Mycologia Iranica 9(1): 97–103, 2022 Short Article DOI: 10.22043/MI.2022.357797.1210

First report of melon decline caused by *Plectosphaerella cucumerina* in Iran

M. Mirtalebi ✉

F. Sabahi

Z. Banihashemi

Shiraz University, College of Agriculture, Department of Plant Protection. Shiraz, Iran.

Abstract: In spring 2019, melon plants (*Cucumis melo* CV. Janna) with collapse and decline symptoms were collected from fields in Dashti county, Bushehr province in southern Iran. Twelve morphologically similar isolates from infected root and crown tissues were recovered. Fungal isolates were identified based on morphological characteristics and molecular data of the internal transcribed spacer (ITS-rDNA) region. According to the morphological and phylogenetic analysis, the isolates were identified as *Plectosphaerella cucumerina*. The pathogenicity test was performed on healthy seedlings of melon plants (CVs. Kharboze-Mashhadi and Shahde-Shiraz). Three weeks after inoculation, the melon seedlings showed root rot, necrosis and wilting symptoms then eventually death. To complete the Koch's postulate, *P. cucumerina* was re-isolated from inoculated plants. This is the first report of *P. cucumerina* causing decline on melon in Iran.

Key words: Pathogenicity, ITS, wilting, root rot

INTRODUCTION

Melon (*Cucumis melo* L.) is one of the most important species in the *Cucurbitaceae* which has been cultivated for at least 5000 years in Iran (Zohary & Hopf 2000). The Annual production of cantaloupe and other melons in Iran has been estimated at around 1,615,642 tons cultivated in more than 82,000 ha (FAOSTAT, 2016). *Fusarium oxysporum* f. sp. *melonis* (Banihashemi 1968, Banihashemi 1982, Sarpeleh & Banihashemi 2000, Mirtalebi et al. 2013), *Fusarium solani* f. sp. *cucurbitae* (Alymanesh et al. 2006, Mirtalebi & Banihashemi 2010), *Macrophomina phaseolinae* and *Phytophthora melonis* (Mansoori & Banihashemi 1980, Banihashemi 1983) are the *major* soil–borne colonies were transferred to PDA and single spore colonies were derived prior to morphological and *fungal pathogens* that pose severe threats to melon production fields in Iran.

Root rot and decline of the melon plants is also an economically important disease that has been reported from different regions throughout the world, including Japan, Israel, Spain, Italy, Brazil and the USA (Yan et al. 2016). *Monosporascus cannonbalus* and *Acremonium cucurbitacearum* are the main causes of the disease (Reuveni et al 1983, Armengol 1998). In addition to the aforementioned fungal pathogens, *Plectosphaerella cucumerina* was frequently isolated from cucurbits that showed collapse and decline symptoms (Carlucci et al. 2012). *Plectosphaerella* was introduced by Kleban (1929), who described *Plectosphaerella cucumeris* from young cucumber in Germany. *Plectosphaerella* species are causal agents of root and collar rot, vascular and leaf symptoms in different hosts (Raimondo & Carlucci 2018).

Previously, *Monosporascus cannonballus* has been reported in Iran as the causal agent of melon collapse (Sarpeleh 2008). To date, there is no available information related to *Plectosphaerella* as the causal agent of melon decline in Iran. This study was therefore carried out to identify and characterize the *Plectosphaerella* isolates recovered from melon plants in Iran in terms of morphological and molecular characteristics and test them for pathogenicity.

MATERIALS AND METHODS

Fungal isolation and morphological characterization

In spring 2019, decline and collapse was observed in melon plants (*C. melo* CV. Janna) growing in Dashti county, Bushehr province in southern Iran. Disease symptoms included decayed areas on roots, yellowing of older leaves, general wilting and death of plants. The infected root and crown tissues were cut into 3-4 mm segments and surface disinfested with 0.5 % sodium hypochlorite for 2 min, rinsed in sterile distilled water three times, plated on potato dextrose agar (PDA; potato extract 300 g/L, dextrose 20 g/L, agar 15 g/L) and incubated at 25 °C for seven days.
molecular identification (Dhingra and Sinclair, 1995). Cultural characteristics and morphological features of

Submitted 10 April 2022, accepted for publication 31 June 2022
✉ Corresponding Author: E-mail: mmirtalebi@shirazu.ac.ir

http://mij.areeo.ac.ir

the isolates were determined on PDA (Domsch et al. 2007, Carlucci et al. 2012).

