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Araştırma Makalesi

Rhizoctonia Türleri ile İlişkili Nohut Kök Çürüklük Hastalık Etmenlerinin Tanılanması ve Hastalık Parametreleri

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Anahtar Kelimeler

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Rhizoctonia bataticola
Rhizoctonia solani

Öz: Nohut protein açısından zengin baklagillerden birisi olup, beslenme dengesi ve vejetaryen diyetler için çok önemlidir. Ancak, fungal kökenli patojenler nedeniyle önemli verim kayıpları yaşanmaktadır ve dünya çapında yaklaşık 25'ten fazla fungal hastalık etmeni nohutu etkilemektedir. Bu durum, nohut hastalıklarının bölgesel etiyolojisinin ve epidemiyolojisinin kapsamlı bir şekilde anlaşılmasını gerektirmektedir. Bu çalışma, toprak kaynaklı *Rhizoctonia* spp. ile mücadele stratejileri geliştirmek için bu hastalık etmeninin bölgesel epidemiyolojisini ve etiyolojisini anlamayı amaçlamıştır. Bu sebeple, nohut kök çürüklük hastalığına neden olan *Rhizoctonia* türlerini belirlemek için Yozgat'ın 14 ilçesinde survey çalışması yürütülmüştür. Yozgat genelinde toplam 138 nohut tarlasından toplanan 690 adet bitkide izolasyon çalışması yapılmıştır. Survey çalışmaları sonucunda Yozgat genelinde hastalık yaygınlığı %7-35 arasında belirlenmiştir. Diğer taraftan, hastalık şiddeti ise %13-38 arasında değişiklik göstermiştir. Elde edilen *Rhizoctonia* izolatları morfolojik özelliklerinin yanı sıra moleküler yöntemler vasıtasıyla tanılanmıştır. İzolasyon çalışmalarında toplam 137 *Rhizoctonia* izolatı toplanmış ve bu izolatlardan 67'si *R. solani*, 70'i *R. bataticola* olarak belirlenmiştir. Patojenisite çalışmaları sonucunda *R. solani* ve *R. bataticola* patojenlerinin virülenslik düzeylerinin bir birine oldukça benzer olduğu ortaya konulmuştur. Çalışma, nohut yetiştiriciliğinde *Rhizoctonia* kök çürüklüğünün yaygınlığını belirlemek için düzenli survey çalışmalarının yapılmasını önermektedir. Böylece, nohut üretim alanlarında sürdürülebilirlik ve hastalık izleme için zamanında önlem alınması sağlanacaktır.

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Disease Parameters and Identification of Chickpea Root Rot Disease Associated with *Rhizoctonia* Species

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Abstract: Chickpea is a legume rich in protein and is very important for nutritional balance and vegetarian diets. However, it experiences significant yield losses due to fungal-based pathogens, and more than 25 fungal disease agents worldwide affect chickpeas. This situation requires a comprehensive understanding of the regional etiology and epidemiology of chickpea diseases. This study aimed to understand the regional epidemiology and etiology of this disease agent in order to develop strategies to combat soil-borne *Rhizoctonia* spp. For this reason, a survey study was carried out in 14 districts of Yozgat to determine the *Rhizoctonia* spp. that cause chickpea root rot disease. Isolation studies were carried out from 690 plants from a total of 138 chickpea fields throughout Yozgat. As a result of survey studies, disease incidence throughout Yozgat varied between 7% and 35%. On the other hand, disease severity varied between 13% and 38%. The obtained *Rhizoctonia* spp. were identified by molecular methods as well as morphological characteristics. A total of 137 *Rhizoctonia* spp. were collected in isolation studies, and 67 of these isolates were identified as *R.solani* and 70 as *R. bataticola*. As a result of pathogenicity studies, it was revealed that the virulence levels of *R. solani* and *R. bataticola* pathogens are quite similar to each other. The study recommends conducting regular survey studies to determine the incidence of *Rhizoctonia* root rot in chickpea cultivation. Thus, timely measures will be taken for sustainable and disease monitoring in chickpea production areas.

