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Short Article

Pathogenicity of *Pythium aphanidermatum* on sugarcane: First record from Iran

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ABSTRACT

Sugarcane is one of the most important industrial crops in Iran, particularly in Khuzestan Province, where soilborne pathogens can threaten crop establishment and productivity. During surveys of sugarcane fields in Khuzestan, plants showing severe root rot, yellowing, and stunting were collected along with samples of rhizosphere soil and irrigation water samples. In total, 526 oomycete isolates were obtained using CMA-PARP selective medium, of which 11 isolates were identified as *Pythium aphanidermatum* based on morphological characteristics, including filamentous inflated sporangia, globose smooth oogonia, aplerotic oospores, and intercalary antheridia. Molecular identification of a representative isolate was carried out using sequence data from the ITS region and the *cox1* gene. Phylogenetic analyses showed that this isolate belongs to Clade A of *Pythium sensu stricto* and groups with reference isolates of *P. aphanidermatum* with strong support. Pathogenicity tests demonstrated that this species caused severe root rot, yellowing, stunting, and wilting on sugarcane four weeks after inoculation, reproducing symptoms similar to those observed in the field. This study reports the first confirmed occurrence of *P. aphanidermatum* causing sugarcane root rot in Iran. The detection of this aggressive oomycete in sugarcane fields of Khuzestan highlights the need for continuous monitoring and integrated management of soilborne diseases in Iranian sugarcane production systems.

KEYWORDS
Pathogenicity, Phylogeny, *Pythium aphanidermatum*, Root rot, Sugarcane.

INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is an economically important industrial crop in Iran, particularly in Khuzestan Province, where most national sugarcane production is concentrated. Its cultivation is affected by several biotic stresses, among which soilborne pathogens are important because they can reduce root function, impair plant establishment, and contribute to yield losses (Magarey 1996; Patel et al. 2018).

Root rot diseases of sugarcane are commonly associated with soil borne fungi and oomycetes, including species of *Pythium*. These pathogens are favored by moist soil conditions and can infect roots, setts, and young seedlings, causing poor germination, stunting, and root necrosis. *Pythium aphanidermatum* (Edson) Fitzp. is a widespread and aggressive oomycete that causes damping-off and root rot in many crops (Van der Plaäts-Niterink 1981). Its association with sugarcane has been known for nearly a century, with early reports documenting pathogenicity on this host (Edgerton et al. 1929).

Despite the long history and economic importance of sugarcane cultivation in Iran, *P. aphanidermatum* has not previously been reported on sugarcane in the country. Therefore, the present study aimed to identify *P. aphanidermatum* associated with diseased sugarcane roots in Iran and confirm its pathogenicity.

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MATERIALS AND METHODS

During a comprehensive survey of sugarcane fields in Khuzestan Province, Iran, symptomatic sugarcane plants exhibiting severe root rot, yellowing, and stunting were collected along with rhizosphere soil and irrigation water. A total of 526 oomycete isolates were recovered from the samples using CMA-PARP selective medium (Jeffers and Martin 1986). Among these, 11 isolates were morphologically identified as *P. aphanidermatum*.

The isolates were initially identified based on standard morphological criteria, including inflated filamentous sporangial structures and the production of globose, smooth oogonia, large, aplerotic oospores, and intercalary antheridia (van der Pläats-Niterink 1981). To confirm the morphological identification, one representative isolate was selected for molecular analysis. DNA extraction was performed using a CTAB-based protocol according to Mirsoleimani and Mostowfizadeh-Ghalamfarsa (2013), and the internal transcribed spacer (ITS) region of rDNA and cytochrome c oxidase subunit I (*cox*1) were amplified using universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'), as well as COXF4N (5'-GTATTTCTTCTTTATTAGGTGC-3') and COXR4N (5'-CGTGAACTAATGTTACATATAC-3') (Villa et al. 2006). The obtained sequences were analyzed and compared with reference sequences retrieved from GenBank using Bayesian inference and Maximum Likelihood analyses.

