Development of Resistance to Hymexazol Among *Pythium* Species in Cucumber Greenhouses in Oman

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Abstract

A study was conducted to characterize the common *Pythium* spp. in greenhouses in Oman and their level of resistance to hymexazol, a widely used fungicide in the country. *Pythium* isolates were obtained from soil samples, cocopeat bags, and cucumber roots collected from seven regions in the country. Identification of 80 *Pythium* isolates to the species level using sequences of the internal transcribed spacer region of the ribosomal RNA showed that they belong to four species: *Pythium aphanidermatum* (77 isolates), *P. spinosum* (1 isolate), *P. myriotylum* (1 isolate), and *P. catenulatum* (1 isolate). Investigating the aggressiveness of three *Pythium* spp. on cucumber showed that *P. aphanidermatum*, *P. myriotylum*, and *P. spinosum* are pathogenic. Phylogenetic analysis

of *P. aphanidermatum* isolates showed that most of the isolates obtained from cocopeat clustered separately from isolates obtained from soil and roots. This may indicate a difference in the origin of the cocopeat isolates. Evaluating the resistance of 27 *P. aphanidermatum* isolates to hymexazol showed that most isolates were sensitive (0.9 to 31.2 mg liter⁻¹) whereas one isolate was resistant (142.9 mg liter⁻¹). This study is the first to report *P. myriotylum* and *P. catenulatum* in Oman. It is also the first to report the development of resistance to hymexazol among *P. aphanidermatum* populations from greenhouses. Growers should use integrated disease management strategies to avoid further development of resistance to hymexazol.

Cucumber is a member of the economically important family Cucurbitaceae (gourd family). It is native to India or South Asia, where it has been cultivated for at least 3,000 years (Rubatzky and Yamaguchi 1997). The world total production of cucumber in 2015 was 57 million metric tons (FAO 2015). Depending on cultivars and location, the cultivation of cucumber is done in either open fields or greenhouses. Greenhouse cucumber are parthenocarpic, with seedless fruit. Today, most cucumber plants are grown in soil-based systems and in various types of soilless culture techniques under greenhouse conditions (Farrag and Fotouh 2010; Herrero et al. 2003).

Greenhouse cucumber production is a fast-growing sector in Oman. Over 90% of greenhouses in Oman are used for cucumber production. Most of cucumber greenhouse owners adapted soil-based production system (Al-Sadi et al. 2011b). A few growers use compost or cocopeat bags for the production of cucumber. This is mainly done by placing these bags in greenhouses and then raising cucumber plants in the bags to avoid contact of cucumber gourds with soil, which is usually contaminated with *Pythium* spp. and other pathogens. This practice usually narrows the potential ways of contamination of cocopeat or even cucumber with *Pythium* spp. Growers can grow cucumber in three seasons per year. This exclusivity and continuity of growing a single crop makes cucumber production a monoculture farming system, leading to excessive use of irrigation water, chemical fertilizers, and pesticides. In addition, it increases the incidence of diseases (Deadman et al. 2007).

Damping-off is a serious epidemic disease affecting the production of cucumber in Oman. It can cause over 75% mortality in cucumber seedlings (Al-Sadi et al. 2011b). This disease is caused by a number of pathogenic fungi, the most important of which are *Pythium* spp. Previous studies in Oman indicated the association of *Pythium aphanidermatum*, *P. spinosum*, and *P. splendens* with greenhouse cucumber. Among them, *P. aphanidermatum* is the most dominant species (Al-Sadi et al. 2011b). Fungal pathogens are introduced into greenhouses via a number of sources, including soil, potting media, and fertilizers (Sanchez et al. 2001).

A number of management techniques have been used to manage *Pythium* spp.-induced diseases in greenhouses. In Oman, the common techniques used to reduce *Pythium* inoculum in greenhouses are solarization and fungicides; namely, mefenoxam, hymexazole, and propamocarb. The application of the same fungicides in large amounts for a long period of time has been found to encourage the buildup of resistance in the fungal isolates to fungicides (Chung et al. 2009; Moyano et al. 2004; Staub and Sozzi 1983). Previous studies in Oman evaluated the resistance of *Pythium* spp. to mefenoxam (Al-Sa'di et al. 2008a). However, no attempts were made to evaluate resistance to the second most commonly used fungicide, hymexazol.

