

Short Paper

First record of *Botryosphaeria dothidea* associated with pistachio (*Pistacia vera* L.) panicle blight in Iran

Hamid Mohammadi^{1*}, Mehdi Sarcheshmehpour² and Ebrahim Mafi¹

1. Department of Plant Protection, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman Iran.

2. Department of Soil Science, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran.

Abstract: During spring and summer of 2012, a disease characterized by panicle blight was observed on some pistachio (*Pistacia vera* L.) trees grown in Kerman province. Eighteen isolates of a Botryosphaeriaceae were obtained from affected panicles. Based on morphological and molecular characteristics, isolates were identified as *Botryosphaeria dothidea*. To our knowledge, this is the first report of *B. dothidea* associated with pistachio panicles in Iran.

Keywords: Botryosphaeriaceae, internal transcribed spacer, Kerman province, *Pistacia vera*

Introduction

Panicle and shoot blight of pistachio caused by *Botryosphaeria dothidea* (Moug.: Fr.) Ces. & De Not. is regarded as one of the most important threats to commercial production of pistachio. The disease was first reported in commercial California pistachio orchards in 1984 (Rice *et al.*, 1985). Panicle and shoot blight of pistachio is characterized by infections of fruit clusters, buds, and twigs, and can result in significant yield reduction (Michailides *et al.*, 1999). During a field survey similar symptoms to panicle blight were observed in some pistachio orchards in Kerman province. The present study was therefore undertaken to isolate and identify fungal isolates associated with symptomatic pistachio panicles in some orchards of this province.

Materials and Methods

During spring and summer of 2012, blighted pistachio clusters and fruits with epicarp lesions

were collected from Kabootarkhan, Rafsanjan and Zarand orchards in Kerman province (south-eastern Iran). Fungal isolations were made from symptomatic tissues on potato dextrose agar (PDA, Merck, Germany) plates. All isolates showing typical morphology of the Botryosphaeriaceae (fast growing, dark, greenish brown or grayish cultures) were selected and stimulated to produce pycnidia and conidia, by growing them on PDA with sterilized pine needles (Fig. 1) placed onto the medium surface (Slippers *et al.*, 2004). Morphological identifications of *Botryosphaeriaceae* isolates were confirmed by sequence analysis of the internal transcribed spacer (ITS) nrDNA region using the primers ITS1 and ITS4 (Fig. 2) (White *et al.*, 1990) as described by Úrbez-Torres *et al.* (2008).

Results

In the current study, 32 pistachio cluster samples showing blight symptoms and red to black lesions on the fruits were collected from 11 pistachio orchards in Kabootarkhan (4 orchards), Zarand (3 orchards) and Rafsanjan (4 orchards). Eighteen fungal

Handling Editor: Vahe Minassian

*Corresponding author, e-mail: hmohammadi@uk.ac.ir
Received: 21 April 2014, Accepted: 17 September 2014
Published online: 21 September 2014

isolates resembling Botryosphaeriaceae (13.33% of total isolates) were obtained only from 14 clusters (43.8% of total samples) as the positive samples. These isolates were obtained from nuts (6 isolates) and rachises (12 isolates) showing lesion symptoms. A total of 135 fungal isolates were obtained from samples collected. All isolates resembling Botryosphaeriaceae produced aerial and highly dense mycelium on PDA. Cultures were initially white but becoming grey to olivaceous-grey and darkening to black with age. These isolates had a regular colony shape and colour (whitish-cream in 48 h), abundant aerial mycelium and a fast growth rate [mean radial growth of 12.4 mm (SD = 0.13, SE = 0.23) at 30 °C as optimum temperature in 48 h] that turned dark-green in

7-10 days (Fig. 1). All isolates sporulated within 25-30 days of incubation on pine needles on PDA agar. Based on cultural and morphological characters (Fig. 1) the isolates well fitted with the description of its anamorph by Slippers *et al.* (2004). The other fungal isolates including *Cladosporium* sp., *Pestalotiopsis* sp., *Penicillium* spp., *Aspergillus niger*, *A. flavus*, *Alternaria* spp. and *Paecilomyces* sp. were isolated from symptomatic tissues which were not considered further in this study. The ITS sequences of the *Botryosphaeriaceae* isolates from this study had 100 % identity with isolates previously identified as *Botryosphaeria dothidea* in GenBank (GenBank Accession no. AY786322.1, Phillips *et al.*, 2005).