Pathogenicity tests were conducted under greenhouse conditions to establish the causal relationship between *P. aphanidermatum* and the observed symptoms in sugarcane. Inoculum preparation was carried out using inoculated vermiculite containing hemp extract (Salmaninezhad et al. 2024). The prepared inoculum was used to induce pre-emergence seedling damping-off by incorporating it into the potting mixture (1:1 sand-to-soil proportion) at a 1:10 ratio. Control pots were supplemented with noninoculated vermiculite containing hemp extract (Salmaninezhad et al. 2024). The pots were then fully saturated with water for 24 hours. Pathogen detection in inoculated pots was performed using floating citrus leaf baits placed in drainage water. The baited leaves were washed with sterile distilled water after 24 hours, dried, and cultured on CMA-PARP selective medium to monitor the pathogen's viability. The pots were maintained in a greenhouse at 25–35 °C. Four weeks post-inoculation, plant health assessments were conducted, recording the number of healthy and dead plants as well as the severity of shoot wilting, stunting, and primary and secondary root rot symptoms.

RESULTS AND DISCUSSION

All recovered *P. aphanidermatum* isolates produced filamentous inflated sporangia, aplerotic oospores, and intercalary antheridia. Based on analyses of the *cox1* and ITS loci, as well as the concatenated dataset, isolate HTP2a was placed in Clade A of *Pythium sensu* Lévesque and de Cock (2004), clustering with the reference isolate *P. aphanidermatum* CBS 28779 with maximum Bayesian posterior probability (1.0) and bootstrap support (100%) (Figs. 1–3). In pathogenicity assays, inoculated plants developed severe root rot, yellowing, and stunting four weeks after inoculation (Fig. 4). Pathogenicity was confirmed by the consistent re-isolation of *P. aphanidermatum* from symptomatic tissues. In contrast, no isolates were recovered from control plants. The re-isolated cultures were morphologically identical to the original isolates, thereby fulfilling Koch's postulates.

The confirmation of *P. aphanidermatum* as a pathogen of sugarcane in Iran marks a significant finding. This species is recognized as an aggressive necrotrophic oomycete that thrives under warm and humid conditions (van der Pläats-Niterink 1981), which are characteristic of the climate in Khuzestan Province. The ability of *P. aphanidermatum* to infect sugarcane raises concerns regarding its potential impact on crop health and productivity.

This study presents the first confirmed record of *P. aphanidermatum* infecting sugarcane in Iran, highlighting its pathogenic potential and the need for effective disease management strategies. Continuous monitoring of sugarcane fields and early detection of oomycete pathogens will be crucial in preventing further disease outbreaks. This study contributes to the growing body of knowledge on oomycete diversity in Iran and underscores the need for sustainable disease management in sugarcane agroecosystems.