DNA extraction, amplification, and phylogenetic analysis

The internal transcribed spacer (ITS) region of the ribosomal RNA gene (rDNA) of Plct1 and Plct2, as representative isolates, were sequenced. DNA was extracted from the fresh mycelium of representative isolates according to the method detailed in Amer et al. (2011). The ITS region of rDNA was amplified using primer set ITS1 and ITS4 (White et al. 1990). The reaction mixture and cycling conditions were the same as described by Mirtalebi et al. (2013). DNA sequences were edited with DNASTAR (Seq Man II) and aligned with ClustalX 1.8 (Larkin et al. 2000). Manual adjustment of sequence alignments was performed to accommodate insertions/deletions. BLASTn search (which is available at https:// blast.ncbi.nlm.nih.gov/) was conducted to compare newly obtained sequences against the NCBI database. All sequences used in this study are listed in Table 1. Phylogenetic analyses were performed in MEGA5 (Tamura et al. 2011) using the Maximum likelihood method (Saitou & Nei 1987). *Gibellulopsis nigrescens* (EF543854) were used as an outgroup in the analysis.

Table 1. Details and GenBank accession numbers of *Plectosphaerella* isolates included in this study.

Species	Isolate	Host	country	Accession number
P. cucumerina	Plct1	*Cucumis melo*	Iran	OM100947[1]
	Plct2	*Cucumis melo*	Iran	OM100948[1]
	Pa1	*Cucumis sativus*	Iran	KX371080[2]
	Pa4	*Solanum lycopersicum*	Iran	KX371083[2]
	CCTU	*Bambusa vulgaris*	Iran	KC845226[3]
	CBS131739	*Cucumis melo*	Italy	KY662258[4]
	Plect4	*Cucumis melo*	Italy	HQ238977[5]
	Plect22	*Cucumis melo*	Italy	HQ238981[5]
	Plect216	*Cucumis melo*	Italy	HQ238992[5]
	HLDT15	*Solanum lycopersicum*	China	KC894931[6]
P. alismatis	GAMS2	*Alisma* sp.	Netherlands	AY572021[7]
P. citrullae	Plect189	*Citrullus lanatus*	Italy	HQ238964[5]
P. delsorboi	MAFF 238958	*Curcuma alismatifolia*	Japan	AB264788[8]
P. melonis	Plect228	*Cucumis melo*	Italy	HQ238967[5]
P. pauciseptata	Plect135	*Solanum lycopersicum*	Italy	JQ246958[5]
P. plurivora	Plect32	*Solanum lycopersicum*	Italy	HQ238969[5]
P. ramiseptata	Plect464	*Solanum lycopersicum*	Italy	JQ246955[5]
P. oratosquillae	CBS408.95	*Oratosquilla oratoria*	Japan	AB425975[9]
Gibellulopsis nigrescens	CBS 387.35	_	_	EF543854[10]

[1]Isolated in this study; [2]Mirtalebi and Banihashemi 2016; [3]Arzanlou et al. 2013; [4]Su and Niu 2017; [5]Carlucci et al. 2012; [6]Xu et al. 2014; [7] Pitt et al. 2004; [8] Unpubl. data (Nagao,H., Sato,T. & Kakishima, M.); [9] Duc et al. 2009; [10]Zare et al. 2007.

Pathogenicity test

To determine pathogenicity, isolates Plct1 and Plct2 were used to inoculate healthy melon (CVs. Kharboze-Mashhadi and Shahde-Shiraz). Using potato dextrose broth on a shaker (60 rpm), the conidial suspension was prepared. spores were washed and counted with a hemocytometer and 300 ml of a 10^6 conidia/ml suspension was used for plant inoculation via a root dip method (Wellman 1939). Seedlings were grown in vermiculite for ten days and then removed and rinsed with water. The roots were dipped in the spore suspension for 1 min. For each isolate, 15 seedlings of each cultivar were inoculated, transferred to plastic pots filled with sterilized soil and incubated under greenhouse conditions (25–28 °C, 14 h photoperiod). Similarly, control melon seedlings were dipped into sterilized water.