1. Introduction

Chickpea (*Cicer arietinum* L.) has an annual production of 16.6 million tons and is the third most produced legume crop globally after beans and peas. World chickpea production has increased steadily from 1975 to the present (Faostat 2023). Research programs, improved germplasm, disease resistance and environmental adaptation have contributed to this increase. According to the Food and Agriculture Organization (FAO), chickpeas are produced in more than 50 countries around the world. India accounts for approximately 74.1% of total world chickpea production and is the leading country in world chickpea production (Faostat 2023). Australia and Türkiye, the next most important producing countries, account for 5.6% and 3.5% of world chickpea production, respectively (Faostat 2023). Other major chickpea producing countries such as Russia and Ethiopia account for 3.2% and 2.7% of world production, respectively. In addition, Myanmar, Pakistan, the USA and Iran are among the major chickpea producing countries (Faostat 2023).

Although chickpea has an important place among food legumes worldwide, its production and yield are not at the desired level (Endes et al. 2024). One of the most important reasons for this is biotic and abiotic stress factors that cause yield losses in chickpea planting areas. Stress factors affecting chickpea yield include diseases (45%), drought (30%), high temperature (6.25%), frost (6.25%), insect damage (6.25%) and other (6.25%) stress factors (Endes 2023). One of the stress factors affecting chickpea yield in the world and in our country is fungal diseases. Fungal diseases reduce both the amount of production and the market value of the product. Chickpea anthracnose and root rot diseases, which have a cosmopolitan distribution in the world, cause yield losses ranging from 10% to 100% in chickpea planting areas when environmental and climatic conditions are suitable for the development of the pathogen (Endes and Atmaca 2022; Endes 2023).

Fungal agents that cause chickpea root rot disease include: *Rhizoctonia solani* (Root rot) and *Rhizoctonia bataticola* (Dry root rot) (Mazur et al. 2002). Symptoms of root rot disease can be observed in every stage of chickpea vegetation (Nene et al. 2012). Chickpea plants affected by the disease turn yellow in clusters due to root and root collar rot, and in the later stages, plant deaths occur due to drying in these plants (Haware et al. 1990). In disease-susceptible varieties, leaves that show wilting, dull green colour and drying within 25 days after planting (Early Wilt) cause the plant to collapse completely (Landa et al. 2004). However, disease symptoms (Late Wilt) are generally more noticeable 6 - 8 weeks after seed planting, at the beginning of the flowering period (Jendoubi et al. 2017). In plants with late wilt, wet or dry blackish or brown lesions in the bark and wood tissue of the roots, yellowing or light brown necrotic lesions in the leaves and petioles, and complete collapse in the entire upper part of the plant are observed (Jiménez-Díaz et al. 2015; Jendoubi et al. 2017; Basbagci and Dolar 2020).

Rhizoctonia species, which are widespread worldwide, cause various diseases affecting various ecosystems in various species such as Solanaceae, Fasciaceae, Asteraceae, Poaceae and Brassicaceae, as well as ornamental and forest trees (Dubey et al. 2014). Species in the genus *Rhizoctonia* form large and complex groups that vary in themselves according to the number of nuclei in hyphal cells (Carling and Summer 1992). When these groups come together, they form subgroups called anastomosis groups (AG), which are compatible hyphae that can fuse (Bayram et al. 2022).

Rhizoctonia solani, an important species in the genus *Rhizoctonia*, was first named by Kühn in 1858 and has become the most studied and known species within the genus *Rhizoctonia*. In 1921, Matsumoto reported the presence of hyphal anastomosis in *R. solani*, and in 1936, Schulz classified the isolates according to their anastomosis abilities. *R. solani* is a soil-borne plant pathogen that infects various agricultural plants, including legumes, cereals, and cucurbits (Dubey et al. 2014; Atmaca et al. 2025). Currently, there are 14 anastomosis groups in *R. solani*, namely AG 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 and AGB1 (Bayram et al. 2022). AG-4 and AG-5 groups of these groups are also present in chickpea planting areas in our country (Basbagci et al. 2019; Basbagci and Dolar 2020).

