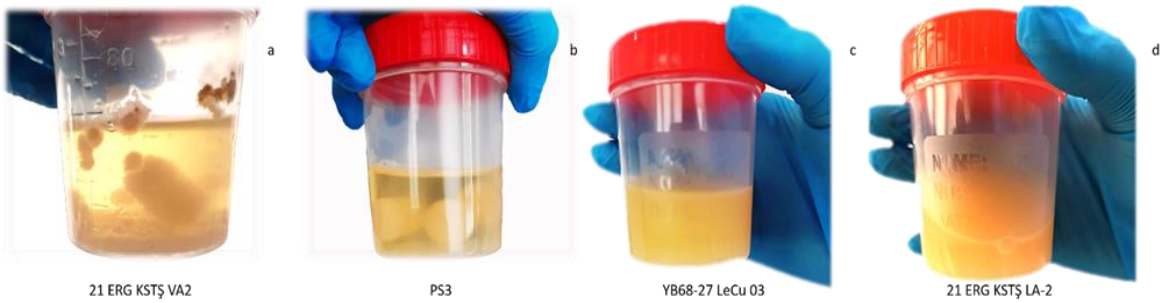

Figure 5. Growth morphology of *Ascochyta* spp. in PDB medium
(Hyphal growth of fungal explants added to PDB medium after incubation (~10 days) - **a:** mycelial and yellowish growth similar to the bacillus form, **b:** cocci-like mycelial and white growth, **c**, **d:** conidia formation in yellow–orange blurred image without any hyphal development)

4. Conclusion

In the study carried out to examine the hyphal and conidial growth of 107 *Ascochyta* spp isolates isolated from *Vicia* spp., *Pisum sativum*, *Lens* spp. and *Lathyrus* spp, 32 for *Vicia* spp. 10 for *P. sativum*, 7 for *Lens* spp., and 8 for *Lathyrus* spp. in PDA medium was expressed to observe hyphal development. In addition, no developmental findings were found in 10 isolates. Moreover, it was determined that 18, 5, 10 and 6 of the isolates used in the study had conidial development together with hyphal growth, respectively. In the second stage of the study, in 97 isolates used in CSMDA, which is a special medium for *Ascochyta* spp., 24 from *Vicia* spp., 5 from *P. sativum*, 10 from *Lens* spp. and 10 from *Lathyrus* spp. to hyphal growth, and 26, 10, 7 and 4 of them had conidial growth as well as hyphal growth, respectively. In addition, 11, 3, 5 and 2 isolates, formed conidial growths together with hyphal growth simultaneously in PDA and CSMDA media. Moreover, it was explained that there were 39, 12, 12 and 12 hyphal growths on the genus basis in 75 isolates simultaneously on PDA and CSMDA media, but no conidial growth was observed, and 31 isolates did not show conidial growth on both media. Of the 65 isolates with a total of 65 conidial and hyphal growths, 18 only in PDA, 26 only in CSMDA, and 21 in both media were found to have conidial and hyphal growth. Accordingly, it was determined that 32 out of 50 isolates that showed hyphal growth in CSMDA also showed hyphal growth in PDA medium, and 18 of them had conidial development as well as hyphal development. It was found that 14, 5, 8 and 5 of the 54 isolates [*Vicia* spp. (25), *P. sativum* (9), *Lens* spp. (13) and *Lathyrus* spp. (7)] taken into the PDB, which is also described as the liquid medium used in the last stage, showed hyphal growth, in addition, 11, 4, 5 and 2 of them had only conidial growth, respectively, without hyphal growth being observed. Within the scope of the study in which PDB medium was used, 20 of 32 isolates with hyphal growth showed conidial growth in PDA, and 22 of them showed conidial growth in CSMDA medium. It was concluded that 10 of the 32 isolates examined in terms of hyphal growth in PDB had hyphal development in PDA and 8 in CSMDA. Similarly, in terms of conidial growth in PDB, 15 of 22 isolates were found to have conidial growth in PDA and 12 in CSMDA. Moreover, it was stated that 31 of the 58

