Research Article

Sugar beet root rot caused by oomycetous pathogens in Turkey and their control by seed treatment

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Abstract: The aim of this study was to determine the oomycetous pathogens causing root rot on sugar beet and test their control by seed treatment in Konya Province, Turkey. Oomycetous fungus-like pathogens of sugar beet were investigated using 866 plant samples collected at 2 growth stages, early seedling and late root, from fields in the Konya region of Turkey and 1 sample from the Thrace region. Herein, 10 oomycetous species belonging to 3 genera: *Aphanomyces cochlioides*, *Phytophthora cryptogea*, *Ph. pseudocryptogea*, *Ph. megasperma*, *Ph. inundata*, *Pythium aphanidermatum*, *Py. helicoides*, *Py. heterothallicum*, *Py. sylvaticum*, and *Py. ultimum* var. *ultimum* (*Globisporangium ultimum* var. *ultimum*) were discovered at various times with in the 2 growth periods, all of which were the first records for Turkey. *A. cochlioides* was the most serious pathogen, both in terms of its wide distribution and aggressiveness. The pathogen produced more than 90% disease severity when tested by soil infestation at the seedling stage, although it also occurred at the late root growth stage. *Pythium* species were also ascommon, such as *A. cochlioides*, the majority of which were very aggressive, producing more than 84% disease severity at the seedling stage, except for *Py. aphanidermatum*. Half-strength potato dextrose agar medium was found to be very useful for the isolation of all of the pathogens from the plant samples at both stages. Morphological features of all of the pathogens were abundantly produced when the pathogens were grown on amended grated carrot agar medium and culture disks of fungal growth of this medium were submerged in sterile and nonsterile soil extracts. Out of the 15 fungicide mixes tested, 2 mixes, thiram+metalaxyl+hymexazole and thiram+metalaxyl+hymexazole+ pyraclostrobin reduced seedling root rot caused by both *A. cochlioides* and *Pythium ultimum* var. *ultimum*, while the standard seed treatment fungicide mix of thiram+hymexazole was not effective against either of the pathogens.

Key words: Oomycetous, rot, seed, sugar beet, Turkey

1. Introduction

Turkey occupies the fifth rank after France, the Russian Federation, USA, and Germany in terms of sugar beet production in the world[1]. About 29% of the 17,436,100 tons of total sugar beet production in Turkey, which is 6,007,777 tons, is produced in Konya Province[2]. Root rot of sugar beets, mostly caused by soil-borne fungi and some bacteria, occur in almost all of the production areas in the world, as well as in Turkey. Some affect sugar beets at all growth stages, while others occur at the late growth stages before harvest. Among the root rot pathogens, 3 oomycetous fungus-like agents, such as

Aphanomyces cochlioides, *Pythium* spp., and *Phytophthora drechsleri*, cause important disease complex on sugar beets everywhere in the world.These 3 pathogens have been listed as major causal agents of sugar beet root rot in many texts (Jacobsen, 2006; Harveson et al., 2009). Jacobsen (2006)also reported that *A.cochlioides*,which causes black root rot, was found in sugar beet growing areas of the North Central and High Plains regions of the USA, Canada, England, Europe, Chile, and Japan. In all of these areas, the disease occurred in 2 phases: acute seedling blight and chronic root rot. Losses can up to be 100% depending on environmental factors and the degree

of soil infestation (Windels, 2000). Poindexter[3] pointed out that *A. cochlioides* could significantly reduce the yield and quality of sugar beet in Michigan and Ontario. He also stated that significant Aphanomyces root rot might not occur every year in Michigan because of its high dependence on the environment. Aphanomyces root rot was also found as the main root rot pathogen in central Poland in 2001–2003, the cultivar Arthur being the most susceptible (Pizczek, 2004).

Many *Pythium* species have been reported to cause root rot on sugar beet. Van der Plaats-Niterink (1981) listed 7 species, *Py. adhaerens*, *Py. aphanidermatum*, *Py. intermedium*, *Py. irregulare*, *Py. ultimum* var. *ultimum*, and *Py. betae*, occurring on sugar beet. In addition to the above mentioned species, *Py. acanthicum* and *Py. deliense*have also been isolated from sugar beet by some researchers (Rush, 1987; Kuznia and Windels, 1993; Jacobsen, 2006). Among *Pythium* species, *Py.ultimum* var. *sporangioforum* and *Py. aphanidermatum* were found as the most widespread and aggressive species (Leach, 1986). Brantner and Windels (1998) identified 72 isolates as *Py. ultimum* var. *sporangiiferum* out of 76 *Pythium* spp. and all of the isolates of *Py. aphanidermatum* were pathogenic, effecting sugar beet stand seriously. The latter species was also found in some states of the USA, Canada, Austria, and Iran (Jacobsen, 2006).

The least known sugar beet oomycetous pathogens are *Phytophthora* species. In his review, Jacobsen (2006) mentioned that Phytophthora root rot caused by *Phytophthora drechsleri* Tucker had been observed in the states of California, Colorado, Idaho, Montana, Oregon, and Utah in the USA and in Iran, and *Ph. megasperma* in England, causing root rot similar to that of *Ph. drechsleri*.

There have been few reports describing the causal agents of root rot on sugar beet in Turkey. Pythium root rot has drawn the least attention and, in many cases, it was reported as *Pythium* spp. from sugar beet growing areas of the Alpullu refinery region and Isparta Province, respectively (Yorgancı and Turhan, 1988; Özgönen and Çulal Kılıç, 2009). The only pathogenic species citation in Turkey was made by Erzurum et al. (1995) as *Py. ultimum* from the Kastamonu and Turhal refinery regions.