RESULTS AND DISCUSSION

In this study, twelve morphologically similar isolates from infected tissue were recovered. Colonies of all 12 isolates were buff or salmon pink after seven days on PDA at 25 °C; the mycelium was slimy with sparse or absent aerial hyphae; The conidiophores were found to be solitary, unbranched or rarely irregularly branched and the Conidiogenous cells were monophialidic, hyaline, solitary, straight or crooked. Occasionally the conidiogenous cells showed one septum near the base. Conidia hyaline, elliptical to ovoid, 0–1 septate, 5.0–

9.2 × 2.3–4.3 µm, aggregated in slimy heads. Chlamydospores were absent (Fig. 1). The morphological characteristic of the fungus was similar to the description of *Plectosphaerella cucumerina* (Lindf.) W. Gams (Carlucci et al. 2012) (anamorph: *Plectosporium tabacinum* (J. F. H. Beyma) M. E. Palm, W. Gams & Nirenberg) (Palm 1995).

DNA fragments, about 641 bp in length, were generated and deposited in GenBank (accession numbers: OM100947 and OM100948). (Table 1). BLASTn searches in GenBank showed 99.8% identity to sequences of *P. cucumerina* (KY662258: CBS131739 neotype of *P. cucumerina*) (Su & Niu 2017). Phylogenetic analysis based on the ITS gene region indicated an excellent distinction between *P. cucumerina* among the other *Plectosphaerella* species (Fig. 2). In the phylogenetic tree, the two ITS sequences of the representative isolates showed 99 to 100 % identity to Pa4 and Pa1 isolates previously recovered from tomato and cucumber in Iran, respectively (Mirtalebi et al. 2016). Representative isolates were deposited in the Culture Collection of the Iranian Research Institute of Plant Protection, Tehran, Iran (IRAN 4565C, IRAN 4566C).

The results of the pathogenicity test demonstrated that *P. cucumerina* is the causal agent of melon decline. Three weeks after inoculation, inoculated plants showed symptoms of wilting. Roots were necrotic and seedlings produced fewer fibrous roots (Fig. 1). No symptoms were observed on the control plants. The characteristics *of the* re-isolated strains from inoculated melon seedlings matched well with the description of *P. cucumerina* (Carlucci et al. 2012). Thus, Koch's postulates were accomplished and it showed that *P. cucumerina* was the causal agent of the disease.

Species of *Plectosphaerella* are distributed in a various habitats and have wide geographic distribution (Carlucci et al. 2012, Zhang et al. 2015). Some species are pathogens on the fruit, root or collar in the *Cucurbitaceae* plant, causing significant losses of melon, pumpkin and zucchini crops (Alfaro-García et al. 1996; Toyozo et al. 2005; Raimondo and Carlucci 2018). *P. cucumerina* has been reported on many crops, such as tomato, fennel, soybean, muskmelon, potato, pepper, and other critical economic crops (Abad et al. 2000, Cai et al. 2021).