Dry root rot caused by *Rhizoctonia bataticola* is a serious disease threatening chickpea production worldwide, causing 10-25% yield loss in tropical arid and semi-arid regions (Manjunatha et al. 2013; Basbagci and Dolar 2020). The disease has a high level of morphological variability among different hosts and geographical regions (Aghakhani and Dubey 2009). *R. bataticola* is highly variable in sclerotial characters, which makes it an important taxonomic feature for identification (Aghakhani and Dubey 2009). The presence of this pathogen in chickpea has been reported by researchers worldwide, and its morphological variations have been studied by researchers in different countries (Aghakhani and Dubey 2009; Khan et al. 1999; Nene et al. 2012; Basbagci and Dolar 2022). The pathogen has a cosmopolitan distribution and damages more than 400 host plant species (Manjunatha et al. 2013).

It is known that root rot disease has a highly complex structure in chickpea (Yimer et al. 2018). Therefore, the present study was carried out with the following objectives: (i) There are many studies on *Rhizoctonia* species, one of the etiological agents of this disease, worldwide. However, in our country, studies on the status of root rot disease caused by *Rhizoctonia* species in chickpea have been limited, and there has been no study on this subject in Yozgat province (Basbagci et al. 2019; Basbagci and Dolar 2020; Basbagci and Dolar 2022; Bayram et al. 2022). If the economic importance of chickpea root rot disease is not fully understood, studies and researches on this disease may lack value for production and make an economic contribution. For this reason, it was aimed to determine the incidence and severity of root rot disease for Yozgat province in this study. (ii) In order to control chickpea root rot disease, it is very important to make a definite diagnosis of the pathogenic fungal agents that cause the disease and to have information about its regional epidemiology (Agrios 2005). It was aimed to determine the *Rhizoctonia* species causing root rot in chickpea fields of Yozgat province with morphological and cultural characteristics as well as molecular studies. (iii) While some anastomosis groups of *Rhizoctonia* species are found on plants as epiphytes or saprophytes, some of them cause disease as facultative or aggressive plant pathogens. Since the number of individuals is

high and some of these individuals are found on the plant as epiphytic or saprophytic, it was aimed to carry out pathogenicity tests to determine the virulence of *Rhizoctonia* spp. to be obtained.

2. Materials and Methods

2.1. Survey and disease parameters

The survey conducted in 2024 in chickpea fields in Yozgat province to determine the incidence and severity of root rot disease was carried out in regions where disease symptoms were intense and in May-June (Endes 2023). Sampling was carried out from 138 different chickpea fields in Yozgat province. Chickpea fields selected for sampling were randomly selected during the survey. During the survey, stopping approximately every 5-10 km along the main road from the first sampling point, chickpea fields on the right or left of the road were observed for root rot disease symptoms. At each sampling point, walking towards the middle of the field by drawing zigzags from the diagonals or edge of the selected field, a 1 m^2 frame was thrown randomly to at least three (3) different points. Then, the percentage of diseased plants was determined by comparing the diseased plants showing yellowing, wilting, root and root collar rot disease symptoms in the frame to the total number of plants in the frame. The disease percentage found for each field was multiplied by the area of that field, and the products obtained were added up. This total was divided by the maximum disease probability (total area examined×100), and the result was multiplied by 100 to find the average disease percentage of the districts (Bora and Karaca 1970). The total number of plants and the number of diseased plants were noted for each chickpea field, and the disease rate (%) of each field was determined (Bora and Karaca 1970). The prevalence of the disease in Yozgat province was found by taking the average of the disease percentage in all surveyed fields in the districts.