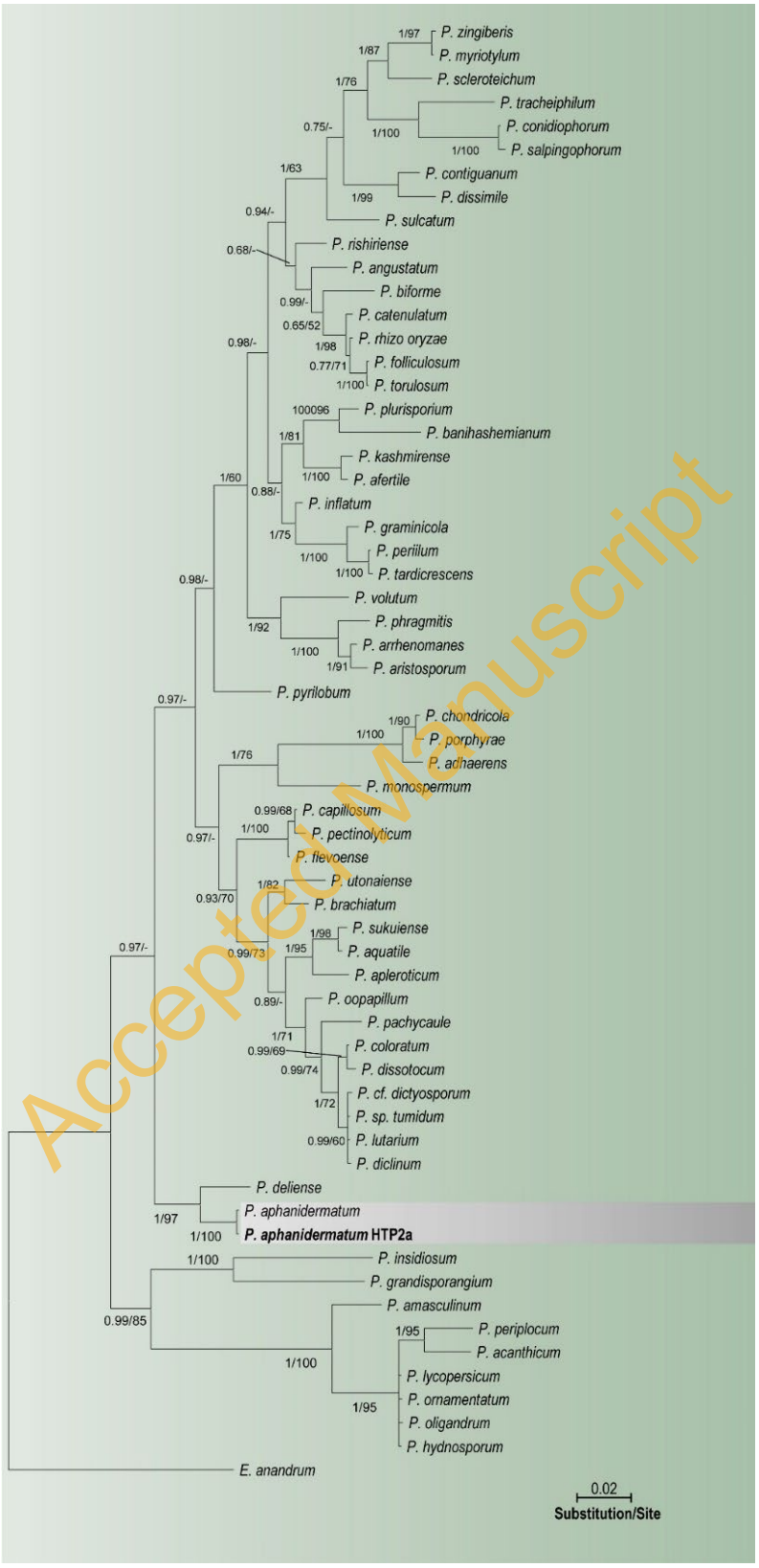

Fig. 1. Phylogenetic relationships of *Pythium aphanidermatum* from sugarcane (Ahvaz County, Iran) among 62 *Pythium sensu stricto* species based on Bayesian analysis of multiple genealogies of ITS rDNA and *cox*1 sequences. Numbers on branches represent posterior probability based on Bayesian analysis and the bootstrap support based on Maximum Likelihood analysis, respectively. *Pythium aphanidermatum* isolate HTP2a is shown in bold. *Elongisporangium anandrum* was considered as an outgroup.