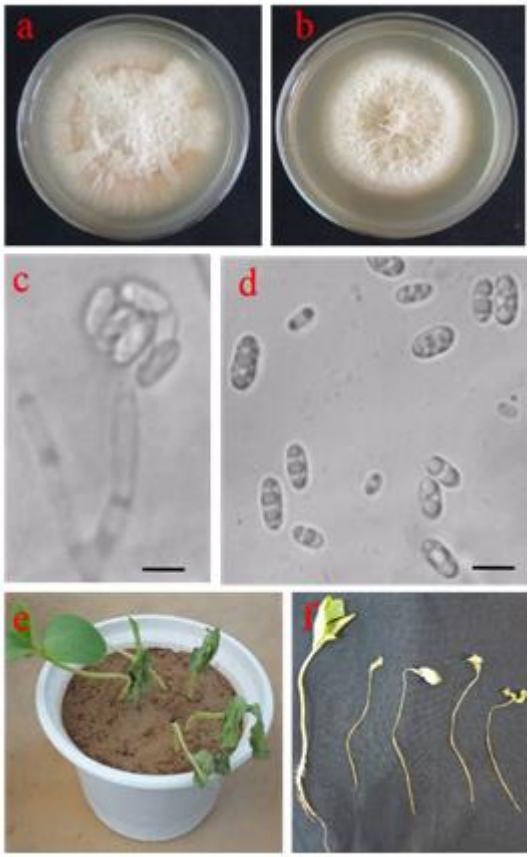

Fig. 1. *Plectosphaerella cucumerina*. Salmon pink colony on PDA after seven days on 25 °C (a–b).; monophialidic and hyaline conidiogenous cells with one septum near the base; hyaline, elliptical to ovoid, septate conidia aggregated in the slimy head (c–d); symptoms on melon seedlings caused by *P. cucumerina* including wilting and necrosis on the root three weeks after inoculation (e–f). — Scale bars = 10 µm.

In Iran, it was reported on Bamboo as an endophytic fungi (Arzanloo 2013), on leaves and pods of common bean, cowpea and soybean plants as the causal agent of anthracnose (Atghia et al. 2014) and on tomato and cucumber with collapse symptoms (Mirtalebi & Banihashemi 2016). Our results showed that this pathogen could cause a decline in melon. Due to the economic importance of melon, the management of the disease should be considered. To the best of our knowledge, this is the first report of *P. cucumerina* causing a decline on melon in Iran. In recent years, *P. cucumerina* has become more visible worldwide as a new or emerging pathogen on plants (Abad et al. 2000, Cai et al. 2021). The wide distribution and broad host range of *P. cucumerina* can present it as a severe threat to crops such as melon production.