In calculating disease severity (DS), each plant obtained from chickpea fields was evaluated according to the 1–9 disease scale used in the studies of Yimer et al. (2018). In the disease scale, 1 = 0–10% of the infected plant, 3 = 11–20%, 5 = 21–30%, 7 = 31–50%, 9 = values indicating that more than 50% of it is affected by the disease. The disease severity percentage was calculated using the Townsend and Heuberger (1943) formula given below with the obtained scale values.

$DS\ (\%) = [\Sigma i(ni \times vi\ ) / (V \times N)] \times 100$

In the formula: ni = number of plants in the scale value, vi = scale value, V = Highest scale value, N = total number of plants observed, i = indicates the number of classes.

2.2. Fungal isolation and morphological characterization

Infected pieces of 4-5 cm in length from the roots and/or root collars of 5-10 plants showing characteristic yellowing, wilting and root rot disease symptoms taken from each sampling area were first washed in tap water to remove coarse residues (Endes 2024a). Then, smaller pieces of 3-5 mm in length were obtained from these large plant pieces, with infected and healthy parts together. Surface disinfection procedures of these pieces were carried out according to Endes (2023). Following this, the infected pieces were placed on Potato Dextrose Agar (PDA, Merck; 1.10130) medium containing 0.01% tetracycline (Sigma-Aldrich), and the petri dishes were incubated in the dark at 23±2 °C for 6-8 days and fungal development was observed.

All fungal isolates were temporarily classified according to their colony characteristics. In the study, *Rhizoctonia* spp. obtained from different chickpea fields were incubated on PDA in the dark at 25±1°C for 48 hours, and then the mycelial growth diameter was measured with a digital caliper. Isolates were categorized as slow growing (up to 60 mm diameter), medium growing (60-80 mm diameter) and fast growing (80 mm diameter). However, 15 days after incubation, the sclerotia formed on the plates were divided into three groups: those producing 1-10 sclerotia per plate, those producing more than 100-200 sclerotia per plate, and those producing more than 200 sclerotia per plate. The

sizes of the sclerotia were measured using an electronic digital caliper and a calibrated compound microscope. Sclerotial pattern was recorded and classified as peripheral, central and scattered groups. Then, the micromorphological characteristics of *Rhizoctonia* species were determined by considering the method of Carling and Summer (1992). Nuclei were stained to determine multinucleate and binucleate isolates. Safranin O solution was prepared by adding Safranin O, 3% KOH solution, glycerin and water. Hyphal tips taken from *Rhizoctonia* spp. were placed on the solution, and the number of nuclei in the hyphae was determined by considering the number of nuclei in at least 10 cells.

2.3. Molecular identification

Total genomic DNA isolation of *Rhizoctonia* isolates was performed according to the protocol described by Endes (2024b). The ITS1/ITS4 primer pair was used in PCR studies conducted according to the protocol specified by Aras and Endes (2023). The obtained PCR products were electrophoresed on 1% agarose gel prepared in 1×TAE (Tris-Acetic Acid–EDTA) buffer solution at 90 volts for 1.5 hours. The gels were stained with 0.5 µg mL^{-1} ethidium bromide, visualized on a UV transilluminator, and visually checked (Endes and Kayım 2022). In order to examine the phylogenetic relationship between *Rhizoctonia* spp., the base sequences of the ITS gene obtained by PCR were synthesized bidirectionally (5´-3´ and 3´- 5´) to Molgentek (Adana, Türkiye) company. Base sequences were compared with the base sequences of the ITS gene of other *Rhizoctonia* spp. in the world by using the Blastn program with the gene data on the NCBI (National Center of Biotechnology Information) site, and thus the isolates were identified at the species level.