isolates, which were found to have hyphal growth only in PDA medium, also had hyphal growth in CSMDA, 27 of them formed conidial growth, 10 of them showed hyphal growth in PDB medium, and 7 of them had conidial growth. Six isolates (PS3, PS5, 15 YŞV VC, 47 MDYT SRKY VN, 47 MDYT GRCŞ LeCu, YB67–27 LeCu 02) had conidial growth, and 1 isolate (YB73-27 LeCu 08) had hyphal growth in all three media used within the scope of the study. It was also determined that 7 isolates from *Vicia* spp., 1 isolate from *P. sativum*, 3 isolates from *Lens* spp. and 2 isolates from *Lathyrus* spp., which had conidial growth in PDA and CSMDA, produced hyphal growth in PDB. In addition to the importance of the study for plant pathology, it is thought that the findings obtained will form a basis for studies to be carried out in this context, and the importance of the quality and suitability of the media in terms of examination is emphasized.

Declaration of Author Contributions

The authors declare that they have contributed equally to the article. All authors declare that they have seen/read and approved the final version of the article ready for publication.

Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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References

Ahmed, H., Chang, K.F., Hwang, S.F., Fu, H., Zhou, Q., Strelkov, S., Conner, R., Gossen, B., 2015. Morphological characterization of fungi associated with the ascochyta blight complex and pathogenic variability of Mycosphaerella pinodes on field pea crops in central Alberta. *The Crop Journal*, 3(1): 10-18.

Ahmed, S., Abang, M.M., Maalouf, F., 2016. Integrated management of Ascochyta blight (*Didymella fabae*) on faba bean under Mediterranean conditions. *Crop Protection*, 81: 65-69.

Akveç, O., Demirel, Ö., Kafadar, F.N., Can, C., 2018. Determination of *Didymella rabiei* as quantitatively in infected chickpea plants. *International Congress on Vocational Technical Sciences-III*, Conference Abstract Book, 21-22 June, Gaziantep, p. 989-990.

Al-Maaroof, E.M., Salih, R.M., 2022. Physiological and molecular characterization of *Ascochyta rabiei* isolates from various chickpea areas across IKR, Iraq. *Iraqi Journal of Agricultural Sciences*, 53(2): 297-314.

Ali, S.R., Iqbal, S.M., Iqbal, U., Ghafoor, A., Akram, A., 2009. Pathogenic diversity in *Ascochyta rabiei* (Pass.) Lib., of chickpea. *Pakistan Journal of Botany*, 41(1): 413-419.

Altınok, H.H., Can, C., Demirel, Ö., Yüksel, G., 2023. Identification and virulence of fusarium wilt and fusarium crown root rot disease agents from tomato greenhouses in mediterranean climate. *International Journal of Agriculture and Wildlife Science.* 9(1): 36-49.

Atik, O., Baum, M., El-Ahmed, A., Ahmed, S., Abang, M.M., Yabrak, M.M., Murad, S., Kabbabeh, S., Hamwieh, A., 2011. Chickpea Ascochyta blight: disease status and pathogen mating type distribution in Syria. *Journal of Phytopathology*, 159(6): 443-449.

Aydın, M.H., 2016. A diseases of lentil of increasing importance in the Southeastern Anatolia Region; *Ascochyta Blight*. *Turkey 6th Plant Protection Congress with International Participation.* Conference Abstract Book, 05-08 September, Konya, pp. 762.

Baite, M.S.D.S., Singh, B., 2016. Morphological variability in the Indian isolates of *Ascochyta rabiei* causing blight in chickpea and evaluation of chickpea cultivars. *Indian Journal of Plant Protection*, 44(1): 74-82.

Barilli, E., Cobos, M.J., Rubiales, D., 2016. Clarification on host range of *Didymella pinodes* the causal agent of pea Ascochyta blight. *Frontiers in Plant Science*, 7: 592.

Basandrai, A.K., Pande, S., Kishore, G.K., Crouch, J.H., Basandrai, D., 2005. Cultural, morphological and pathological variation in Indian isolates of *Ascochyta rabiei*, the chickpea blight pathogen. *The Plant Pathology Journal*, 21(3): 207-213.