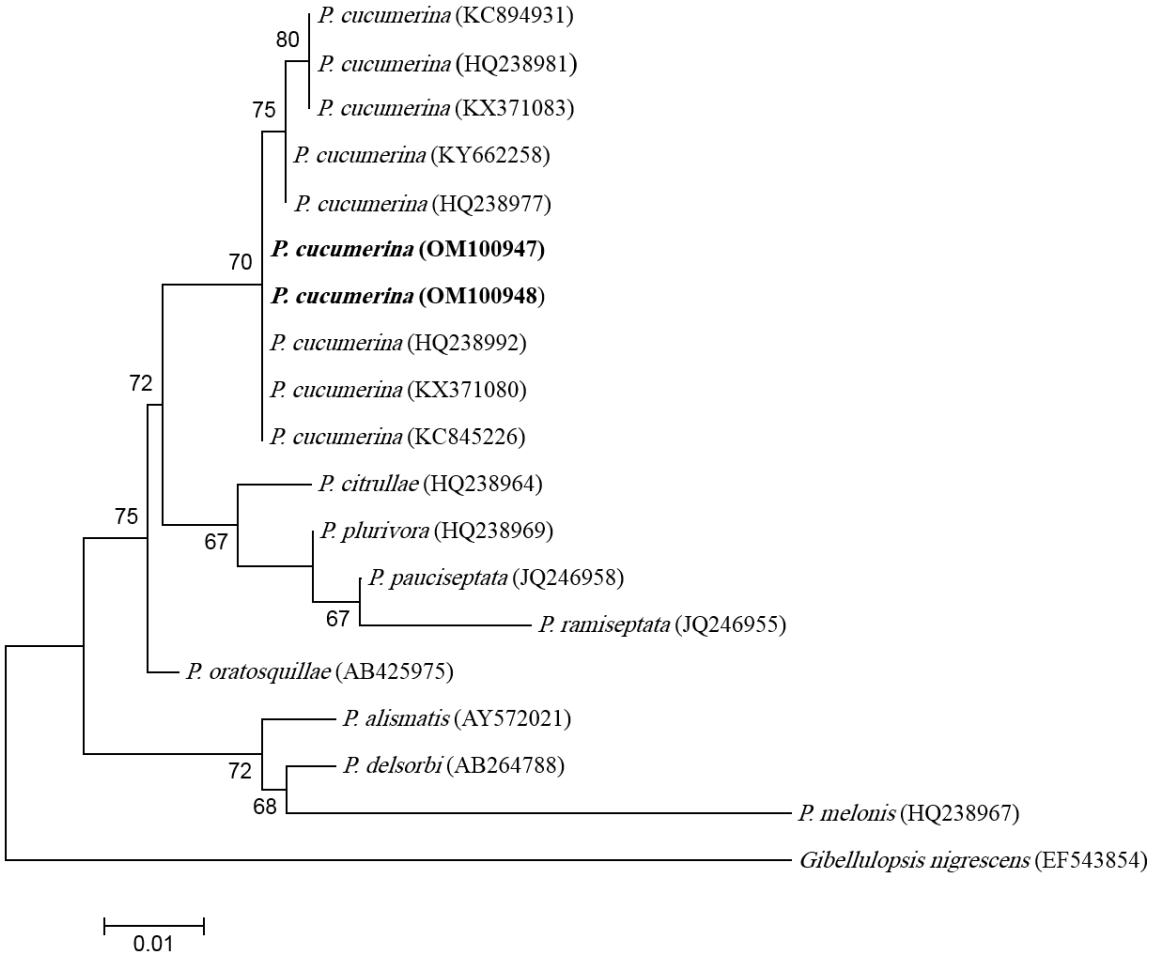

Fig. 2. Maximum likelihood phylogenetic tree inferred from internal transcribed spacer (ITS) region of ribosomal RNA gene sequences of *Plectosphaerella cucumerina* isolates. Bootstrap values (> 60%) are shown as percentages of 1,000 replicates at the branch point. *Gibellulopsis nigrescens* isolate CBS 387.35 is included as an outgroup. *Plectosphaerella cucumerina* isolates obtained in this study are indicated in bold.

ACKNOWLEDGEMENTS

This study was funded by the Iran National Science Foundation (INSF), award number 92033695.

REFERENCES

Abad P, Pérez A, Marqués MC, Vicente MJ, Bruton BD, García-Jiménez J. 2000. Assessment of vegetative compatibility of *Acremonium cucurbitacearum* and *Plectosphaerella cucumerina* isolates from diseased melon plants. European and Mediterranean Plant Protection Organization Bulletin 30(2): 199 204.

Alfaro-Garcìa A, Armengol J, Bruton BD, Gams W, Garcìa-Jimenez J, Martinez- Ferrer G. 1996. The taxonomic position of the causal agent of *Acremonium* collapse of muskmelon. Mycologia 88: 804–808.

Alymanesh M, Falahatirastegar M, Jafarpour B, Mehdikhani Moghadam F. 2006. Detection of *Fusarium solani* f. sp. *cucurbitae* race 1 in cucurbits plants of Khorasan Razavi, Northern Khorasan and Fars provinces. Proceeding of 17th Iranian Plant Protection Congress Karaj, Iran, p 203.

Amer OE, Mahmoud MA, Samawaty ARMAE, Sayed SRM. 2011. Non liquid nitrogen-based-method for isolation of DNA from filamentous fungi. African Journal of. Biotechnology 10: 14337-14341

Armengol J, Sanz E, Martínez–Ferrer G, Sales R, Bruton BD,. García-Jiménez J. 1998. Host range of *Acremonium cucurbitacearum*, cause of *Acremonium* collapse of muskmelon. Plant Pathology 47: 29–35.

Arzanlou M, Torbati M, Khodaei S. 2013. Phenotypic and molecular characterization of *Plectosphaerella cucumerina* on bamboo from Iran. Mycosphere 4(3): 647-651.

Atghia O, Javan-Nikkhah M, Alizadeh A, Stukenbrock EH. 2014. First report of *Plectosphaerella cucumerina* on some host plants in Iran and the

world. Proceeding of 21st Iranian Plant Protection Congress, Oromieh, Iran, p 907.

Banihashemi Z. 1968. The existence of *Fusarium* wilt of melon in Iran. Proc. First Nat. Cong. Plant Med, pp.47-48

Banihashemi Z. 1982. A new physiological race of *Fusarium oxysporum* f. sp. *melonis* in Iran. Iran Journal of Plant Pathology 18: 1-6

Banihashemi Z. 1983. Distribution of mating types of *Phytophthora drechsleri* the incitant of cucurbit root rot in Fars province. Proceeding of 9th Iranian Plant Protection Congress, Karaj, Iran. p 102

Banihashemi Z. 1982. A new physiological race of *Fusarium oxysporum* f. sp. *melonis* in Iran. Iranian Journal of Plant Pathology 18: 1-6.