The aim of this study was to characterize *Pythium* spp. obtained from cucumber greenhouses in Oman and to evaluate the sensitivity of *P. aphanidermatum* to hymexazol. Specific objectives were to (i) characterize the common *Pythium* spp. in greenhouses in Oman, (ii) study the degree of pathogenicity of *Pythium* spp. on cucumber, and (iii) study the degree of sensitivity of *P. aphanidermatum* isolates to hymexazol.

Investigations into these areas will aid understanding of the prevalent *Pythium* spp. in greenhouses in Oman and their level of resistance to hymexazol. This will help growers plan appropriate strategies for management of *Pythium* spp.

Materials and Methods

Collection of samples. A survey was conducted from November 2015 to April 2016 that covered 19 cucumber greenhouses from seven different governorates in Oman: Muscat, Al Batinah North, Al Batinah South, Al Dakiliya, Al Sharqiya North, Buraimi, and Dhofar (Fig. 1). Samples were collected from soil, cucumber roots, and cocopeat bags. Soil samples were collected from the top 15 cm of soil near the dropper of the drip irrigation pipe. The total number of samples was 140 from soil, 20 from cucumber roots, and 30 from cocopeat. Soil samples were collected systematically from all the surveyed greenhouses. However, the cucumber root samples were collected only from 20 plants developing wilt symptoms in six sites. Because some cucumber growers were found to use cocopeat media for raising cucumber plants instead of sowing seed in greenhouse soil, samples of cocopeat were collected from greenhouses in Batinah utilizing cocopeat as growing media. All samples were placed in a sterile plastic bag, labeled, and kept in a refrigerator at 4°C until processed.

Isolation of *Pythium* spp. *Pythium* spp. were isolated from soil and cocopeat using the direct plate method. In this method, 1.7% corn

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meal agar (Oxoid) media amended with rose Bengal (30 µg ml⁻¹), pimaricin (5 µg ml⁻¹), ampicillin (250 µg ml⁻¹), rifampicin (10 µg ml⁻¹), and pentachloronitrobenzene (100 µg ml⁻¹) was prepared (Jeffers and Martin 1986). Then, 0.05 g of the soil or cocopeat sample was spread on a Petri dish.

Roots were washed, cut into small pieces, and placed in 1.5% NaOCl for 1 min. After that, the root sections were transferred to sterilized distilled water for 1 min, then blotted on sterile filter paper to remove excess water. Finally, root sections were placed on the selective media and the plates were incubated at 28°C for up to 7 days. Finally, oomycete growth typical of *Pythium* spp. was subcultured to a new plate of corn meal agar.

Identification of *Pythium* spp. The identity of 80 *Pythium* isolates (Table 1) was confirmed using the sequence of the internal transcribed spacer region of ribosomal RNA (ITS rRNA). *Pythium* mycelium was harvested from corn meal agar media and placed inside 1.5-ml Eppendorf tubes. Then, the mycelium was freeze dried and ground into fine powder. This was followed by lysis, purification, and precipitation of DNA (Lee and Taylor 1990). Finally, DNA pellets were washed using 70% ethanol, dried, and suspended in 100 µl of Tris-EDTA buffer. The DNA was kept at -20°C until used.

Fig. 1. Map indicating the regions from which *Pythium* isolates were collected.

Amplification of the ITS rRNA of *Pythium* isolates was done using ITS1 (forward) (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (reverse) (5'-TCCTCCGCTTATTGATATGC-3') primers (White et al. 1990). The reaction mixture of polymerase chain reaction (PCR) consisted of PuREtaq Ready-To-Go PCR beads (GE Healthcare), 0.5 µl of 20 µM ITS1, 0.5 µl of 20 µM ITS4, 2 µl of DNA sample, and sterile distilled water up to 25 µl. After that, the tubes were centrifuged at short spin speed and placed inside a thermal cycler machine (Applied Biosystems). The condition of the PCR machine was set up as described previously (Al-Sadi et al. 2011a). PCR products were run on 1.5% agarose gel in 5× Tris-borate-EDTA buffer for 40 min at 120 V. PCR products were purified and sequenced at Macrogen using the ITS1 and ITS4 primers.

