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Isolation, identification and occurrence of Plectosphaerella cucumerina (Lindf.) W. Gams, 1968 on sugar beets cultivation areas in Türkiye

Türkiye'de şeker pancarı ekim alanlarında Plectosphaerella cucumerina (Lindf.) W. Gams, 1968'nın izolasyonu, tanımlanması ve bulunuşu

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Abstract

As a result of the research conducted on the detection of severe root rot and drying in sugar beet (*Beta vulgaris* L.) fields in the Konya region of Türkiye in 2015-2017, a fungus named *Plectosphaerella cucumerina* was detected together with many different root pathogens. This fungus, which is known to occur in many hosts and on different substrates, is a new record for Türkiye. There are not many studies on this fungus, which was found to be low virulent in our study, neither in the world nor in our country. However, according to the limited number of studies, it has been found that there are pathogen strains of the pathogen that infect sugar beet in the Netherlands. Furthermore, it is a necrotrophic fungus that is used as a potential biological control agent against potato cyst nematodes, so it is considered a fungus that should be studied for this reason. We define this contribution as a new record for Türkiye mycobiota from *P. cucumerina* sugar beet seedlings.

Özet

2015-2017 yıllarında Türkiye'nin Konya Bölgesi'nde şeker pancarı (*Beta vulgaris* L.) tarlalarında şiddetli kök çürüklüğü ve kurumasının tespiti üzerine yapılan araştırma sonucunda birçok farklı kök patojeni ile birlikte *Plectosphaerella cucumerina* Lindf.) W. Gams, 1968 isimli bir fungus tespit edilmiştir. Birçok konukçuda ve farklı substratlarda bulunduğu bilinen fungus, Türkiye için yeni bir kayıt oluşturmuştur. Çalışmamızda düşük virülensli olarak bulunan bu fungus üzerinde de, dünyada da çok çalışma yapılmamıştır. Fakat yapılan sayılı çalışmalara göre Hollanda'da şeker pancarını enfekte eden patojen suşların olduğu tespit edilmiştir. Bunun dışında patates kist nematodlarına karşı potansiyel biyolojik mücadele ajanı olarak kullanılan nekrotrofik bir fungustur. Bu nedenle de üzerinde çalışılması gereken bir fungus olduğu düşünülmektedir. Bu makale tarafımızca *P. cucumerina* şeker pancarı fidelerinden elde edilen Türkiye mikobiyotası için yeni bir kayıt olarak tanımlanmaktadır.

INTRODUCTION

Sugar beet (*Beta vulgaris* L.) is a dicotyledonous plant that prefers especially temperate regions and is mainly cultivated for sugar production (Trebbi and McGrath 2004). Sugar beet production in Türkiye is 23.5 million tonnes in 2023. About 31% of the sugar beet production, which is approximately 7.5 million tonnes in Türkiye, is produced in the Konya region of Türkiye (TÜİK 2023). Türkiye ranks fifth in the world in sugar beet production. Russia (14.8%), USA (12.0%), France (12.4%), Germany (11.5%) and Türkiye (6.6%) rank first in the world sugar beet production, which totals 287 million tonnes, and these five countries produce 57.2% of the world sugar beet production (FAO 2021).

Plectosporium was introduced for *Fusarium tabacinum*, the anamorph of *Plectosphaerella cucumerina* (Lindfors) W. Gams (Palm et al. 1995). The teleomorph of the agent was identified from the cucumber (*Cucumis sativus* L.) plant (Klebahn 1929, Lindfors 1919) and the anamorph was obtained from an infected tobacco (*Nicotiana tabacum* L.) plant (van Beyma 1933).

The fungus *P. cucumerina*, a necrotrophic fungus, was introduced as a potential biocontrol agent against potato cyst nematodes in 2003 (Atkins et al. 2003). Although widespread, it is a fungus that can be encountered on many hosts and common substrates. This fungus, which can cause many diseases in arable lands, is also isolated from sand dunes and salty beaches (Domsch et al. 1980). It has also been reported that *P. cucumerina* is found in the areas where sugar beet is grown in the Netherlands (van der Spek 1972). In Türkiye, Demirel et al. reported in 2005 that fungi of the genus *Plectosphaerella* were obtained from the agricultural soils of Eskişehir province.