Cai F, Yang C, Ma T. 2021. First report of Plectosphaerella cucumerina causing root rot on fennel in China. Plant Disease 105(11): 3752.

Carlucci A, Raimondo ML, Santos J, Phillips AJL. 2012. *Plectosphaerella* species associated with root and collar rots of horticultural crops in southern Italy. Persoonia 28: 34– 48.

Dhingra OD, Sinclair JB. 1995. Basic plant pathology methods, 2nd ed. CRC Press, Boca Raton, 355 pp.

Domsch KH, Gams W, Anderson TH. 2007. Compendium of Soil Fungi. IHW-Verlag Eching. 627p.

Duc PM, Hatai K, Kurata O, Tensha K, Yoshitaka U, Yaguchi T, Udagawa S. 2009. Fungal infection of mantis shrimp (*Oratosquilla oratoria*) caused by two anamorphic fungi found in Japan. Mycopathologia 167 (5): 229-247.

FAOSTAT. 2016. FAO Statistics Division 2016. Online:http://www.fao.org/faostat/en/#data/QC.

Han KS, Choi SK, Kim HH, Lee SC, Park JH, Cho MR, Park MJ. 2014. First Report of Myrothecium roridum causing leaf and stem rot disease on *Peperomia quadrangularis* in Korea. Mycobiology 42: 203-205

Klebahn H. 1929. Vergilbende junge Treibgurken, ein darauf gefundes *Cephalosporium* und dessen Schlauchfrüchte. Phytopathologische Zeitschrift 1: 31–44.

Larkin MA, Blackshields G, Brown NP, Chenna R, Mc Gettigan PA, William Mc H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG. 2000. Clustal W and X version 2.0. Bioinformatics 23: 2947–2948.

Mansoori B, Banihashemi Z. 1980. Prevalence of homothallic and heterothallic isolates of *Phytophthora drechsleri* in cucurbit fields. Phytophthora Newsletter 8:53.

Mirtalebi M, Banihashemi Z. 2010. Isolation and pathogenicity of some *Fusarium* species isolated from crown and root of melon. Proceeding of 19th Iranian Plant Protection Congress. Tehran, Iran. p 151.

Mirtalebi M, Banihashemi Z. 2016 Identification and characterization of collapse on tomato and cucumber caused by *Plectosphaerella cucumerina* in Iran. Iranian Journal of Plant Pathology 52:489-502.

Mirtalebi M, Banihashemi Z, Linde CC. 2013. Phylogenetic relationships of *Fusarium oxysporum* f. sp. *melonis* in Iran. European Journal of Plant Pathology 136: 749-762.

Palm ME, Gams W, Nirenberg HI. 1995. *Plectosporium*, a new genus for *Fusarium tabacinum*, the anamorph of *Plectosphaerella cucumerina*. Mycologia 87: 397–406.

Pascoe IG, Nancarrow RJ, Copes CJ. 1984. *Fusarium tabacinum* on tomato and other hosts in Australia. Transactions of the British Mycological Society 82: 343–345.

Pitt WM, Goodwin SB, Ash GJ, Cother NJ, Cother EJ. 2004. *Plectosporium* alismatis comb. Nov. a new placement for the *Alismataceae* pathogen *Rhynchosporium alismatis*. Mycological Research 108: 775-780.

Raimondo ML, Carlucci A. 2018. Characterization and pathogenicity assessment of *Plectosphaerella* species associated with stunting disease on tomato and pepper crops in Italy. Plant pathology 67: 626–641.

Reuveni R, Krikun J, Shani U. 1983. The role of *Monosporascus eutypoides* in collapse of melon plants in an arid area of Israel. Phytopathology 73: 1123–1126.

Saitou N, Nei M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4: 406–425.