Seed treatment by metalaxyl-containing pesticides is the most frequently applied control measure against root rot caused by oomycetous pathogens. There have been reports mentioning the insufficient control of Pythium root rot with the usual rate of 0.65 g/kg seed of metalaxyl in the USA due to the resistance acquired by some isolates (Brantner and Windels, 1998). Variations

in sensitivity to metalaxyl have also been reported among and within the species of *Pythium* (Cook and Zhang, 1985; White et al., 1988), and *Phytophthora* (Coffey and Bower, 1984; Csinos and Bertrand, 1994; Goodwin and McGrath, 1995). For this reason, various fungicides or their mixes, including thiram+metalaxyl, fludioxonil, hymexazole, difenoconazole+metalaxyl-M+sedaxane, penthiopyrad, thiamethoxam+metalaxyl+fludioxonil, metconazole+tolclofos-methyl, have been proposed by some companies to control *Pythium* spp. and other pathogens that cause root rot[4].

Since only *Pythium ultimum* and *Py.* spp. have been reported in Turkey thus far, and taking the importance of the other oomycetous pathogens, such as *A. cochlioides* and *Phytophthora* spp., into consideration in all of the sugar beet growing areas in world, and due to the difficulty of their isolation, this study was undertaken in places where severe root rot has been reported. Along with the occurrence of oomycetous pathogens at the seedling and harvest stages of sugar beet, their identification, pathogenicity, and distribution in Konya sugar beet growing areas, and control by seed treatment, were also studied.

2. Materials and methods

2.1. Collection of plant samples

Disease samples were collected in 2 consequent years, 2015–2017, from all of the sugar beet growing areas in Konya Province, from an area of about 30.000 ha, which had 4 sugar refineries, 2 of which belonged to the General Directorate of Sugar Corporation and the other 2 to belonged to Türk Şeker A.Ş. Plants showing root rot symptoms, like wilting and marginal necrosis of the leaves, at the seedling (in spring) and mature plant stages (in autumn), were uprooted and the samples were brought to the laboratory in ice boxes for isolation. The total number of samples collected at the 2 stages is given in Table 1. Since the study started in August of 2015, samples for the seedling stage were collected in the spring of 2016 and 2017. A single sample with symptoms of dark, watery lesions resembling Phytophthoraroot rot was obtained from Kırkkavak Village, in the Thrace region of Turkey.

2.2. Isolation of oomycetous fungi-like organisms from the root tissues

For the isolation of the 151 samples collected at the root stage 2015; Aphanomyces medium of Pfender et al. (1984) (APHM) (as g/L: DifcoBacto agar 10, Difco cornmeal agar 10, metalaxyl 0.030, benomyl 0.005, and vancomycin 0.2), half-strength potato dextrose agar (HSPDA) (as g/L; PDA (Merck) 20, agar agar 10) and water agar (WA) (as g/L:

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[3] Poindexter S (2004). *Aphanomyces cochlioides* can significantly reduce yield and quality of sugar beets in Michigan and Ontario [online]. Website https://www.canr.msu.edu/news/ [accesses 12 December 2018].

[4] Germains Seed Technology (2019). Basics of sugarbeet seed treatments for North America [online]. Website https://germains.com/us/basics-of-sugarbeet-seed-treatments-north-america/ [accessed 16 October 2019].

Table 1. Number of root samples analyzed at the seedling and root stages and number of diseased and disease-free samples in the 2 years of study.

Oomycetous pathogens	Occurrence of oomycetous pathogens				Total
	Seedling stage (9–14 BBCH[1])		Root stage (31–49 BBCH[1])		
	2016	2017	2015	2016	
Root samples analyzed[2]	107	254	224	281	866 (691)[3]
Aphanomyces cochlioides	4 (4.25%)	10 (5.43%)	23 (15.7%)	13 (4.9%)	50 (7.23%)[3]
Phytophthora spp.	1 (1.1%)	0 (0.0%)	6 (4.1%)	0 (0.0%)	7 (1.01%)[3]
*Pythium*s pp.	17 (18.1%)	12 (6.52%)	5 (3.4%)	8 (2.9%)	42 (6.22%)[3]
No of total oomycetous pathogens and their percentages	22 (23.35%)	22 (11.95%)	34 (23.2%)	21 (7.8%)	99 (14.46%)

[1]Sugarbeet growth stages published by Meier et al. (1993).
[2]The number of root samples represents the number of fields.
[3]The number of diseased fields and their percentages were calculated based on the 691 fields found to be infected out of 866 fields examined.

agar 20) were used. For the isolation of the remaining root samples over the 2 years, only HSPDA was used, since it was found to be the most suitable medium for the first 151 fleshy root isolations. Various symptoms were observed at the late stage of root growth, and small sections of about $10 \times 10 \times 10$ mm in size were taken from the borders of the intact and rotting tissues and disinfected in 2% NaOCl for 3 min. Smaller sections of about 2–3 mm were removed aseptically and plated on the isolation media.

Samples collected at the seedling stage were washed under tap water and disinfected in 1% NaOCl for 2 min, dried on blotter paper, and plated in HSPDA for probable oomycetous pathogens.