The alignment of forward and backward ITS sequences for each *Pythium* isolate was done using ChromasPro V.1.41. The generated sequences were compared with sequences deposited at the National Center for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool. Sequences of reference isolates were obtained from GenBank for comparison purposes. Most of the reference isolates used in phylogenetic analysis came from the study by Lévesque and de Cock (2004). Phylogenetic trees were constructed using Mega 6 (Tamura et al. 2013). Bootstrap support values were generated based on 1,000 replicates.

Pathogenicity of *Pythium* isolates. Two *P. aphanidermatum*, one *P. spinosum*, and one *P. myriotylum* isolates were tested for their pathogenicity on cucumber seedlings (Al-Sadi et al. 2011b). First, the potting mixture was autoclaved two times and placed inside sterilized pots. Then, seven 'Beit Alpha' cucumber seeds were sown and irrigated daily. The emerged seedlings were thinned to five. For each isolate, five replicate pots were used. After that, 7-day-old seedlings were inoculated with 10-mm agar plugs of *Pythium* spp. (3 days old), which were placed near the crown area of each seedling. Control seedlings received a 10-mm agar plug without inoculum. The pathogenicity level for each isolate was measured as percent mortality of cucumber seedlings. The pots were incubated in a growth chamber in a completely randomized design under 70% relative humidity at 28°C. The experiment was repeated two times. A pathogenicity test was not done for *P. catenulatum* because it was lost during subculturing.

Sensitivity of *P. aphanidermatum* isolates to hymexazol. The degree of sensitivity of 27 randomly selected isolates of *P. aphanidermatum* to hymexazol (Sankyo Co., Ltd.) was tested as described previously (Al-Sadi et al. 2015a). Corn meal agar was amended with five concentrations of hymexazol: 0, 20, 50, 500, and 3,000 µg ml⁻¹. Agar plugs (5 mm) of 3-day-old *Pythium* isolates were placed at the edge of each plate. Three replicates were used for each isolate-concentration combination and the plates were incubated at room temperature (23°C) in a completely randomized design. Linear growth of mycelium was measured after 24 and 48 h. The hymexazol concentration that results in 50% growth inhibition of *Pythium* isolates (EC₅₀) was estimated from the regression line of growth reduction plotted against log-transformed fungicide concentration. The experiment was repeated once.

Means values of the pathogenicity and fungicide tests were analyzed using the Statistical Analysis System (SAS). Separation of means was based on Tukey's Studentized range test (SAS).

Table 1. *Pythium* isolates detected in seven governorates in Oman

Governorate	Sample size	Source of isolates	<i>Pythium aphanidermatum</i>	<i>P. spinosum</i>	<i>P. myriotylum</i>	<i>P. catenulatum</i>
Batinah North	24	22 Cocopeat, 1 soil, 1 root	24	0	0	0
Batinah South	10	Soil	13	0	0	1
Muscat	6	Soil	5	0	1	0
Buraimi	10	Soil	9	1	0	0
Dakiliya	11	Soil	11	0	0	0
Sharqiyah	7	Soil	7	0	0	0
Dhofar	12	5 root, 7 soil	12	0	0	0
Total	80	...	77	1	1	1

Results

Isolation and identification of *Pythium* spp. Isolation from 140 soil samples, 30 cocopeat samples, and 20 cucumber root samples using selective medium for *Pythium* spp. resulted in 109 *Pythium* isolates. PCR of 80 randomly selected *Pythium* isolates (52 from soil, 22 from cocopeat, and 6 from roots) using ITS1 and ITS4 primers produced DNA fragments in the range of 700 to 800 bp.