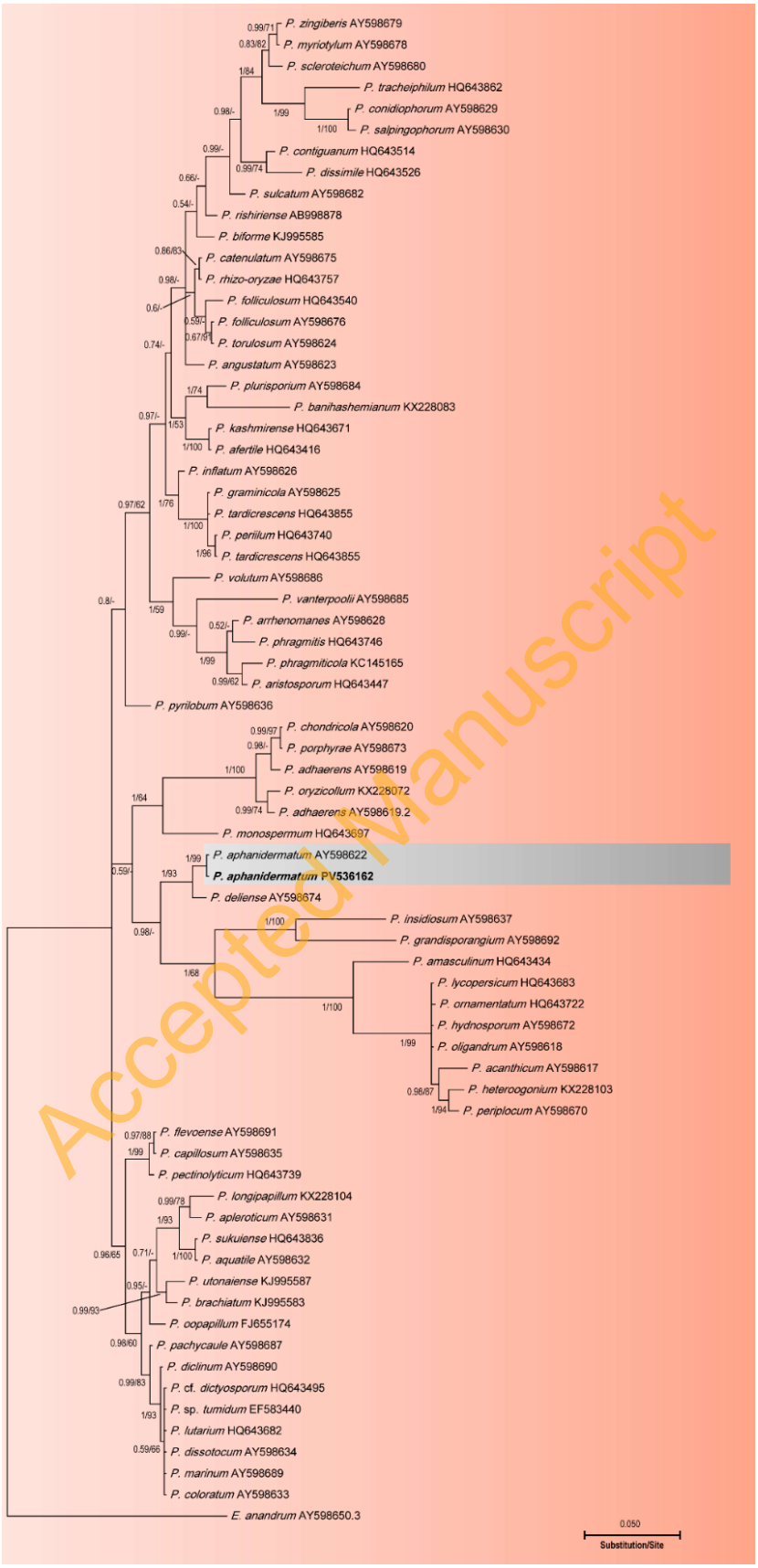

Fig. 2. Phylogenetic relationships of *Pythium aphanidermatum* from sugarcane (Ahvaz County, Iran) among 71 *Pythium sensu stricto* species based on Bayesian analysis of internal transcribed spacers 1, 2 and 5.8S gene of rDNA sequences. Numbers on branches represent posterior probability based on Bayesian analysis and the bootstrap support based on Maximum Likelihood analysis, respectively. *Pythium aphanidermatum* isolate HTP2a is shown in bold. *Elongisporangium anandrum* was considered as an outgroup.

