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plant disease

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DISEASE NOTES

First Report of Alfalfa (*Medicago sativa* L.) Seed Rot, Seedling Root Rot, and Damping-Off Caused by *Pythium* spp. in Sudanese soil

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Alfalfa (*Medicago sativa*) is an important forage crop in Sudan but has relatively low biomass yields ([Abdel-Rahman and Abu-Suwar 2012](#)). In September 2016, bulked soil samples were collected from three commercial alfalfa production fields (designated as 94, 97, and 98) near Khartoum, Sudan, with poor seedling establishment and rapid stand decline. Pathogens were baited from wet soil samples using alfalfa seedlings ([Altier and Thies 1995](#)). Over a 10-day period, seedlings with chlorosis and damping-off were removed from soil samples, surface disinfested, transferred to 1.5% water agar plates, and incubated at 21°C. Mycelial tips were removed and subcultured on cornmeal agar until 145 pure cultures were obtained. DNA was extracted from 72 cultures, and the rDNA amplified with primers ITS1 and ITS4 ([White et al. 1990](#)) and sequenced. BLASTn searches of the NCBI GenBank database identified 39% of the isolates as *Fusarium* spp., 25% as *Pythium aphanidermatum*, 15% as *P. myriotylum*, 10% as *Rhizoctonia solani*, 7% as other fungi, 3% as *P. irregulare*, and 1% as *P. carolinianum*. *Pythium* species were recovered from all the fields sampled. Isolates from field 94 corresponded to *P. aphanidermatum* (94A1), *P. myriotylum* (S94A1), and *P. irregulare* (94S1), having 100% sequence identity with accessions KU211462.1, KX671068.1, and

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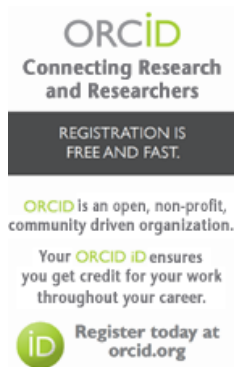
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HQ643616.1, respectively. Two isolates from field 97 were identified as *P. aphanidermatum* (97A1) and *P. myriotylum* (S97SPARP1), having 100% sequence identity with accession KX788823.1 and KX671095.1, respectively. Sequences were deposited under accessions MF948664 to MF948669 and cultures submitted to the University of Minnesota Mycological Culture Collection. Pathogenicity of four isolates (94A1, 94S1, 97A1, and S97SPARP1) was tested using a culture plate method (Altier and Thies 1995). After 5 days the 'Saranac' alfalfa seedlings were rated on a 1 to 5 disease scale with 1 = healthy plant and 5 = rotted ungerminated seed. All *Pythium* species were able to cause disease on alfalfa seeds or seedlings. Symptoms of seed rot and root rot were observed, with *P. aphanidermatum* (94A1 and 97A1), *P. irregulare* (94S1), and *P. myriotylum* (S97SPARP1) having a disease severity \pm standard error (SE) of 4.0 ± 0.2 , 4.3 ± 0.1 , 4.1 ± 0.1 , and 3.8 ± 0.1 , respectively. A high frequency of seed rot (disease score 5) of 45% (94A1), 52% (94S1), 37% (97A1), and 75% (S97SPARP1) was observed. Sterilized potting mix was infested with mycelium of each isolate and used to germinate 25 Saranac alfalfa seeds, with four replicate pots per isolate (Berg et al. 2017). Germination rate \pm SE in the infested mixture at 7 days after planting (DAP) at 21°C was $32 \pm 2\%$ (94A1), $12 \pm 2\%$ (94S1), $37 \pm 6\%$ (97A1), and $52 \pm 2\%$ (S97SPARP1) compared with $80 \pm 5\%$ germination in the control mixture. Damping-off was 0% (94A1), 13% (94S1), 8% (97A1), and 59% (S97SPARP1) at 18 DAP. Surviving plants examined at 18 DAP all showed disease symptoms including chlorosis of cotyledons, necrosis of roots, root rot, and stunted growth. Cultures isolated from the inoculated plants were identified as *P. aphanidermatum* (94A1 and 97A1), *P. myriotylum* (S97SPARP1), and *P. irregulare* (94S1), completing Koch's postulates. The presence of *P. aphanidermatum* and other *Pythium* spp. in Sudanese soils was reported from fields with alfalfa and *Sorghum vulgare* (Nour 1956). However, pathogenicity of these isolates was not documented. To our knowledge, this is the first report of seed rot, seedling root rot, and damping-off of alfalfa by *P. aphanidermatum*, *P. myriotylum*, and *P. irregulare* isolated from Sudanese soil.



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Section:

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