The resulting sequences were compared with previously deposited sequences in GenBank. Phylogenetic analysis showed that the

sequences of 77 isolates from this study clustered with *P. aphanidermatum* isolates from GenBank (HM008882, KT336808, KC438411, KR095341, KP331545, EU095017, KM102739, and KF667387). Clustering of 77 *P. aphanidermatum* isolates was found associated with the source of inoculum, with most isolates coming from cocopeat from Batinah North making a separate cluster apart from the other *P. aphanidermatum* isolates obtained from soil and roots (Fig. 2). One isolate (M4) from Muscat clustered with *P. myriotylum* (KT364295), one isolate (BR-7) from Al Buraimi governorate

Fig. 2. Phylogenetic tree based on sequence analysis of the internal transcribed spacer region of *Pythium* isolates using the neighbor-joining method. Bootstrap support values $\geq 50\%$ are shown (1,000 replicates). Sequences were deposited in GenBank under the accession numbers shown in the figure.

clustered with *P. spinosum* (LN867319), and one isolate (BS-2) from Al Batinah South governorate clustered with *P. catenulatum* (HM134861) (Fig. 2). Sequences from this study were deposited in GenBank (NCBI) under the accession numbers indicated in Table 2 and Figure 2.

All the root and cocopeat isolates were *P. aphanidermatum*. The 52 *Pythium* isolates from soil were found to be 49 isolates of *P. aphanidermatum* and 1 isolate each of *P. myriotylum*, *P. spinosum*, and *P. catenulatum* (Table 2; Fig. 2).

Pathogenicity test. Inoculation of cucumber seedlings with *P. aphanidermatum*, *P. spinosum*, and *P. myriotylum* resulted in typical damping-off disease symptoms. Water-soaked areas were observed on the stem base of cucumber plants followed by collapse of seedlings. *Pythium* spp. caused varying levels of mortality in the inoculated seedlings (82 to 100%), with isolates all being aggressive on cucumber seedlings (Fig. 3). No mortality was observed in the control seedlings, which were inoculated using agar plugs.

Fungicide test. Evaluating the sensitivity of 27 *P. aphanidermatum* isolates to hymexazol showed significant variation among isolates. The EC₅₀ values of the different isolates ranged from 0.9 to 142.9 mg liter⁻¹ (average = 10.9 mg liter⁻¹). The isolate with the highest level of sensitivity was from Buraimi while the one with highest level of resistance was from Muscat. The majority of *P. aphanidermatum* isolates (*n* = 21) were very sensitive to hymexazol

(Fig. 4). Five isolates were found to be moderately sensitive to hymexazol and one isolate was found to be resistant to hymexazol. The resistant isolate came from Muscat, from a greenhouse which has been using hymexazole for at least the last 5 years. The EC₅₀ values of the two independent tests for the resistant isolate (M-6) were 132.8 and 153 mg liter⁻¹. All isolates were deposited at Sultan Qaboos University Fungal Collection and the ITS sequence of the resistant isolate (M-6) was deposited in GenBank under accession number KY095193. Despite the presence of significant differences between individual isolates, no significant differences were observed between populations of *P. aphanidermatum* from different regions in their level of sensitivity to hymexazol (*P* > 0.05; Table 3).

Discussion

Pythium spp. are a major problem in greenhouse cucumber in different parts of the world (Al-Mawaali et al. 2012; Al-Sa'di et al. 2008a; Al-Sadi et al. 2011b; El-Tarabily et al. 2009; Vestberg and Kukkonen 2014). In addition, they attack other vegetable crops, ornamental plants, fruit crops, and grasses. Losses due to these pathogens have been reported to reach 75% of seedlings, especially in the first 2 weeks of planting (Al-Sadi et al. 2011b).

Findings from our surveys showed the presence of four different species of *Pythium* in greenhouses in Oman: *P. aphanidermatum*, *P. spinosum*, *P. myriotylum*, and *P. catenulatum*. Among them,

Table 2. GenBank accession numbers of *Pythium* isolates detected during the study