2.4. Pathogenicity test

Rhizoctonia spp. were made using the ILC – 482 chickpea variety, which is known to be susceptible, based on Endes (2023)'s soil inoculation method. For the inoculum, 1000 g of sterile field soil mixture (field soil: peat: perlite; 1: 1: 1; v: v: v) containing 25% chickpea flour was transferred to 5'L transparent plastic bags, and 25 disks of 10 mm diameter from 10-day-old fungal cultures grown on PDA were left into the bags for each isolate. The plastic bags prepared in this way were incubated for 25 days in climate chambers with controlled conditions containing 12 fluorescent light (light/dark) periods and 23±1 °C temperature. At the end of this period, 5 g of sterile field soil mixture containing 25% chickpea flour was added to each well of 5 cm diameter, 10 cm deep and 24-well black plastic vials containing fungal cultures, and after being slightly watered, the vials were placed in the climate chamber with the conditions specified above. It was waited for 15 days for the inoculum to cover the soil. At the end of this period, chickpea seeds that did not show disease symptoms were shaken in 1% sodium hypochlorite (NaOCl) solution for 5 minutes and then rinsed 3 times with sterile water for 10 minutes each. Then, to promote germination, the seeds were kept for 5 days on drying papers moistened with sterile water at +4 °C. To the control vials, 5 g of sterile field soil mixture containing 25% sterile chickpea flour without inoculum was added.

The study was established with 3 replications according to the randomized complete block design. For each isolate, one 24-eyed viol was used (one viol=one replication: In other words, there are 24 chickpeas in each replication and disease severity was obtained from these plants). The evaluation of pathogenicity tests was carried out 8 weeks after seed sowing, using the 1-9 disease scale used in survey studies.

3. Results and Discussion

3.1. Determination of disease parameters

During the survey studies, 138 chickpea fields in Yozgat were evaluated for root rot disease (Table 1). A total of 1538 da area was examined in Yozgat province. It was determined that the prevalence rate of root rot disease in Yozgat in general was 20.5%, and the disease was not found in 15 of the examined fields. The disease severity in Yozgat province varied between 15.3% and 38.6%,

and the average disease severity for Yozgat was determined as 24.2% (Table 1). In the Central and Sorgun districts of Yozgat, the incidence rate of root rot disease in chickpea fields was determined as 35.8% and 32.3%, respectively. On the contrary, the disease incidence rate in chickpea fields in Çekerek and Aydıncık districts was determined as 7.8% and 9.3%, respectively. In this study, the incidence rate of the disease varied between 7.8-35.8% on a district basis. However, Endes (2023) reported that the rate of occurrence of root rot and wilt disease in Yozgat in their survey study could be up to 90% on a provincial basis. It is thought that this difference is due to more rainfall compared to previous years, the characteristics of the varieties used in production, and the differences in farmer practices. On the other hand, it was reported by Dubey et al. (2014) that the average occurrence rate of the disease varied between 6.8-22.2% in the survey study conducted in India for root rot and wilt disease.

Isolation studies were carried out by collecting 690 plants showing disease symptoms from chickpea fields examined for root rot (Table 2). Fungal isolates showed distribution within four major groups. While *R. solani* was classified in the first group, *R. bataticola*, *Fusarium* spp. and other were grouped as pathogens or saprophytes. While the isolation rate of *R. solani* isolates was 9.7% for Yozgat in general, it was obtained the most in Kadışehri district with an isolation rate of 13.1%. On the contrary, Sarıkaya district was determined as the district with the least contamination with an isolation rate of 4.2% (Table 2).

Table 1. Number of fields examined for chickpea root rot disease in Yozgat province and disease incidence and severity rates (%)

County	Number of Field	Surveyed Sowing Area (Decare)	Disease Incidence (%)	Disease Severity (%)
Akdağmadeni	6	63	14.8	21.9
Aydıncık	5	38	9.3	15.3
Boğazlıyan	17	128	24.9	26.2
Kadışehri	5	35	11.4	19.5
Merkez	22	247	35.8	38.6
Saraykent	5	95	19.6	20.7
Sarıkaya	11	145	25.2	28.3
Sorgun	17	193	32.3	32.4
Yenifakılı	5	40	13.5	23.8
Yerköy	13	147	17.7	26.4
Çandır	7	73	18.5	17.5
Çayıralan	8	112	29.6	23.6
Çekerek	5	39	7.8	13.7
Şefaatli	12	183	27.1	30.3
Overall	**138**	**1538**	**20.5**	**24.2**