MATERIAL AND METHODS

Sampling, Fungal Isolation and Phenotypic Characterization

Plectosphaerella cucumerina isolate PC-16-648 was obtained from a sample of sugar beet seedling collected in Konya province (Konya province and its surroundings were examined in the cultivation areas of 4 factories, namely Konya and Çumra sugar factories of Türkiye Şeker A.Ş.), the main sugar beet growing area of Türkiye during 2015-2017. Plants showing symptoms of root rot such as wilting and marginal necrosis of leaves at the seedling (spring) and mature plant (autumn) stages were included in the sampling, and the samples were taken to the laboratory in ice boxes for isolation. Symptomatic tissues (2-4 mm in length) were surface sterilised by soaking in 1% sodium hypochlorite solution for 2 min, then rinsed in distilled water and air dried. They were then transferred to petri dishes containing potato dextrose agar (PDA) medium (Merck, Darmstadt, Germany). Petri dishes were incubated at 25 °C for 7 days with a 12 h dark/light cycle. Phenotypic characterization of the fungus was made by taking into account its color, shape, colony structure, conidia and sclerotia structures in the petri dish.

DNA Sequence Analysis

Isolate was also confirmed by DNA sequencing analysis of the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA. Total genomic DNA was extracted from the fresh mycelia of 10-day-old colonies using the GeneJet Plant Genomic DNA purification mini extraction kit (Thermo Fisher Scientific Inc., Waltham, MA, USA) by following the manufacturer's instructions. DNA isolation of such isolates was based on the Lee and Taylor (1990) method. The ITS region of the DNA was amplified using ITS 1 (5' TCC GTA GGT GAA CCT GCGG 3') and ITS 4 (TCC TCC GCT TAT TGA TATGC 3') primers described by White et al. (1990). PCR reaction was performed in a 50 µL mixture containing 1.5 mM of MgCl2, 0.2 mM of dNTPs, 0.4 µM of each primer, 10x PCR buffer, 1 U of Taq DNA polymerase (Thermo Fisher Scientific Inc.), and approximately 10–15 ng of genomic DNA. PCR amplification was performed with cycling conditions consisting of 2 min at 94 °C, 35 cycles of 30 s at 94 °C, 30 s at 56 °C, 1 min at 72 °C, and 8 min at 72 °C. Amplification products were analyzed by electrophoresis through 1.0% agarose in TAE buffer. They are described and their sequences of the 5.8S and 28S ribosomal DNA and internal transcribed spacers 1 and 2 of the ribosomal RNA gene cluster analyzed (Gilgado et al. 2005). They were sequenced by Macrogen Biotechnology. ITS sequenced analysis was performed using BLAST via http://www.ncbi.nlm.nih.gov. Sequences of each isolate were deposited in GenBank.

Pathogenicity Test

Pathogenicity of the fungus was determined in vitro conditions using sugar beet seeds of the universally susceptible Aranka variety and was assesed in the test using the inoculum layer technique described by Brantner and Windels (Brantner and Windels 1998) by placing 10 cm diameter culture disks of the fungus on the soils in half filled 15 cm diameter pots having a sterilized soil mix of 1:1:1, soil, sand and manure. 10 sugar beet seeds of cv. After Aranka was disinfected with 2% NaOCl for 3 min., it was placed in a circular manner on the culture disks of the fungus. For the control group, seeds were planted in sterile soil mix. The experiment was performed in four

replicates. Then, 2 cm of soil mixture was added onto the pots and incubated at 25-26 °C in a 12 h dark/light cycle, and watered regularly. Plants that died before and after emergence were recorded.

In the final assessment, at the end of the 6th week, the remaining plants were removed and scored according to the Hanson scale of 0-4, where: 0=no lesions; 1=mild growth retardation and wilting of leaves; 2=there are chlorotic leaves and necrotic appearance at the leaf margins; 3=drying of roots, browning, death of leaves; 4=dead plant (Hanson et al. 2006).