2.3. Identification of the oomycetous pathogens

The oomycetous pathogens growing on the isolation media, with nonseptate mycelia, were subcultured on amended grated carrot agar (AGCA) containing (as µg mL⁻¹: β-sitosterol 30, thiamine hydrochloride 1, and tryptophan 20; 40 g of grated carrots and 20 g of agar; 1 L water) by removing small pieces from the mycelialtips under a stereomicroscope, and kept in the dark at 22±2 °C to stimulate oospore and sporangia production, especially for *Aphanomyces* spp. and *Phytophthora* spp. (Wilcox and Ellis, 1989). Sporangial characteristics of *Aphanomyces* spp. and *Phytophthora* spp. were observed on culture disks grown on AGCA medium and incubated in sterile and nonsterile soil extracts (Jeffers, 2006). Oospore formations of *Phytophthora* spp. and the other pathogens were observed on 4-week-old cultures on AGCA medium. Identification of *A. cochlioides* was done based on the morphological characteristics of the isolates (Windels, 2000; Avan et al., 2019). Identity of *Phytophthora* spp., and *Pythium* spp. at the genus level was accomplished using the

morphological aspects described in the literature (Plaats-Niterink, 1981; Stamps et al., 1990; Erwin and Ribeiro, 2005; Gallegly and Hong, 2008).

Morphological identification of the selected 5 *Phytophthora* spp. and 18 *Pythium* spp. isolates 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. The ITS region was amplified with primer pairs ITS1 and ITS4 (White et al., 1990). PCR reaction was performed in a 50 µL mixture containing 1.5 mM of $MgCl_2$, 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. The amplicons were sequenced in both directions by the Macrogen Inc. Sequencing Center (Seoul, South Korea). Sequences of the representative isolates were deposited in GenBank under the following accession numbers: *Pythiumultimum* var. *ultimum*: MN541097 to MN541106, *Pythium heterothallicum* MN541110-MN541111, *Pythium aphanidermatum* MN541107, *Pythium sylvaticum* MN541112 to MN541114, *Phytopythium helicoides* MN541108-MN541109, *Phytophthora pseudocryptogea* MN089654-MN089655, *Phytophthora cryptogea* MK789592, *Phytophthora megasperma* MN217536, and *Phytophthora inundata* MN089653.

2.4. Pathogenicity of the oomycetous pathogens

The pathogenicity of some isolates of *A. cochlioides*, *Phytophthora* spp., and *Pythium* spp. was determined using aslightly modified soil layer technique described by Brantner and Windels (1998), by placing10-cm-diameter culture disks of the organisms on the soils in half-filled 15-cm-diameter pots that contained a pasteurized soil mix of soil, sand, and manure (1:1:1). Next, 10 sugar beet seeds of cv. Aranka, disinfected with 2% NaOCl for 3 min, were sown into the culture disks and covered by 2 cm of soil mix. A total of 4 pots were used for each isolate. The seeded pots were incubated in a growth chamber adjusted to 25 ± 2°C and watered when necessary. The emerging seedlings were regularly observed and the dead seedlings were recorded. At 40 days after sowing, the remaining seedlings were uprooted and evaluated for disease intensity using the following scale: 0, no disease; 1, one-third of the root was necrotic; 2, two-thirds of the root was necrotic; 3, the whole root was necrotic, or the seedling was completely dead.

The disease index value for each replicate was calculated using the formula below:

$$\text{Disease index} = \frac{\Sigma \ (\text{Number of seedlings} \times \text{scale value})}{\text{Total seedlings} \times \text{maximum scale value}}$$

2.5. In vitro effectiveness of some fungicide mixes on the oomycetous pathogens

The effectiveness of 16seed treatment fungicide mixtures was investigated under the same conditions as mentioned for the pathogenicity tests. Seeds were coated with fungicide mixtures with a polymer (Sesvanderhave Vinamylpolyvil) at a rate of 7 g per kg of seed by mixing with the fungicidesin jars, and were sown on top of the pathogen culture disks with 4 replicates. Disease rates were calculated as mentioned for the pathogenicity tests above.

2.6. Data analysis

To compare the aggressiveness of the isolates obtained by the inoculation tests and the effectiveness of the fungicide mixes, ANOVA was performed on the calculated disease index values using Minitab 17 statistical software (Minitab Inc., State College, PA, USA). Statistical differences between the isolates were assessed with the Tukey multiple range test. For all of the tests, statistical significance was accepted as $P \leq 0.05$ (Mathews, 2005).

The sequence files were edited manually using SeqMan Pro (DNASTAR, Inc., Madison, WI, USA) and run through BLAST[5] to determine the sequence identity. The consensus sequences were aligned together with the reference sequence data from GenBank by MEGA6 (Tamura et al., 2013) and phylogenetic trees were constructed using the neighbor-joining method (Tamuara-Nei as a substitution model, 1000 replicates).

3. Results

3.1. Occurrence of oomycetous pathogens in sugar beet fields in Konya Province

The following oomycetous pathogens were determined at various frequencies in the 2 growth periods: *A. cochlioides*, *Ph. cryptogea*, *Ph. pseudocryptogea*, *Ph. inundata*, *Py. aphanidermatum*, *Py. helicoides*, *Py. heterothallicum*, *Py. sylvaticum*, and *Py. ultimum* var. *ultimum*. All of the above mentioned species were the first records on sugar beet for Turkey. A single isolate of *Ph. megasperma* was obtained from a root sample sent from Kırkkavak Village in the Thrace region of Turkey.

A. cochlioides was isolated from 50 field samples out of 691, with 36 at the late growth stage and 14 at the early growth stage. However, for the *Pythium* species, this was the other way around, with 13 and 29 for the late growth and early growth stages, respectively. The occurrence of *Phytophthora* spp. was very scarce, with 6 samples (fields) at the root stage in 2015 and 1sample at seedling stage in 2016 (Table 1).

A. cochlioides was found together with a *Pythium* sp. in 1 field, while its coinfections with other root rot pathogens, such as *Rhizoctoniasolani* and *Phoma betae* (not taken into consideration at this paper), were very common. No other pathogens were found from the plant samples with *Ph. cryptogea* and *Ph. pseudocryptogea*.