The isolation rate of *R. solani* in Yozgat in general varied between 4.2% and 13.1%. The isolation rate of the second group, *R. bataticola*, was determined as 10% on average for Yozgat. The isolation rate of *R. bataticola* in Yozgat in general varied between 4.3% and 14.3%. While Kadışehri district had the lowest isolation rate with 4.3%, Çayıralan district became the district where *R. bataticola* was isolated the most with an isolation rate of 14.3%. The 3rd group, where *Fusarium* isolates were collected in isolation studies, has an approximate average isolation rate of 75%. However, this third group will be evaluated in later studies because the current study focuses on *Rhizoctonia* species in the province of Yozgat.

Basbagcı et al. (2019) obtained 75 *R. solani* isolates from 268 infected chickpea plants in their study on chickpea root rot in the provinces of Denizli, Isparta, Kütahya and Uşak. In addition, Basbagcı and Dolar (2022) reported that 15 *R. bataticola* isolates were obtained from chickpea fields in Denizli, Isparta, Kütahya and Uşak provinces. On the other hand, Bayram et al. (2022) carried out isolation

studies from 204 infected chickpea plants in their study conducted in chickpea fields in Konya province, and as a result of the isolation studies, they reported that they obtained 11 *R. solani* isolates belonging to different anastomosis groups.

Table 2. Information on fungi isolated from infected chickpea plants in Yozgat province

County	Number of Plant Used for Isolation	Isolation Frequence (%)			
		R. solani	*R. bataticola*	*Fusarium* spp.	Other
Akdağmadeni	30	9.3	5.70	77.0	8.00
Aydıncık	25	10.60	11.30	75.4	2.70
Boğazlıyan	85	9.50	13.80	72.8	3.90
Kadışehri	25	13.10	4.30	78.5	4.30
Merkez	110	10.50	9.30	73.8	6.40
Saraykent	25	7.30	12.80	72.5	7.40
Sarıkaya	55	4.20	13.70	77.0	5.10
Sorgun	85	8.50	13.40	72.1	6.00
Yenifakılı	25	11.30	12.90	71.1	4.70
Yerköy	65	9.70	6.40	73.1	10.80
Çandır	35	10.80	7.40	76.2	5.60
Çayıralan	40	11.90	14.30	70.4	3.50
Çekerek	25	8.70	7.20	80.2	3.90
Şefaatli	60	10.40	8.60	76.7	4.30
Overall	**690.00**	**9.70**	**10.08**	**74.8**	**5.42**

The results obtained in previous studies are in line with the results of the current study. However, the fact that *Rhizoctonia* species have a higher isolation rate in the current study can be explained by the number of infected plant samples used in fungal isolation. While a total of 137 *Rhizoctonia* isolates were obtained in the current study, it was determined that 67 of these isolates consisted of *R. solani*; 70 of them consisted of *R. bataticola*. In Yozgat province, wheat, barley, sugar beet, chickpea and lentil cultivation are generally preferred as crop patterns. These products are among the hosts of *R. solani* and *R. bataticola* species. This situation may be the reason for the increase in the population of *Rhizoctonia* isolates in the soil. Because *Rhizoctonia* species are soil-borne fungal agents that form a single-cycle epidemic, and these agents form one or a few reproductive structures per year. For this reason, the population of *Rhizoctonia* species may increase slowly from year to year.