Fungal species	Isolate number ^a	Accession
<i>Pythium aphanidermatum</i>	D-1	KY095118
<i>P. aphanidermatum</i>	D-2	KY095119
<i>P. aphanidermatum</i>	D-3	KY095120
<i>P. aphanidermatum</i>	DK-1	KY095121
<i>P. aphanidermatum</i>	BS-1	KY095122
<i>P. aphanidermatum</i>	BN-1	KY095123
<i>P. aphanidermatum</i>	BN-2	KY095124
<i>P. aphanidermatum</i>	BN-3	KY095125
<i>P. aphanidermatum</i>	BS-3	KY095126
<i>P. aphanidermatum</i>	BS-4	KY095127
<i>P. aphanidermatum</i>	D-4	KY095128
<i>P. aphanidermatum</i>	DK-2	KY095129
<i>P. aphanidermatum</i>	DK-3	KY095130
<i>P. aphanidermatum</i>	DK-4	KY095131
<i>P. aphanidermatum</i>	DK-5	KY095132
<i>P. aphanidermatum</i>	DK-6	KY095133
<i>P. aphanidermatum</i>	D-5	KY095134
<i>P. aphanidermatum</i>	D-6	KY095135
<i>P. aphanidermatum</i>	BN-4	KY095136
<i>P. aphanidermatum</i>	BN-5	KY095137
<i>P. aphanidermatum</i>	BN-6	KY095138
<i>P. aphanidermatum</i>	BN-7	KY095139
<i>P. aphanidermatum</i>	BN-8	KY095140
<i>P. aphanidermatum</i>	BN-9	KY095141
<i>P. aphanidermatum</i>	BN-10	KY095142
<i>P. aphanidermatum</i>	BN-11	KY095143
<i>P. aphanidermatum</i>	BN-12	KY095144
<i>P. aphanidermatum</i>	BN-13	KY095145
<i>P. aphanidermatum</i>	BN-14	KY095146
<i>P. aphanidermatum</i>	BN-15	KY095147
<i>P. aphanidermatum</i>	D-7	KY095148
<i>P. aphanidermatum</i>	D-8	KY095149
<i>P. aphanidermatum</i>	BS-5	KY095150
<i>P. aphanidermatum</i>	DK-7	KY095151
<i>P. aphanidermatum</i>	DK-8	KY095152
<i>P. aphanidermatum</i>	BS-6	KY095153
<i>P. aphanidermatum</i>	BS-7	KY095154
<i>P. aphanidermatum</i>	D-9	KY095155

(continued)

^a Isolates in bold were included in the hymexazol sensitivity test.

Table 2. (continued)

Fungal species	Isolate number ^a	Accession
<i>P. aphanidermatum</i>	D-10	KY095156
<i>P. aphanidermatum</i>	DK-72	KY095157
<i>P. aphanidermatum</i>	BS-8	KY095158
<i>P. aphanidermatum</i>	D-11	KY095159
<i>P. aphanidermatum</i>	D-12	KY095160
<i>P. aphanidermatum</i>	BN-16	KY095161
<i>P. aphanidermatum</i>	BN-17	KY095162
<i>P. aphanidermatum</i>	BN-18	KY095163
<i>P. aphanidermatum</i>	BN-20	KY095164
<i>P. aphanidermatum</i>	BN-22	KY095165
<i>P. aphanidermatum</i>	BN-23	KY095166
<i>P. aphanidermatum</i>	BN-24	KY095167
<i>P. aphanidermatum</i>	BR-1	KY095169
<i>P. aphanidermatum</i>	SN-1	KY095170
<i>P. aphanidermatum</i>	BR-2	KY095171
<i>P. aphanidermatum</i>	BS-9	KY095172
<i>P. aphanidermatum</i>	BR-3	KY095173
<i>P. aphanidermatum</i>	M-1	KY095174
<i>P. aphanidermatum</i>	BS-10	KY095175
<i>P. aphanidermatum</i>	M-2	KY095176
<i>P. aphanidermatum</i>	BR-4	KY095177
<i>P. aphanidermatum</i>	SN-2	KY095178
<i>P. aphanidermatum</i>	M-3	KY095179
<i>P. aphanidermatum</i>	BR-5	KY095180
<i>P. aphanidermatum</i>	DK-9	KY095181
<i>P. aphanidermatum</i>	BR-52	KY095182
<i>P. aphanidermatum</i>	BR-6	KY095183
<i>P. aphanidermatum</i>	BR-8	KY095184
<i>P. aphanidermatum</i>	SN-3	KY095185
<i>P. aphanidermatum</i>	SN-4	KY095186
<i>P. aphanidermatum</i>	SN-5	KY095187
<i>P. aphanidermatum</i>	SN-6	KY095188
<i>P. aphanidermatum</i>	SN-7	KY095190
<i>P. aphanidermatum</i>	M-5	KY095191
<i>P. aphanidermatum</i>	M-6	KY095193
<i>P. aphanidermatum</i>	BS-100	KY095192
<i>P. aphanidermatum</i>	BN-21	MF356253
<i>P. aphanidermatum</i>	BN-19	MF356254
<i>P. catenulatum</i>	BS-2	KY095194
<i>P. myriotylum</i>	M-4	KY095189
<i>P. spinosum</i>	BR-7	KY095168