Four pots were used for each isolate in all pathogenicity tests. Pots containing seeds were placed in a growth chamber in four replicates according to a completely randomised experimental design. Disease index values were calculated using the following formula;

Disease severity index =

$$\frac{\sum (\text{no of the seedlings at each scale} \times \text{scale value})}{\text{The highest scale value} \times \text{total seedling evaluated}}$$

(Towsend and Heuberger 1943)

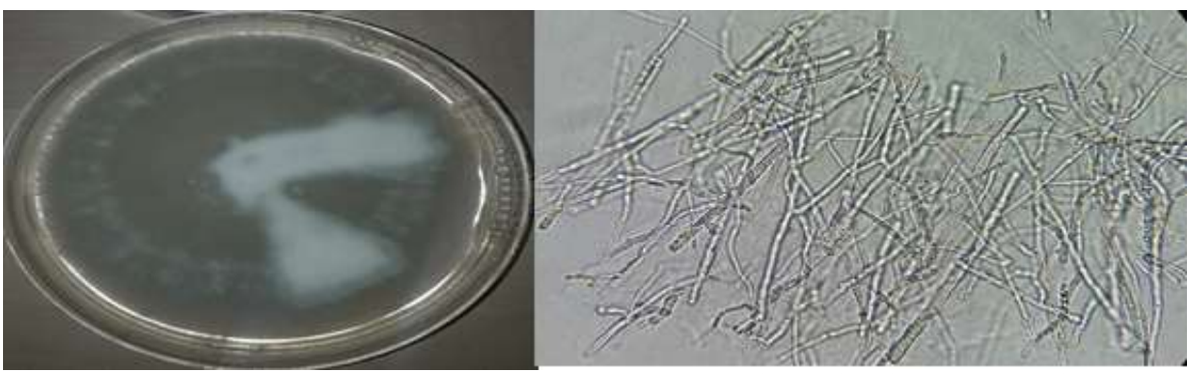
In this way, the percentage of disease in the plant caused by the isolate was determined. Disease severity is calculated using the Townsend-Heuberger (1943) formula. The percentage of disease severity was calculated by multiplying the Disease Severity Index value by 100. The pathogenicity of these isolates is expressed as a percentage of disease severity.

RESULT AND DISCUSSION

Phenotypic Characterization

Plectosphaerella-like colonies growing from infected tissues were subcultured on PDA, purified and microscopically examined for morphological characteristics (Figure 1).

Plectosphaerella colonies were salmon pink in colour. They had aerial micelles and these were rare. Conidia were smooth, thin-walled and appeared elliptical. No chlamidospores were found (Carlucci et al. 2012).



Plectosphaerella cucumerina colony growth on PDA medium — *Plectosphaerella cucumerina mycelium with hyphae and budding of hypha*

Figure 1. Microscopic features of *Plectosphaerella cucumerina*

DNA Sequence Analysis

The resulting *P. cucumerina* isolate sequences (MN900536) were approximately 99% similar to other isolates on GenBank (HQ248206). To our knowledge, these two new species are the first to be reported from sugar beet in Türkiye.

Pathogenicity Test

According to the pathogenicity test under in vitro conditions, the *P. cucumerina* isolate was found to be low virulent in our study (Figure 2).

Inoculated pot with *P. cucumerina* isolate for pathogenicity test

Appearance of seedlings as a result of pathogenicity test

Control group

Figure 2. Pathogenicity test steps of *Plectosphaerella cucumerina* and pathogenicity results

The characteristics of the strains re-isolated as a result of inoculation of sugar beet seedlings were consistent with the description of *P. cucumerina* (Carlucci et al. 2012). Studies have shown that *P. cucumerina* is an agent that is not considered a pathogen, but causes partial disease symptoms in sugar beet leaves.

Plectosphaerella species are widely distributed and are known as root pathogens in a wide variety of hosts (Zazzerini and Tosi 1987). Previous studies have also shown that *P. cucumerina* is a very common fungus in many different crops and soils, especially in decaying plant residues (Abad et al. 2000). In Türkiye, Demirel et al. reported in 2005 that fungi of the genus *Plectosphaerella* were obtained from the agricultural soils of Eskişehir province. According to the study conducted by van Spek in the Netherlands in 1972, he observed that *P. cucumerina* has pathogenic races in sugar beet and causes disease in the plant. However, Atkins et al. concluded in 2003 that the fungus could be successfully used as a biological control agent against potato cyst nematodes. The fungus is widespread and occurs on many hosts and substrates. It has been isolated from arable soils as well as sand dunes and salty beaches (atkins
et al. 1980) and has been shown to cause a number of diseases.

According to the pathogenicity test under in vitro conditions, the *P. cucumerina* isolate was found to be low virulent. Detection of the disease is important because it can also cause root rot and wilt, and in combination with other pathogens it can cause greater problems. To our knowledge, this new species is the first reported on sugar beet in Türkiye.

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