The rates of stand loss due to root rot caused by the oomycetous pathogens was roughly estimated during the surveys and the percentages varied between 1% and 15%, 3% and 30%, and 3% and 50% for *A. cochlioides*, *Pythium* spp., and *Phytophthora* spp., respectively.

3.2. Detection of oomycetous pathogens on various isolation media

The recovery of oomycetous pathogens out of the 151 plant samples collected at the late root stagein 2015 from the various media is outlined in Table 2. HSPDA medium was the most suitable isolation medium for the root rot samples. AGCA medium provided profuse growth of sporangia and oospores when present, for all of the pathogens. The production of zoospores was profuse for *A. cochlioides* and *Phytophthora* spp., but not for *Pythium* spp.

APHM medium was not sufficiently selective for *A. cochlioides*, since various pathogenic and nonpathogenic fungi were also recovered from the 224 plant samples in the following order: *Phomabetae* 9, *Rhizoctonia solani* 10, *Rhizopus* sp. 10, *Geotrichum* sp. 16, *Fusarium* spp. 3, bacterial growth 3, zygomycetou ssp., 3, and *Alternaria alternata* 2.

3.3. Identification of the oomycetous pathogens

Preliminary identification of the pathogens was performed using their morphological characteristics. *A. cochlioides*

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[5] National Center for Biotechonolgy Information (2020). Basic Local Alignment Search Tool (BLAST) [online]. Website http://blast.ncbi.nlm.nih.gov/Blast.cgi [accessed 14 May 2019].

Table 2. Number of isolates obtained from the 151 root samples collected at the fleshy root stage from September to October, 2015.

Media/pathogens	APHM	HSPDA	WA	Total
Aphanomyces coclioides	7	9	8	24*
Phytophthora spp.	0	4	2	6
Pythium spp.	1	2	2	5

APHM, Aphanomyces selective medium of Pfender et al. (1984); HSPDA, half-strenght PDA; WA, water agar. *From 1 sample, *Aphanomyces cochlioides* was obtained from both of HSPDA and WA.

was identified solely based on the host specificity and growth characteristics obtained from the used media (Avan et al., 2019).

A. cochlioides not only grew well on all of the APHM, HSPDA, and WA isolation media (Figure 1a), but also produced a lot of oospores, and abundant sporangia and zoospores when the culture disks taken from the AGCA were incubated in sterile and nonsterile soil extracts (Figures 1b and 1c). Along with these microscopic features, mycelial branching and the other aspects were also in accordance with the description of Windels (2000).

Ph. Cryptogea and *Ph. pseudocryptogea* also produced abundant sporangia and zoospores when incubated under the same conditions as given above. Microscopic characteristics of *Ph. cryptogea* and *Ph. pseudocryptogea* were also typical of the species; being heterothallic, not producing oospores, and having nonpapillate, noncaducous, ellipsoid sporangia with a crosswall at the tapered bottom, as described by Gallegly and Hong (2008) (Figure 1d). As stated by Safaiefarahani et al. (2015), these morphological features did not distinguish these 2 species from *Ph. drechsleri*, which belongs to Phytophthora clade 8a.

The BLASTn search showed that the ITS sequences of the *Phytophthora* spp. isolates from this study had 99.73%–100% identity with isolates of *Phytophthora* species previously recorded in GenBank. The sequence dataset had 786 characters, of which 113 characters were parsimony-informative, and 114 characters were variable. The phylogenetic tree clustered the *Phytophthora* species into 4 well-supported clades (Figure 2). The isolates of *Ph. cryptogea* and *Ph. pseudocryptogea* were clustered into distinct clades together with reference GenBank sequences reported by Safaiefarahani et al. (2015). The other 2 isolates were clustered together with reference GenBank sequences of *Ph. inundata* and *Ph. megasperma*.

Many of the *Pythium* species growing on AGCA medium produced sporangia and oospores. None of the *Pythium* spp. showed vesicle formation or produced zoospore discharge when incubated on the soil extracts,

but some produced oospores and spherical sporangia only. Identification of *Pythium* species, for this reason, was mostly based on a comparison of ITS sequencesvia the BLAST analysis and *Pythium* spp. showed 99.05%–100% identity with the isolates previously described in GenBank. The sequence dataset had 901 included characters, of which 486 were parsimony-informative, and 504 were variable. A phylogenetic tree (Figure 3) was prepared using the relevant sequences clustered into 5 species(*Py. aphanidermatum*, *Py. helicoides* (*Phytopythium helicoides*), *Py. heterothallicum*, *Py. sylvaticum*, and *Py. ultimum* var. *ultimum*)together with the reference GenBank sequences into 5 well-supported clades (100% bootstrap support).

3.4. Aggressiveness of the isolates of the oomycetous pathogens
The randomly selected 8 isolates of *A. cochlioides*, 9 isolates of *Pythium* spp., and 5 isolates of *Phytophthora* spp. showed varying rates of aggressiveness (Table 3). The majority of *A. cochlioides* and *Phytophthora* spp. isolates were highly aggressive, producing more than 90% disease intensity, while the aggressiveness of *Pythium* spp. was quite variable (Table 3).

3.5. Effectiveness of the seed treatment fungicides against the 2 most widespread oomycetous pathogens
When the fungicide-treated seeds were sown onto the inoculated soil mixtures, various percentages of disease severities were obtained. Out of 16 fungicide mixes, only 5 mixes protected the seedlings from the infections of 1 aggressive isolate of *A. cochlioides* sufficiently, and the mix of boscalid 8 + pyraclostrobin 2 + metalaxyl 3.2 was the most effective. The same fungicide mix showed high effectiveness against an aggressive isolate of *Pythium ultimum* var. *ultimum*. The standard seed treatment fungicide mixture, thiram 3.2 + hymexazole 3.5, did not provide sufficient protection against the 2 pathogens.