3.2. Morphological and molecular characterization

The present study revealed that there are significant differences between *Rhizoctonia* spp. in terms of growth diameter, size, and number of sclerotia. The colony color of *R. solani* varied among 67 isolates. The colony color of *R. solani* was determined as whitish brown, light or dark brown, and the hyphae were septate and multinucleate. All of these characteristics were parallel to the characteristic morphological characteristics of *R. solani*. 27% of *R. solani* were determined as slow-growing, 53% as medium-growing, and 20% as fast-growing isolates. The sclerotium size of *R. solani* varied between 0.14 and 6.1 mm. On the other hand, *R. bataticola* showed morphological characteristics such as gray to black colored colony, black colored microsclerotia. The colony texture of 46 isolates of *R. bataticola* was velvety mycelium, and the remaining isolates formed aerial and fluffy mycelium. 23% of *R. bataticola* were determined as slow-growing, 47% as medium-growing, and 30% as fast-growing isolates. It was determined that *R. bataticola* had a faster growth than *R. solani* isolates.

Dubey et al. (2014) reported that *R. solani* were multinucleate and septate, in parallel with this study. In addition, these researchers classified the colony development of *R. solani* as slow, medium, and fast growing, as in this study. Furthermore, they reported that the sclerotium sizes of *R. solani* varied between 0.10 to 5.4 mm. In the present study, sclerotium sizes were determined to be larger.

This difference may reveal that geography and climate are effective on the morphological characteristics of *R. solani*.

Basbagcı and Dolar (2022) reported that *R. bataticola* were in colors ranging from gray to black and reported that microsclerotia were produced in old colonies. In addition, the researchers divided the growth rate of *R. bataticola* into three classes, slow, medium, and fast, along with the formation of velvety, aerial, and fluffy mycelia on PDA. In the present study, R. bataticola were grouped into three growth classes, and it was revealed that the growth rate of *R. bataticola* was higher than that of R. solani in terms of mycelial growth.

As a result of PCR studies of *Rhizoctonia* spp., a DNA fragment of approximately 700 bp for *R. solani* and approximately 500 bp for *R. bataticola* was produced using the ITS1/ITS4 primer. Bidirectional (5´-3´ and 3´-5´) base sequences obtained from Molgentek company (Adana, TÜRKİYE) were compared with other *Rhizoctonia* spp. in the NCBI gene bank using the Blastn program. *R. solani* showed 97.5% - 100% nucleotide sequence homogeneity with India (JF701712) and China (FJ440192) isolates. *R. bataticola* showed 99-100% nucleotide sequence homogeneity with Türkiye (MN640983) and Hungary (OQ304119) isolates. Later, the genetic relationship between *Rhizoctonia* spp. was determined with the phylogenetic tree obtained according to the Maximum Parsimony (MP) method using the Mega 11 program (Figure 1). In the MP phylogenetic tree, 791 nucleotide characters, including gaps, were used, and 401 of these nucleotides were determined as parsimonious informative regions. MP analyzes gave one of the most parsimonious trees (Figure 1; Tree Length: 364; Consistency Index (ConI): 0.953; Retention Index (RI): 0.991 and Rescaled Index (RC: 0.944].