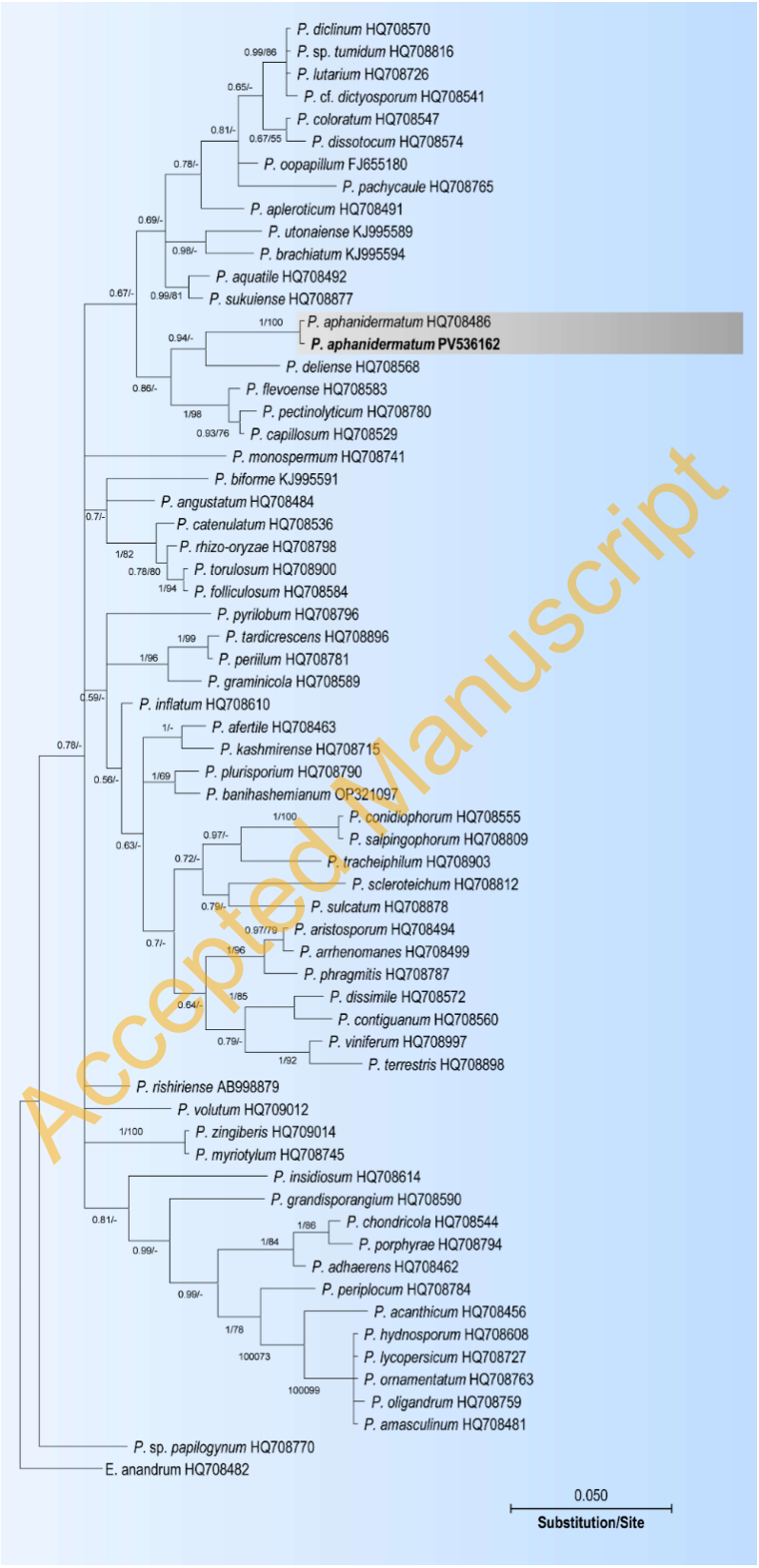

Fig. 3. Phylogenetic relationships of *Pythium aphanidermatum* from sugarcane (Ahvaz County, Iran) among 65 *Pythium sensu stricto* species based on Bayesian analysis of cytochrome c oxidase subunit I sequences. Numbers on branches represent posterior probability based on Bayesian analysis and the bootstrap support based on Maximum Likelihood analysis, respectively. *Pythium aphanidermatum* isolate HTP2a is shown in bold. *Elongisporangium anandrum* was considered as an outgroup.