Benzohra, I.E., Bendahmane, B.S., Labdi, M., Benkada, M.Y., 2013. Sources of resistance in chickpea germplasm to three pathotypes of *Ascochyta rabiei* (Pass.) Labr. in Algeria. *World Applied Sciences Journal*, 21(6): 873-878.

Bessada, S.M., Barreira, J.C., Oliveira, M.B.P., 2019. Pulses and food security: dietary protein, digestibility, bioactive and functional properties. *Trends in Food Science & Technology*, 93: 53-68.

Bradley, D.J., Gilbert, G.S., Martiny, J.B., 2008. Pathogens promote plant diversity through a compensatory response. *Ecology Letters*, 11(5): 461-469.

Bulat, F., Sarı D., Sarı, H., Eker, T., Özay, H., Toker, C., 2023. Molecular identification of ascochyta blight of Cicer montbretii Jaub. & Spach. *Mediterranean Agricultural Sciences*, 36(1): 7-12.

Chasti, F., Wani, T.A., Bhat, F.A., Khan, M.A., Maqbool, S., Bhat, M.A., 2022. Evaluation of pea (*Pisum sativum* L.) genotypes against *Ascochyta pisi*.

Çapa, B., 2022. Elma mavi küf *(Penicillium expansum)* hastalık etmeninin glukoz, sukroz ve vitamin C'ye karşı duyarlılıklarının belirlenmesi. Doktora Tezi, Harran Üniversitesi, Fen Bilimleri Enstitüsü, Şanlıurfa.

Demirel, Ö., Akveç, O., Can, C., 2022. A current overview of plant biotechnology. *Euroasia Journal of Mathematics, Engineering, Natural Medical Sciences.* 9(20): 110-149.

Endes, A., 2021. Influence of culture media, temperature, pH and light regime on mycelial growth of *Ascochyta rabiei*. *International Journal of Agriculture Forestry and Life Sciences*, 5(1): 87-93.

FAOSTAT, (2021). Food and agricultural organization of the United Nations. (https://www.fao.org/faostat/en/#home), (Erişim tarihi: 12.07.2023).

Foresto, E., Carezzano, M.E., Giordano, W., Bogino, P., 2023. Ascochyta blight in chickpea: an update. *Journal of Fungi*, 9(2): 203.

Gikas, G.D., Parlakidis, P., Mavropoulos, T., Vryzas, Z., 2022. Particularities of fungicides and factors affecting their fate and removal efficacy: a review. *Sustainability*, 14(7): 4056.

Gilbert, G.S., Webb, C.O., 2007. Phylogenetic signal in plant pathogen–host range. *Proceedings of the National Academy of Sciences*, 104(12): 4979-4983.

Gowen, S.R., 1986. Investigation into variability in *Ascochyta rabiei* and resistance to the disease in chickpea. Report of Project R3712 funded by Overseas Development Administration and Done in Collaboration with the International Centre for Agriculture Research in Dry Areas, University of Reading, UK.

Grewal, J.S., 1984. Evidence of physiologic races in Ascochyta rabiei of chickpea. In: M.C. Saxena, K.B. Singh (Eds), *Proceedings of the Workshop on Ascochyta Blight and Wintering of Chickpeas*. ICARDA, Aleppo, Syria, pp. 55-65.

Güneş, H., Demirel, Ö., Calayır, O., Demir, S., Can, C., 2022. Innovative technologies in sustainable agriculture. *8th International Agriculture Congress*. Conference Abstract Book, 26-29 October, Ankara, pp. 66.

Kahraman, A., 2023. Evaluation of legume farming in Turkey and agricultural sustainability. *Legume Research-An International Journal*, 46(2): 166-170.

Kaiser, W.J., 1973. Factors affecting growth, sporulation, pathogenicity, and survival of Ascochyta rabiei. *Mycologia*, 65(2): 444-457.

Kaur, S., 1995. Phytotoxicity of solanapyrones produced by the fungus *Ascochyta rabiei* and their possible role in blight of chickpea (*Cicer arietinum*). *Plant Science*, 109(1): 23-29.