The effects of various seed treatment fungicide mixes against the 2 more widespread oomycetous pathogens were quite variable. The application of hymexazole mixed with either thiram or pyraclostrobin did not provide sufficient protection for the emerging seedlings (Table
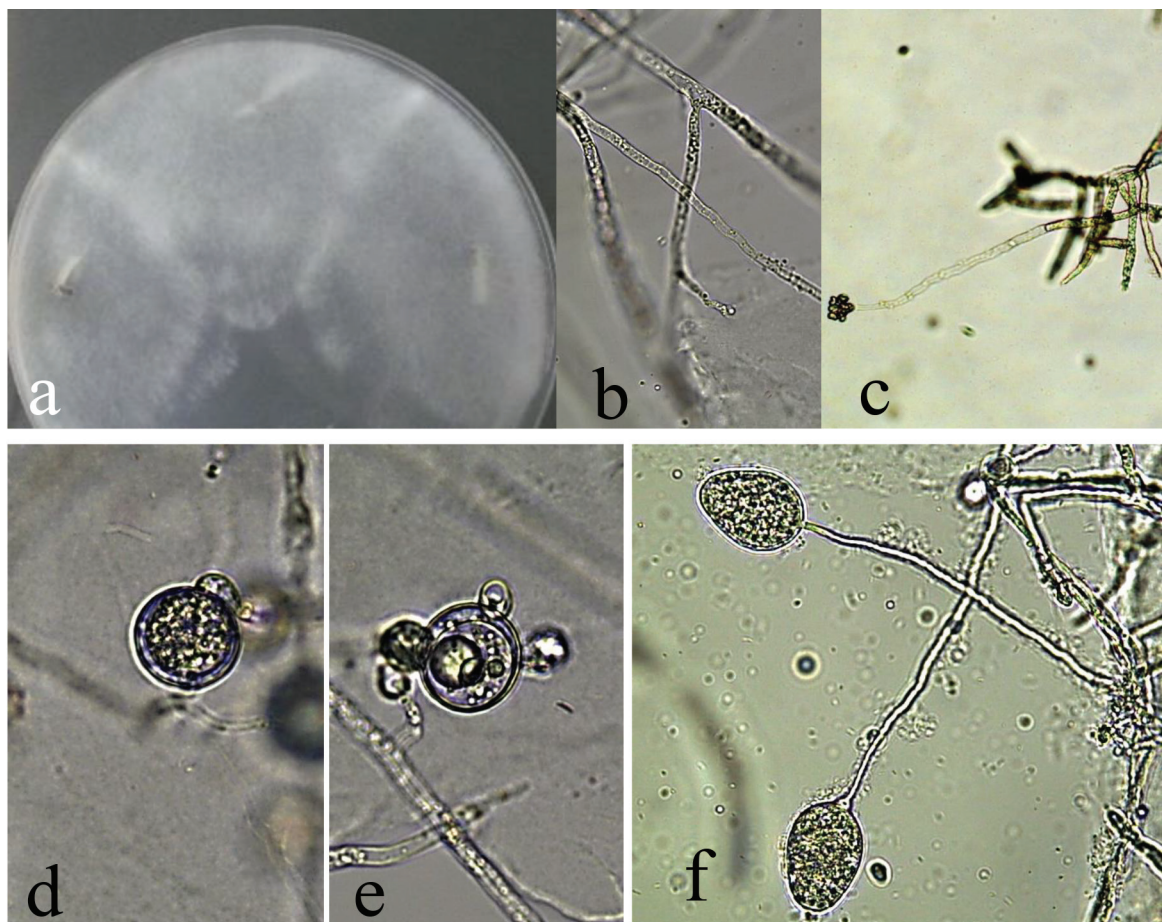
Figure 1. Various aspects of the oomycetous pathogens of sugar beet. a) Growth of *A. cochlioides* on HSPDA. b, c) Zoosporangia of *A. cochlioides* delimited from the vegetative hyphae and formation of zoospores in a vesicle at the end of zoosporangia. d, e) Oospores of *A. cochlioides*. f) Sporangia of *Phytophthora drechsleri*.

4). Combinations of metalaxyl with some fungicides, for example, with fludioxonil and metconazole, which is known to be effective against the 2 pathogens, did not control seedling damping-off. Hymexazole and metalaxyl combinations provided sufficient control of the 2 pathogens.

4. Discussion

Konya Province is an important sugar beet growing region, producing about one-third of the total sugar beet yield of Turkey, and this study presented the importance of oomycetous pathogens on root rot, causing stand losses of upto 50%, which occurred in about 15% of the fields.

Oomycetous pathogens causing root rot on sugar beet have not been investigated extensively in Turkey, except for 2 records mentioned as *Pythium* spp. (Yorgancı and Turhan, 1988; Özgönen and Çulal Kılıç, 2009) and 1 record of *Pythium ultimum* (Erzurum et al., 1995). Whereas 3 pathogens in this group, *A. cochlioides*, *Phytophthora* spp. and, *Pythium* spp., are well-known as important pathogens

in many sugar beet growing areas of the world[3] (Kuznia and Windels, 1993; Brantner and Windels, 1998; Windels, 2000; Pizczek, 2004; Jacobsen, 2006; Harveson et al., 2009). *A. cochlioides*, *Ph. cryptogea*, *Ph. pseudocryptogea*, *Ph. inundata*, *Py. aphanidermatum*, *Py. helicoides*, *Py. heterothallicum*, *Py. sylvaticum*, and *Py. ultimum var. ultimum* were not only the first records for Turkey, but they were also widespread and important pathogens of root rot in the Konya region of Turkey. Their distribution in the other sugar beet growing areas is possible and should be investigated, since anotherspecies of *Phytophthora*, *Ph. megasperma*, was isolated from a root sample sent to the laboratory from the Thrace region of Turkey.