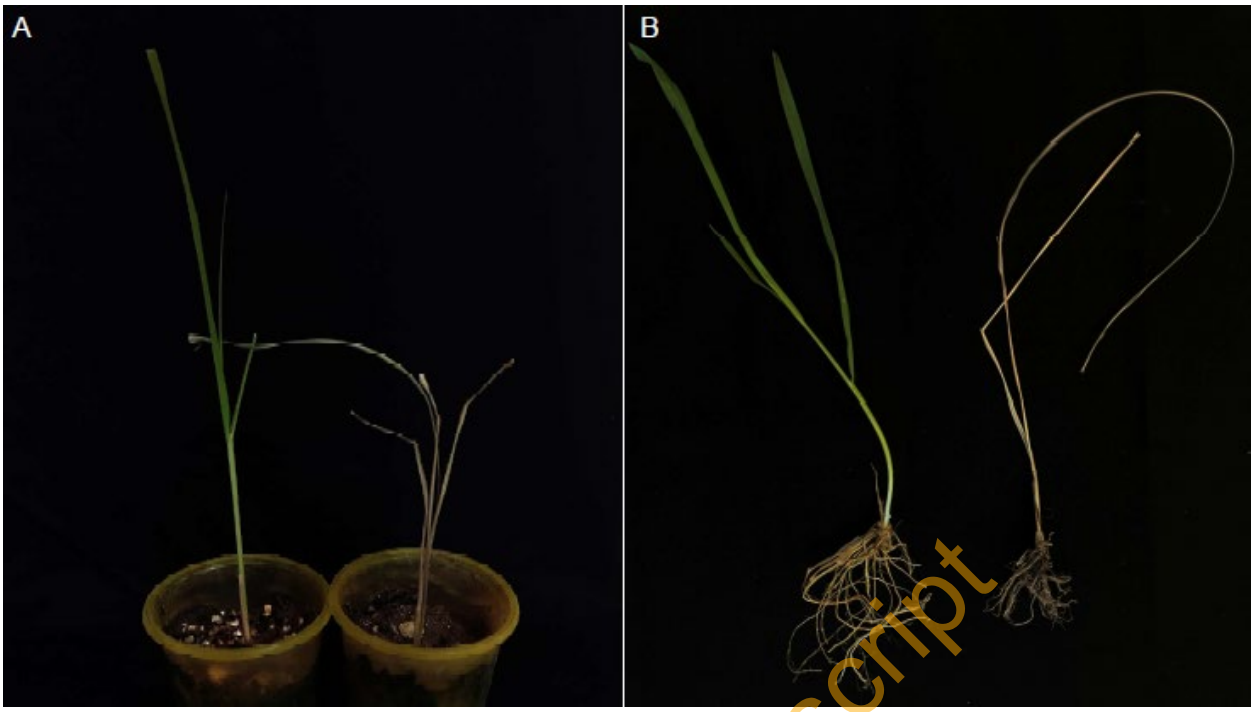

Fig. 4. Pathogenicity of *Pythium aphanidermatum* on sugarcane seedling, causing severe root rot, yellowing, and decline in the plant (right), compared to a healthy control (left), four weeks after inoculation.

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AUTHOR CONTRIBUTION

Nahid Sadat Favazeli: Investigation, Visualization, Validation, Data curation, Formal Analysis; Zahra Mirsoleymani: Conceptualization, Funding acquisition, Formal analysis, Project administration, Resources, Supervision, Validation, Writing, review and editing; Fatemeh Salmaninezhad: Data curation, Formal analysis, Visualization, Validation, Writing, original draft, Writing, review and editing; Reza Mostowfizadeh-Ghalamfarsa**:** Supervision, Validation, Writing, original draft, Writing, review and editing.

DATA AVAILABILITY

All datasets generated during this study are available from the corresponding author upon request.

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DECLARATION

The authors declare no competing interests.

ETHICAL APPROVAL

This article does not contain any studies with human participants or animals by any of the authors.

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مقاله کوتاه

بیماری‌زایی گونه *Pythium aphanidermatum* بر روی نیشکر: نخستین گزارش از ایران

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چکیده

نیشکر یکی از محصولات صنعتی مهم در ایران، به ویژه در استان خوزستان است، جایی که عوامل بیماری‌زای خاک‌بُرد می‌توانند استقرار و بهره‌وری محصول را تهدید کنند. طی بررسی مزارع نیشکر در خوزستان، گیاهانی با علائم پوسیدگی شدید ریشه، زردی و کوتولگی، همراه با نمونه‌های خاک فراریشه و آب آبیاری جمع‌آوری شدند. در مجموع، ۵۲۶ جدایه اُمیستی با استفاده از محیط انتخابی CMA-PARP به دست آمد که از میان آن‌ها، ۱۱ جدایه بر اساس ویژگی‌های ریخت‌شناختی، شامل اسپورانژیوم‌های رشته‌ای آماسیده، اُگونیوم‌های کروی و صاف، اُسپورهای ناپُرساز و آنتریدیوم‌های بین‌ریسه‌ای، *Pythium aphanidermatum* شناسایی شدند. شناسایی مولکولی یک جدایه نماینده با استفاده از داده‌های توالی ناحیه‌ی ITS و ژن *cox1* انجام شد. واکاوی‌های فیلوژنتیکی نشان داد که این جدایه در تبار A از *Pythium sensu stricto* با جدایه‌های مرجع *P. aphanidermatum* با پشتیبانی بالا قرار می‌گیرد. نتایج آزمون‌های بیماری‌زایی نشان داد که این گونه روی گیاه نیشکر چهار هفته پس از مایه‌زنی ایجاد علائم پوسیدگی شدید ریشه، زردی، کوتولگی و پژمردگی، مشابه علائم مشاهده‌شده در مزرعه، ایجاد می‌کند. این نخستین گزارش تأییدشده از بیماری‌زایی گونه *P. aphanidermatum* به‌عنوان عامل پوسیدگی ریشه نیشکر در ایران است. شناسایی این اُمیست مهاجم در مزارع نیشکر خوزستان، ضرورت پایش مستمر و مدیریت تلفیقی بیماری‌های خاک‌بُرد را در صنایع تولید نیشکر ایران نشان می‌دهد.

واژگان کلیدی: بیماری‌زایی، پوسیدگی ریشه، فیلوژنی، نیشکر، *Pythium aphanidermatum*.

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