Keçeli, A., 2023. The changes in susceptibility status of the old and of the newly registered chickpea (*Cicer arietinum* L.) cultivars with respect to the blight disease caused by the pathotypes of *Ascochyta rabiei* (Pass.) Labr. *Poljoprivreda*, 29(1): 20-26.

Kızılok, H., Ceyhan, D.İ., Güneş, S., Talapov, T., Demirel, Ö., Dedecan, O., Can, C., 2019. Fusarium wilting factor in lentils *Fusarium oxysporum* f. sp. *lentis* biological control of lentis. *1th International Congress on Sustainable Agriculture and Technology (INCSAT).* Conference Abstract Book, 01-03 April, Gaziantep, pp. 547.

Kosiada, T., 2012. *In*-vitro growth of some species of *Ascochyta Lib*. *Open Life Sciences*, 7(6): 1076-1083.

Maden, S., 2007. Nohutla Fusarium solgunluğu (*Fusarium oxysporum* f. sp. *ciceris*) ve Ascochyta yanıklığının (*Ascochyta rabiei*) moleküler yöntemlerle hızlı tanısı, patotiplerinin ayrımı, moleküler karakterizasyonları. Ankara Üniversitesi Bilimsel Araştırma Projeleri Sonuç Raporu. 1-24.

Mahiout, D., Bendahmane, B.S., Benkada, M.Y., Rickauer, M., 2015. Physiological characterisation of *Ascochyta rabiei* (Pass.) Lab. isolated from diseased chickpea fields in six regions of northwestern Algeria. *American-Eurasian Journal of Agricultural & Environmental Sciences*, 15: 1136-1146.

Manjunatha, L., Saabale, P.R., Srinivasa, N., Bandi, S., Singh, S., Kumar, K., 2021. Molecular characterization and development of sequence characterized amplified region (SCAR) marker for detection of *Ascochyta rabiei* (Pass.) Labr., infecting chickpea. *Indian Phytopathology*, 1-9.

Manjunatha, L., Saabale, P.R., Srivastava, A.K., Dixit, G.P., Yadav, L.B., Kumar, K., 2018. Present status on variability and management of *Ascochyta rabiei* infecting chickpea. *Indian Phytopathology*, 71: 9-24.

Markell, S., Wise, K., McKay, K., Goswami, R., Gudmestad, N., 2008. Plant disease management. NDSU Extension Service. North Dakota State University Fargo, North Dakota, 58-105.

Morkunas, I., Ratajczak, L., 2014. The role of sugar signaling in plant defense responses against fungal pathogens. *Acta Physiologiae Plantarum*, 36: 1607-1619.

Nalcaci, N., Turan, A., Basbuga, S., Kafadar, F.N., Isler Ceyhan, D., Anay, A., Mart, D., Ogut, E., Sarpkaya, K., Can C., 2021. Virulence and mating type distribution of *Didymella rabiei* in chickpea growing areas of Turkey. *Journal of Agricultural Science and Technology*, 23(1): 209-220.

Nene, Y.L., 1982. A review of Ascochyta blight of chickpea. *International Journal of Pest Management*, 28(1): 61-70.

Obuseng, V.C., Moshoeshoe, M.N., Nareetsile, F.M., Kwaambwa, H., Maina, I., 2022. Plant biomass as potential economic commodities for agricultural purposes. *Frontiers in Chemistry*, 10: 806772.

Oguiba, B., Karkachi, N., Suárez-Estrella, F., Haouhach, S., Kihal, M., López, M. J., 2023. Ascochyta blight (*Ascochyta fabae*) of faba bean (*Vicia faba* L.): phenotypic and molecular characterization, pathogenicity and in vitro biological control by *Bacillus* spp. and *Pseudomonas* spp. *Archives of Biological Sciences*, 75(1): 103-117.

Ozkilinc, H., Frenkel, O., Abbo, S., Eshed, R., Sherman, A., Shtienberg, D., Ophir, R., Can, C., 2010. A comparative study of Turkish and Israeli populations of *Didymella rabiei*, the ascochyta blight pathogen of chickpea. *Plant pathology*, 59(3): 492-503.