Among the 3 pathogens, *A. cochlioides* was not only the most widespread, but the most aggressive as well. Although the aggressiveness test was conducted at the early seedling stage, when the intensity of the pathogen was low, it can be very harmful at late growth stages, since the pathogen prefers considerably higher temperatures to grow, as stated by Windels (2000), who recorded about 100% loss at this
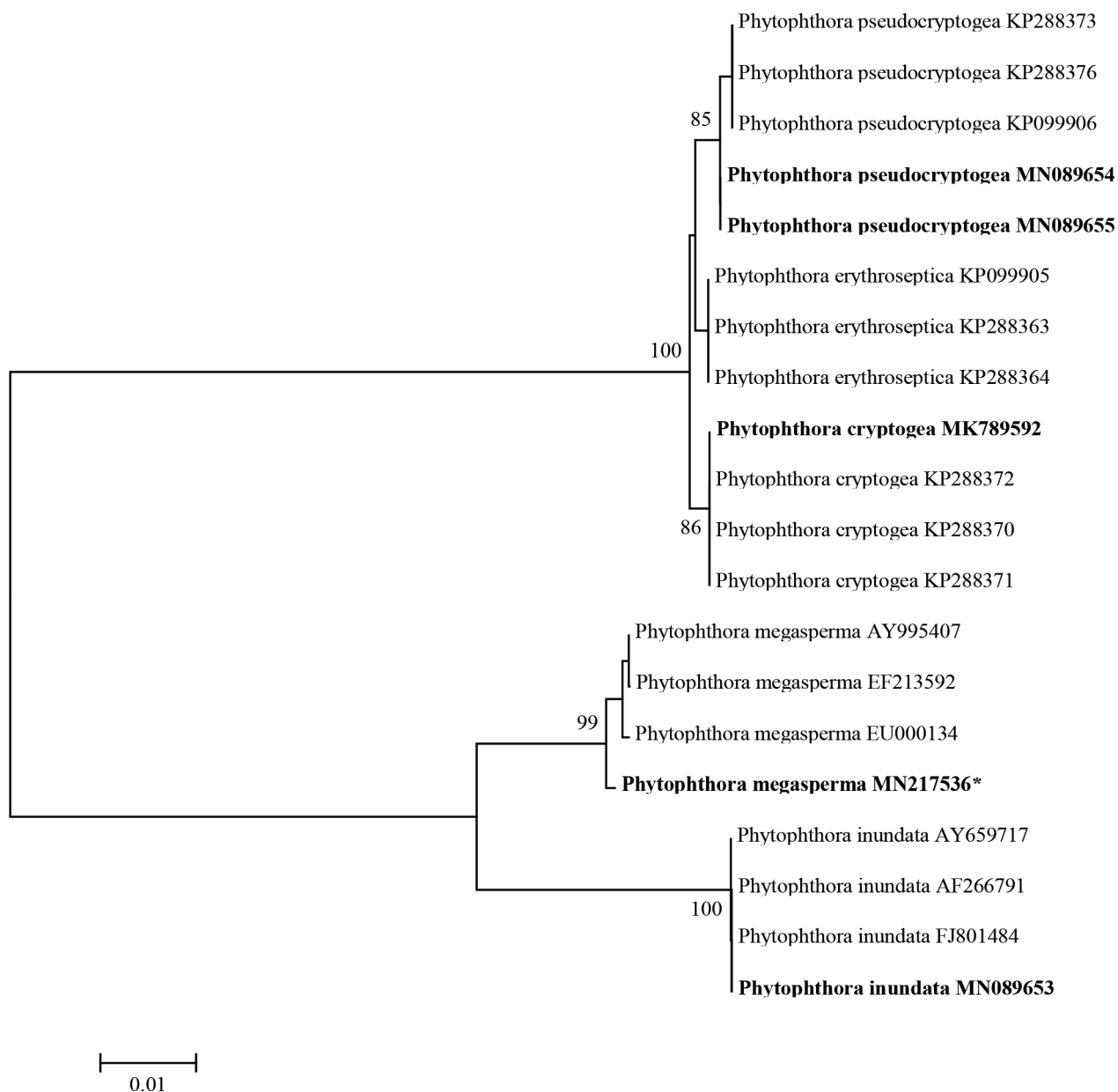
Figure 2. Neighbor-joining tree derived from ITS sequences of the *Phytophthora* species obtained from the sugar beet roots and published sequences in GenBank. The numbers at the branch points indicate bootstrap values based on 1000 replicates. The isolates used in this study are shown in bold letters. *This species was isolated from a sample sent from Kırkkavak Village in the Thrace region.

stage. The same situation was valid for the *Phytophthora* species, even though their occurrences were not so high.

The occurrence of *Pythium* species at high rates at the seedling stage was interesting, since a routine seed treatment with thiram+hymexazole, which was reported to be effective against these pathogens, has been practiced in Turkey for a long time. It was also reported that this seed treatment was also effective for *A. cochlioides*, which should be reevaluated, or alternative seed treatment options should be researched.

No report dealing with the selective isolation of all 3 of the oomycetous root rot pathogens from the infected tissues has been presented thus far, except for 1 medium mentioned to be semiselective for *A. cochlioides* (Pfender et al., 1984). Isolation of the 3 oomycetous pathogenswas performed successfully from the infested plant samples using HSPDA and WA media.