P. aphanidermatum is the most dominant species, making up more than 95% of the isolates. This finding supports previous studies, which showed that *P. aphanidermatum* is the major soilborne pathogen of cucumber in this part of the world (Al-Sadi et al. 2011b; Stanghellini and Phillips 1975). The dominance of *P. aphanidermatum* in greenhouses in Oman and other countries could be attributed to the high temperature there, which favors the reproduction and dominance of high-temperature pathogens, including *P. aphanidermatum* (Bates and Stanghellini

1984; Stanghellini and Phillips 1975). *P. aphanidermatum* has been reported to be the most common *Pythium* spp. on cucumber in other countries, including the United Arab Emirates (Stanghellini and Phillips 1975) and Iran (Hatami et al. 2013).

The occurrence of *P. spinosum*, *P. myriotylum*, and *P. catenulatum* in greenhouses in Oman was very limited. However, pathogenicity tests on cucumber seedlings showed that *P. spinosum*, *P. aphanidermatum*, and *P. myriotylum* were highly pathogenic at temperatures

Fig. 3. Mortality of cucumber seedlings (%) following inoculation with four isolates belonging to three *Pythium* spp. Bars with the same letter are not significantly different from each other at $P < 0.05$ (Tukey's Studentized range test, SAS).

Fig. 4. Range of sensitivity of 27 *Pythium aphanidermatum* isolates to hymexazol.

around 28°C. *P. spinosum* and *P. myriotylum* were found to be more aggressive on cucumber at 28°C compared with the dominant species (*P. aphanidermatum*). *P. catenulatum* and *P. myriotylum* are reported in this study for the first time in Oman. *P. catenulatum* has been isolated in previous studies from different substrates and was shown to be pathogenic on cucumber (Sánchez and Gallego 2002). *P. myriotylum* is a common pathogen on cucumber, ginger, *Glycyrrhiza uralensis* cocoyam, and others (Ben-Yephet and Nelson 1999; Gómez-Alpízar et al. 2011; Ishiguro et al. 2014; Le et al. 2016).

Previous surveys in Oman conducted by our team over a 10-year period showed the association of *P. aphanidermatum*, *P. spinosum*, and *P. splendens* with diseases in greenhouse cucumber (Al-Sadi et al. 2011b). The occurrence of two new species of *Pythium* in Oman (*P. catenulatum* and *P. myriotylum*) may suggest that these pathogens have been recently introduced into the country. This is supported by their limited distribution in greenhouses in Oman. Previous studies have shown that *Pythium* spp. can be introduced into greenhouses via potting media, organic fertilizers, wind-driven dust, and other agricultural material (Al-Sadi et al. 2011a; Sanchez et al. 2001). Alternatively, *P. catenulatum* and *P. myriotylum* could have been introduced into Oman a long time ago but they could have possess a lower degree of fitness in this part of the world compared with the dominant species (*P. aphanidermatum*). Assessing the genetic diversity and degree of fitness of a larger population of *P. catenulatum* and *P. myriotylum* from different areas in Oman may help provide insights about the origin of these species.

Phylogenetic analysis of *P. aphanidermatum* isolates based on the ITS sequences showed separation of the isolates into two main clusters and a few subclusters. Agriculture in Oman relies heavily on the import of different types of growing substrates (e.g., peat moss, cocopeat, and so on) and organic fertilizers. Recent studies in Oman have shown that potting media and organic fertilizers can act as sources of *P. aphanidermatum* and other pathogens in the country due to the difficulty of detecting fungi in these substrates (Al-Sa'di et al. 2008b; Al-Sadi et al. 2011a, 2015b). Variation in the ITS sequence of *P. aphanidermatum* isolates in Oman may indicate that the isolates have originated from different sources.