Pande, S., Siddique, K.H.M., Kishore, G. K., Bayaa, B., Gaur, P.M., Gowda, C.L.L., Bretag, T.W., Crouch, J.H., 2005. Ascochyta blight of chickpea (*Cicer arietinum* L.): a review of biology, pathogenicity, and disease management. *Australian Journal of Agricultural Research*, 56(4): 317-332.

Polatbilek, H., Akveç, O., Kafadar, F.N., Mart, D., Can, C., 2017. Towards determining *Didymella rabiei* from infected chickpea seeds with RT-PCR analyses. *International DNA Day and Genome Congress*, Conference Abstract Book, 24-28 April, Kırşehir, pp. 182.

Rajpal, V.R., Singh, A., Kathpalia, R., Thakur, R.K., Khan, M.K., Pandey, A., Hamurcu, M., Raina, S.N., 2023. The prospects of gene introgression from crop wild relatives into cultivated lentil for climate change mitigation. *Frontiers in Plant Science*, 14: 1127239.

Ray, D.K., Gerber, J.S., MacDonald, G.K., West, P.C., 2015. Climate variation explains a third of global crop yield variability. *Nature Communications*, 6(1): 5989.

Rehman, A., Farooq, M., Lee, D.J., Siddique, K.H., 2022. Sustainable agricultural practices for food security and ecosystem services. *Environmental Science and Pollution Research*, 29(56): 84076-84095.

Rhaiem, A., Chérif, M., Peever, T.L., Dyer, P.S., 2008. Population structure and mating system of *Ascochyta rabiei* in Tunisia: evidence for the recent introduction of mating type 2. *Plant Pathology*, 57(3): 540-551.

Rubiales, D., Khazaei, H., 2022. Advances in disease and pest resistance in faba bean. *Theoretical and Applied Genetics*, 135(11): 3735-3756.

Salgotra, R.K., Chauhan, B.S., 2023. Genetic diversity, conservation, and utilization of plant genetic resources. *Genes*, 14(1): 174.

Schmit, J.P., 2002. Tradeoffs between reproduction and mycelium production in the unit-restricted decomposer Coprinus cinereus. *Mycologia*, 94(1): 40-48.

Sharma, G., Pandey, R.R., 2010. Influence of culture media on growth, colony character and sporulation of fungi isolated from decaying vegetable wastes. *Journal of Yeast and Fungal Research*, 1(8): 157-164.

Singh, R., Kumar, K., Purayannur, S., Chen, W., Verma, P.K., 2022. *Ascochyta rabiei*: a threat to global chickpea production. *Molecular Plant Pathology*, 23(9): 1241-1261.

Stoddard, F.L., Nicholas, A.H., Rubiales, D., Thomas, J., Villegas-Fernández, A.M., 2010. Integrated pest management in faba bean. *Field Crops Research*, 115(3): 308-318.

Talapov, T., Gunes, N., Bagriyanik, Z., Demirel, O., Dedecan, O., Uygun, A.E., Akvec, O., Kahraman, A., Kamphuis, L., Can, C., 2023. The first report of *Ascochyta rabiei* infections on endemic Turkish populations of *Cicer bijugum* and *C. turcicum*. *Plant Disease*.

Treindl, A.D., Stapley, J., Leuchtmann, A., 2023. Genetic diversity and population structure of Epichloe fungal pathogens of plants in natural ecosystems. *Frontiers in Ecology and Evolution*, 11: 1129867.

Udupa, S.M., Weigand, F., 1997. Pathotyping of *Ascochyta rabiei* isolates of Syria. DNA Markers and Breeding for Resistance to Ascochyta Blight in Chickpea. Proceedings of the Symposium on "Application of DNA Fingerprinting for Crop Improvement: Marker-assisted Selection of Chickpea for Sustainable Agriculture in the Dry Areas". *ICARDA, Aleppo, Syria*, pp. 39-48.

Vail, S., Banniza, S., 2008. Structure and pathogenic variability in Ascochyta rabiei populations on chickpea in the Canadian prairies. *Plant Pathology*, 57(4): 665-673.

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