Identification of the *A. cochlioides* and *Phytophthora* species basedon their morphological characteristics did not create any difficulty when their host specificity was
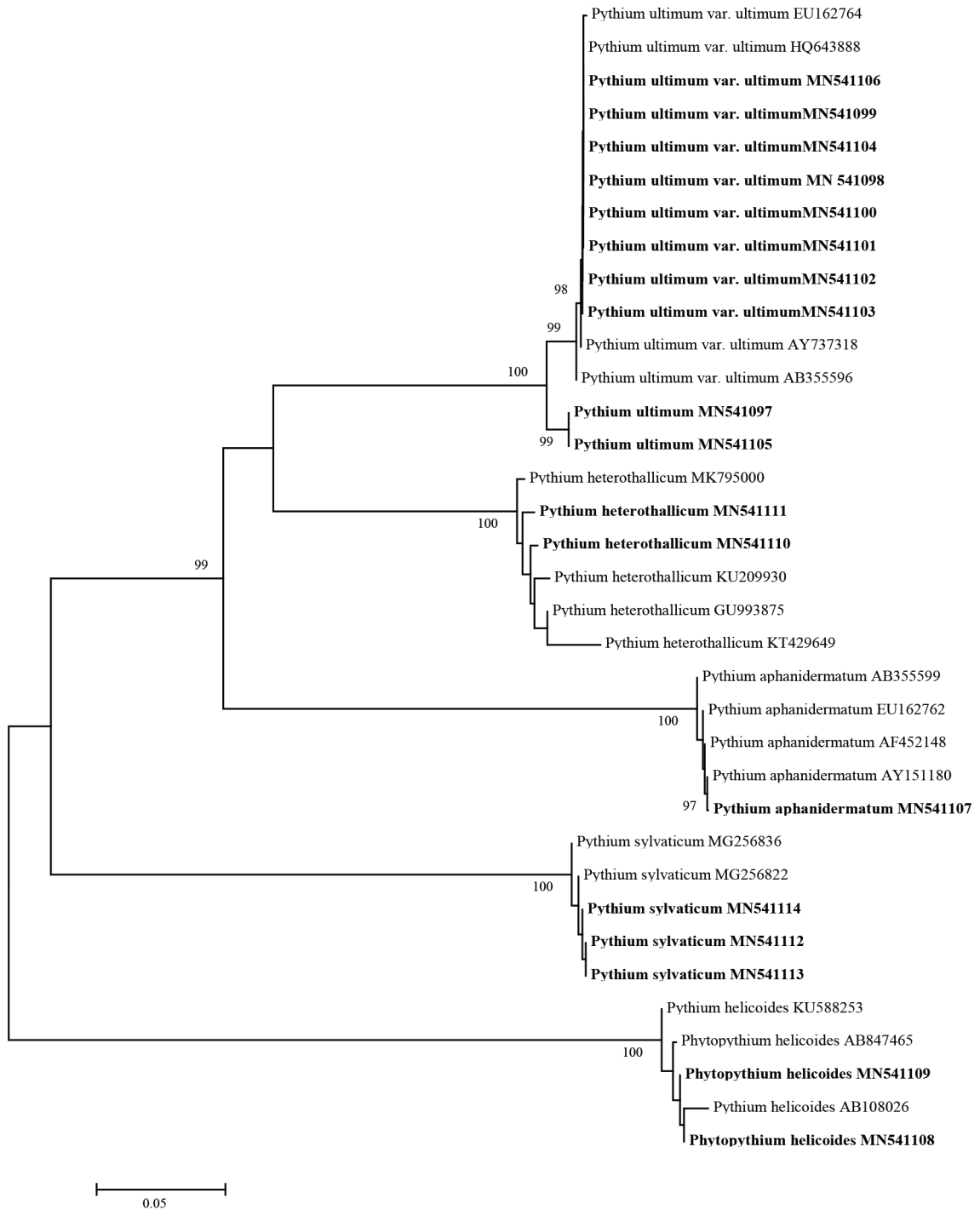
Figure 3. Neighbor-joining tree derived from ITS sequences of the *Pythium* species obtained from the sugar beet roots and published sequences in GenBank. The numbers at the branch points indicate bootstrap values based on 1000 replicates. The isolates used in this study are shown in bold letters.

taken into consideration. Zoosporangia and zoospores by *A. cochlioides* and *Phytophthora* species were characteristic when grown on AGCA and incubated on the soil extracts. AGCA was easily prepared, and it was reported as very

usefulfor the identification of *Phytophthora* spp. from chestnuts (Akıllı et al., 2012) and based on the current findings, it can also be used for *A. cochlioides*. For quite some time, *Phytophthora drechsleri*, which belongs to

Table 3. Percent disease severity values of the 8 *A. cochlioides*, 9 *Pythium* spp. and 3 *Phytophthora* spp.[1] obtained from the infested soil mix at the seedling stage.

A. cochlioides			Pythium spp.			Phytophthora spp.		
Isolate No.	Percent disease severity		Species	Percent disease severity		Species	Percent disease severity	
150	97.50	a[2]	P. heterothallicum	100.00	a	P. cryptogea	99.00	a
122	97.50	a	P. ult.var. ultimum	100.00	a	P. pseudocryptogea	95.75	a
105	97.50	a	P. heterothallicum	100.00	a	P. pseudocryptogea	95.00	a
31	97.50	a	P. ult.var. ultimum	99.15	a	P. inundata	32.50	b
53	92.50	a	P. heterothallicum	98.30	a	Control	28.00	b
58	90.83	a	P.helicoides	96.67	a			
191	90.00	a	P. ult.var. ultimum	94.15	a			
85	87.50	a	P. sylvaticum	84.95	a			
Control	35.80	b c	P. aphanidermatum	50.80	b			
			Control	22.50	c			

[1]The pathogenicity of *Ph. megasperma* was not tested since the sample arrived late. [2]Means that do not share a letter are significantly different (P ≤ 0.05). Grouping information was based on the Tukey test.