Cluster analysis of *P. aphanidermatum* isolates showed separation of 14 isolates obtained from cocopeat in a distinct group. These isolates were obtained from Al Batinah North. The other cluster contained *P. aphanidermatum* isolates that were obtained from all other regions as well as six cocopeat isolates from Al Batinah North and two isolates from soil and roots obtained from Al Batinah North. It is possible that isolates from cocopeat have two origins. The 14 isolates in the first cluster could have been introduced with cocopeat from abroad because they share less similarity with other isolates from Oman. These cocopeat isolates clustered with a *P. aphanidermatum* isolate from China (KF667387). Because there are no previous reports on the infestation of cocopeat with *Pythium* spp., including our recent work on cocopeat and other products (Al-Mazroui and Al-Sadi 2015), future studies should investigate the potential of infestation of cocopeat by *Pythium* isolates, which could be done, in part, through testing contamination of fresh cocopeat with *Pythium* spp. at different production stages. On the other hand, the six other isolates in the second cluster that share high similarity with

isolates from other regions and with two isolates from Al Batinah North could have originated from greenhouse soils. Because cocopeat is usually placed on soil, it is possible that these isolates infested cocopeat when it was used in the greenhouse (Al-Sa'di et al. 2008b). Also, phylogenetic analysis in this study cannot provide a solid conclusion regarding the origin of *P. aphanidermatum* isolates in Oman. Therefore, a genetic study is required to analyze the population structure of *P. aphanidermatum* in order to get a clear picture about the origin and movement of *P. aphanidermatum* in Oman.

Variation was observed in the sensitivity of *P. aphanidermatum* isolates to hymexazol, with the EC₅₀ for different isolates ranging from 0.9 to 142.9 mg liter⁻¹ (average of 10.9 mg liter⁻¹). Although most of the isolates were found to be sensitive to highly sensitive to hymexazol (0.9 to 31.2 mg liter⁻¹), a single isolate was found to be resistant (142.9 mg liter⁻¹). The resistant isolate was collected from a greenhouse in Muscat governorate, where the owner has been using hymexazol fungicide intensively during at least the last 5 years. Hymexazol is an important fungicide in greenhouse cucumber, and it is widely used in Oman and in many other countries. Previous studies indicated the development of resistance to hymexazol among some *Fusarium* spp. and some *Pythium* spp., including *P. ultimum* (Al-Sadi et al. 2015a; Ali-Shtayeh et al. 2003; Chung et al. 2009; Moorman and Kim 2004; Moyano et al. 2004). This study appears to report, for the first time, the development of resistance to hymexazol among *P. aphanidermatum* from greenhouse cucumber. The development of resistance to hymexazol among *P. aphanidermatum* may raise concerns about the reduced efficacy of this fungicide in managing *Pythium*-induced damping-off and wilt of cucumber. Therefore, it is important to take necessary actions to reduce the chance of further development of resistance to hymexazol and other fungicides commonly used for *Pythium* spp. through the use of integrated management strategies. In addition, the level of sensitivity of *Pythium* spp. to hymexazol needs to be examined in countries relying heavily on hymexazol.

This study shows the presence of several *Pythium* spp. in greenhouses in Oman, with *P. aphanidermatum* being the most dominant. The study reports, for the first time, the presence of *P. myriotylum* and *P. catenulatum* in Oman. It also appears to report, for the first time, the development of resistance among *P. aphanidermatum* from greenhouses to hymexazol. Future studies should examine the population structure of *P. aphanidermatum* in Oman and whether the hymexazol-resistant isolate could also be resistant to other fungicides, including mefenoxam.

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Table 3. Mean concentration that results in 50% growth inhibition of *Pythium aphanidermatum* isolates (EC₅₀ values) from five regions in Oman^z

Region	Sample size	Mean EC ₅₀	Standard deviation
Batinah South	4	4.0 a	2.5
Buraimi	4	3.2 a	4.4
Al Dakiliya	6	3.4 a	4.2
Al Sharqiya	7	6.1 a	5.3
Muscat	5	39.9 a	59

^z Isolates with the same letter in the same column are not significantly different from each other at $P < 0.05$ (Tukey's Studentized range test, SAS). Results are presented for 26 isolates because isolate number 27 came from a different region and no statistical analysis was applied on the single isolate.

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