Sarpeleh A. 2008. The role of *Monosporascus cannonballus* in melon collapse in Iran. Australasian Plant Disease Notes 3: 162-164

Sarpeleh A, Banihashemi Z. 2000.Vegetative compatibility groups within races of *Fusarium oxysporum* f. sp. *melonis* in Iran and *Fusarium oxysporum* weeds in Maharloo region of Fars province of Iran. Iran Journal of Plant Pathology 36: 31-45

Su L, Niu Y. 2017. Phylogenetic analysis of *Plectosphaerella* species based on multi-locus DNA sequences and description of *P. sinensis* sp. nov. Mycological Progress 16 (8): 823-829

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28(10): 2731–2739.

Toyozo S, Tadaoki I, Mitsutaka M, Ken W, Keisuke T, Etsuji H. 2005 *Plectosporium* blight of pumpkin and ranunculus caused by *Plectosporium tabacinum*. Journal of General Plant Pathology 71: 127–132.

Wellman FL. 1939. A technique for studying host resistance and pathogenicity in tomato *Fusarium* wilt. Phytopathology29: 945–956.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal

ribosomal RNA genes for phylogenetics, pp. 315–322. In: M. A. Innis, D. H. Gelfand, J. J. Sninsky and T.J. White (Eds). PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, California.

White TJ, Bruns TD, Lee SB, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis N, Gelfand D, Sninsky J, White T (eds). PCR protocols: a guide to methods and applications. Academic Press, New York, pp 315–322.

Xu J, Xu XD, CaoYY, Zhang WM. 2014. First report of greenhouse tomato wilt caused by *Plectosphaerella cucumerina* in China. Plant Disease 98(1): 158.

Yan LY, Zang QL, Huang YP, Wang YH. 2016. First report of root rot and vine decline of melon caused by *Monosporascus cannonballus* in eastern mainland China. Plant Disease 100(3): 651.

Zare R, Gams W, Starink-Willemse M, Summerbell RC. 2007. *Gibellulopsis*, a suitable genus for *Verticillium* nigrescens, and *Musicillium*, a new genus for *V. theobromae*. Nova Hedwigia 85: 463–489.

Zhang Y, Li M, Liang Y, Zhou HY, Zhao J. 2015. First report of sunflower wilt caused by *Plectosphaerella cucumerina* in China. Plant Disease 99: 1646.

اولین گزارش از زوال خربزه ناشی از *Plectosphaerella cucumerina* در ایران

مریم میرطالبی ✉، فاطمه صباحی، ضیاء بنی‌هاشمی
گروه گیاه‌پزشکی، دانشکده کشاورزی، دانشگاه شیراز، شیراز، ایران.

چکیده: در بهار ۱۳۹۸، گیاهان خربزه رقم جانا (*Cucumis melo* CV. Janna) با علائم زوال از مزارع شهرستان دشتی استان بوشهر در جنوب ایران جمع‌آوری شدند. دوازده جدایه شبیه به هم از لحاظ ریخت‌شناختی از بافت ریشه و طوقه آلوده جداسازی شدند. بر اساس خصوصیات ریخت‌شناختی و مولکولی بر پایه‌ی توالی‌یابی ناحیه فاصله ترانویسی شده داخلی (آی‌تی‌اِس)، جدایه‌های قارچی شناسایی شدند. بر اساس خصوصیات ریخت‌شناختی و واکاوی تبارشناسی، جدایه های به‌دست آمده متعلق به *Plectosphaerella cucumerina* بودند. اثبات بیماریزایی با استفاده از گیاهچه‌های سالم خربزه و طالبی رقم خربزه مشهدی و شهد شیراز انجام شد. سه هفته پس از مایه‌زنی، گیاهچه‌های خربزه و طالبی پوسیدگی و بافت مردگی ریشه، پژمردگی و در نهایت مرگ نشان دادند. به منظور اثبات اصول کخ از گیاهان آلوده مجدداً قارچ *P. cucumerina* جداسازی شد. این اولین گزارش از زوال خربزه ناشی از *P. cucumerina* در ایران است.

کلمات کلیدی: بیماریزایی، پژمردگی، ناحیه آی‌تی‌اِس، پوسیدگی ریشه

مکاتبه کننده: مریم میرطالبی Email: mmirtalebi@shirazu.ac.ir
تاریخ دریافت: ۱۴۰۱/۱/۲۱ تاریخ پذیرش: ۱۴۰۱/۴/۱۰