Table 4. Percent disease severities obtained from the treated seeds by 16 fungicide mixtures.

Fungicide mixtures tested	Percent disease severity[1]	
	Aphanomyces cochlioides	Pythium ultimum var. ultimum
Thiram 3.2 + metalaxyl 3.2[2]	**27.25 e**[3]	92.25 abc
Thiram 3.2 + hymexazol3.5	72.25 cd	92.00 abc
Thiram 3.2 + metalaxyl 3.2 + hymexazol3.5	**29.75 e**	**24.00 e**
Thiram 3.2 + pyraclostrobin2	90.75 abc	98.00 a
Thiram 3.2 + metalaxyl 3.2 + pyraclostrobin2	**29.75 e**	64.00 cd
Thiram 3.2 + hymexazol3.5 + pyraclostrobin2	73.00 cd	77.75 abc
Thiram 3.2 + metalaxyl 3.2 + hymexazol3.5 + pyraclostrobin2	**29.75 e**	**20.75 e**
Boscalid 8 + pyraclostrobin2	74.00 bcd	93.00 ab
Boscalid 8 + pyraclostrobin2 + metalaxyl 3.2	**16.00 e**	65.50 bcd
Prothioconazole 0.16 + spiroxamine0.3	97.00 abc	99.00 a
Prothioconazole 0.16 + spiroxamine0.3 + metalaxyl 3.2	73.00 cd	96.00 a
Fludioxonil 2 + metalaxyl 3.2	87.25 abc	96.25 a
Metconazole 1+ metalaxyl 3.2	95.50 abc	91.25 abc
Thiram 3.2 + tolchlofos-methyl 0.42	98.25 ab	96.25 a
Sedaxane 1.35 + fludioxonil 2 + mefenoxam1.35	60.50 d	**46.30 de**
Inoculated control	100.00 a	99.00 a
Noninoculated control	0.00	0.00

[1]Percent disease severities are the averages of 4 replications obtained from the treated seeds. [2]Figures following fungicide mixtures show the active ingredient applied per kg of seed. [3]Means that do not share a letter are significantly different (P ≤ 0.05). All of the noninoculated seeds (40 in total) produced healthy seedlings. Grouping information was based on the Tukey test. All of the treatments contained 9 g of imidacloprid per kg of seed and 7 g of Vinamylpolyvil (Sesvanderhave) per kg of seed for coating.

Phytophthora clade 8a, has been reported as the most widespread and common species causing root rot on sugar beet (Jacobsen, 2006; Harveson et al., 2009). Recently, Safaiefarahani et al. (2015) reevaluated this clade and distinguished 4 species by molecular analysis of some gene sequences. *Ph. drechsleri* was not determined in the surveyed areas of the current study, but *Ph. cryptogea*, *Ph. pseudocryptogea*, and *Ph. inundata* were present in sugar beet fields surveyed, the first 2 belonging *Phytophthora* clade 8a. The current study also indicated that molecular analysis is necessary and time saving for the identification of *Phytophthora* species on sugar beet.

The majority of the isolates of *A. cochlioides* and *Pythium* spp., 2 of the *Ph. cryptogea* isolates, and 1of the *Ph. pseudocryptogea* isolates were highly aggressive, causing more than 85% disease severity at the seedling stage. Moreover, 1 isolate of *Py. aphanidermatum* and 1 *Ph. inundata* isolate produced lower rates of diseases than the other pathogens. Babai-Aharyet al. (2004), on the other hand, found *Py. aphanidermatum* highly pathogenic in Northern Iran. They also recovered *Py. ultimum* var. *ultimum* as adominant species.

Pythium heterothallicum, *Phytopythium helicoides*, and *Py. sylvaticum* all caused seedling root rot and were the first reports on sugar beet, although the first species was reported previously on table beet from Queensland (Scot et al., 2005). Of the *Pythium* species, except for *Py. ultimum*, 4 were the first reports for Turkey. Although the pathogenicity of the oomycetous pathogens was tested at the seedling stage, their damage might be higher at the late growth stages, since old roots are more senescent then than at the early growth stages. The results herein (Table 1) also supported this, since the 2 pathogens, except *Pythium* spp., were isolated more frequently at the late growth stages.

The seed treatment combination of thiram + hymexazole applied for the routine control of root rot of sugar beet in Turkey did not control seedling infections of *A. cochlioides* and *Pythium ultimum* var. *ultimum* sufficiently. When metalaxyl was added to this mixture, the 2 pathogens were highly suppressed (Table 4). Some of the metalaxyl combinations, such as fludioxonil and metconazole, did not control seedling damping-off of the 2 pathogens. This result might have been due to the antagonistic or incompatible reaction of the fungicides with metalaxyl, which is known to be effective against the 2 pathogens. The effects of various seed treatment fungicide mixes against the 2 pathogens were quite variable. Hymexazole, containing mixes with either thiram or with pyraclostrobin, did not provide sufficient protection for the emerging seedlings. The combination of thiram + metalaxyl + hymexazole + pyraclostrobinprovided sufficient control of the 2 pathogens (Table 4.)

Seed treatment fungicides should control not only oomycetous pathogens, but also other root rot pathogens, such as *Phoma betae* and *Rhizoctonia solani*, and a combination of other fungicides are necessary. The new fungicide mix (Cruiser maxx) registered by Syngenta for sugar beet seed treatment, for example, contains metalaxyl and fludioxonil.

The seed treatment fungicides should alsocontain other fungicides effective against *Rhizoctonia*, which is also very common in sugar beet fields.

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