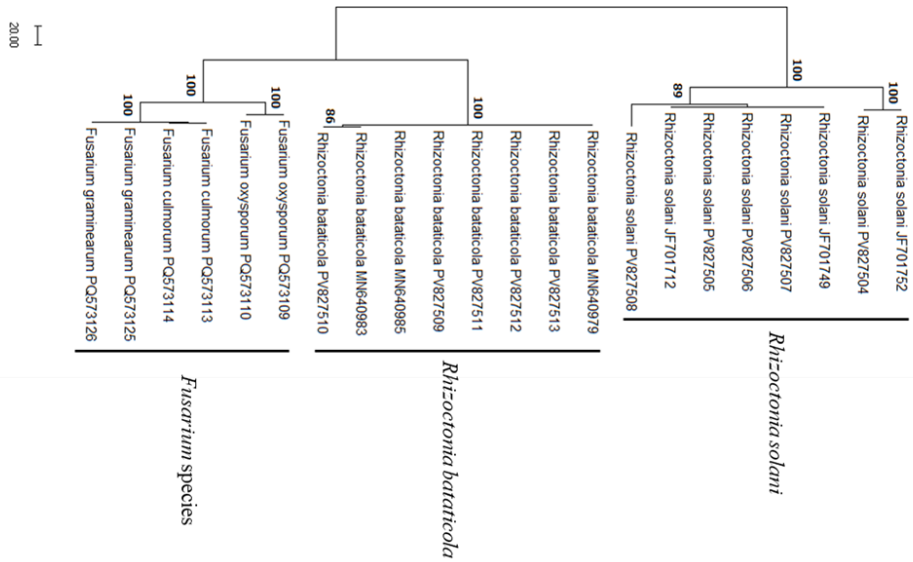

Figure 1. Most parsimonious unrooted tree based on internal transcribed spacer (ITS)1, 5.8S ribosomal DNA and ITS2 of Rhizoctonia and Fusarium species inferred from maximum parsimony analysis using MEGA 11. Numbers on branches are bootstrap values >70% in 1,000 replicates. The isolates obtained in the study are indicated by PV. The other isolates were provided from NCBI GenBank.

When this MP dendrogram of *Rhizoctonia* spp. is examined, the pedigree is primarily divided into 2 main branches. The first of these includes different Fusarium species used as an outgroup supported by a 100% bootstrap value, and the other group includes *Rhizoctonia* spp. *Rhizoctonia* isolates were divided into 2 subgroups within themselves, the first subgroup contained *R. bataticola* with a 100% bootstrap value and the other subgroup contained all of the *R. solani* isolates.

3.3. Determination of virulence levels of *Rhizoctonia* spp.

The pathogenicity study results of *Rhizoctonia* isolates, which were isolated from chickpeas showing root rot and whose molecular identification studies were completed, are summarized in Table 3. In general, all *Rhizoctonia* isolates used in the study showed different levels of disease severity in the ILC-482 chickpea variety. The disease severity caused by *R. solani* isolates in chickpea plants varied

between 75.9% and 95.5%, and the average disease severity was calculated as 89.3%. On the other hand, the disease severity caused by *R. bataticola* isolates varied between 78.1% and 96.9%, with an average disease severity of 88.0%.

Table 3. Disease severity (%) and Re-isolation rate (%) created by different *Rhizoctonia* isolates in ILC-482 chickpea variety

***Rhizoctonia* Species**	**Isolate**	**Disease Severity (%) (Mean ± Standart Error)**	**Re-isolation (%)**
R. solani	YBURs1	87.0 ± 1.5	82.5
	YBURs2	75.9 ± 2.3	75.0
	YBURs3	92.5 ± 3.1	100.0
	YBURs4	95.1 ± 3.9	90.0
	YBURs5	95.5 ± 2.5	95.0
R. bataticola	YBURb1	82.5 ± 2.7	95.0
	YBURb2	93.6 ± 1.6	100.0
	YBURb3	78.1 ± 2.6	87.5
	YBURb4	88.8 ± 3.4	100.0
	YBURb5	96.9 ± 1.8	100.0

Rhizoctonia spp. infections in chickpeas, especially extreme temperatures and rainfall at the end of spring or the beginning of the summer season, can cause very high disease severity in plants. Indeed, Dubey et al. (2014) revealed in their study conducted in different agro-ecological regions of India that the incidence of chickpea root rot varied between 6.8% and 22.2%, with the highest incidence in the rainy season (8.5-25.0%) and the lowest prevalence in the winter season (5-11%). In addition, they reported that high relative humidity and hot climate conditions during the rainy season create a very favorable environment for the development of the disease. On the other hand, Basbagcı and Dolar (2020) reported that hypocotyl formation was not prevented in the majority of seeds infected with *R. bataticola*, but subsequently, infection-related deaths occurred in seeds that formed hypocotyls. In addition, the researchers reported that in pot trials, 100% seed emergence was achieved in the control pots, but in the pots inoculated with *R. bataticola*, emergence could not be achieved in some plants due to the disease, and drying occurred over time in some of the plants that emerged. In addition, they determined that the disease severity value caused by the *R. bataticola* agent varied between 30-100%.

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