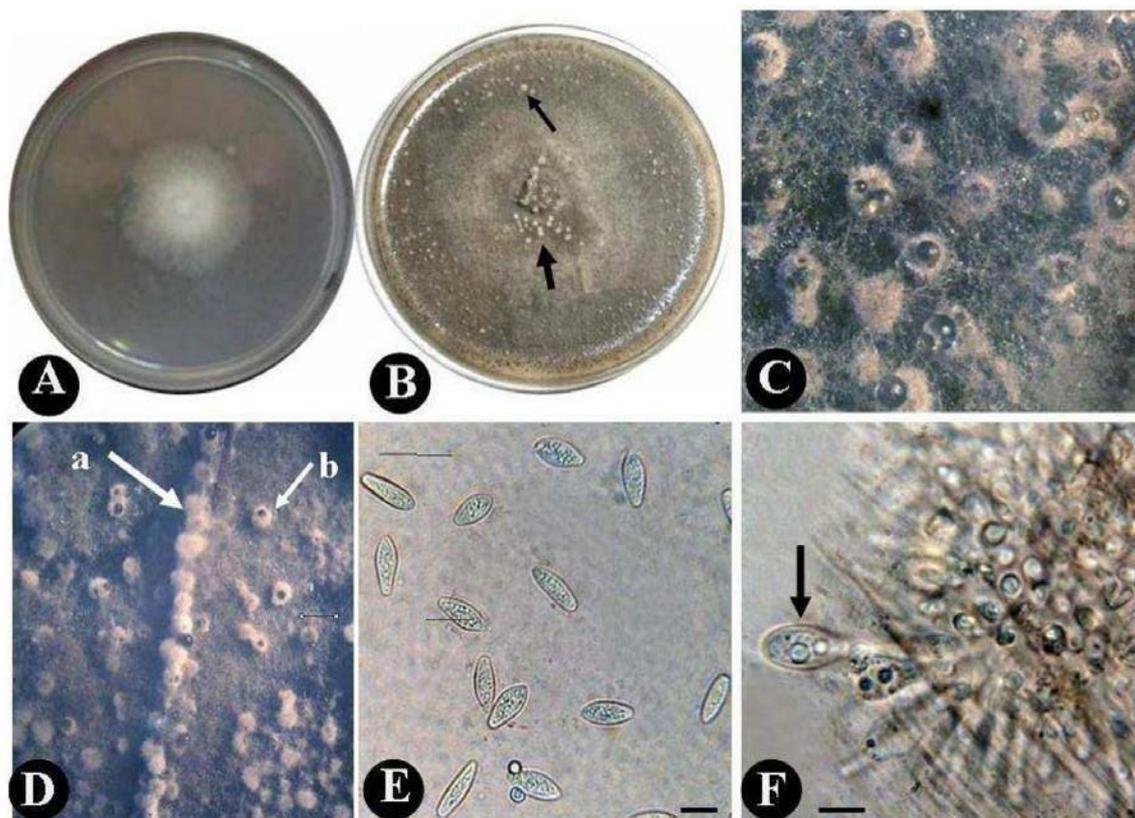


Figure 1 *Botryosphaeria dothidea*. A- 3-days-old colony on PDA (at 30 °C). B: 18-days-old colony on PDA. C: Conidiomata on PDA culture showing oozing of conidia. D: Pycnidia formed on a sterile pine needle (a) and on PDA culture (b). E: Mature, aseptate and hyaline conidia. F: Conidia developing on conidiogenous cells (Bars = 10 µm).

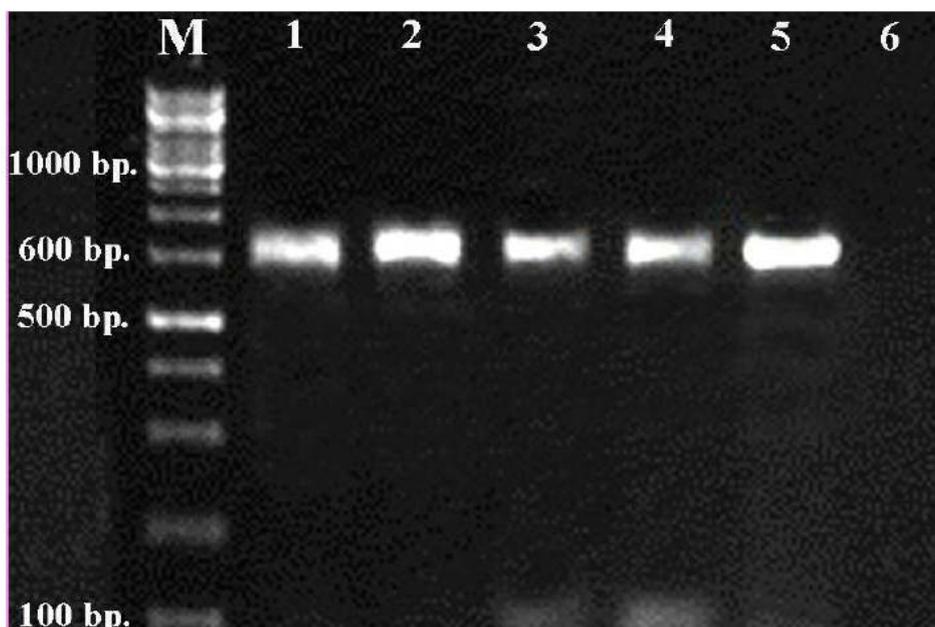


Figure 2 A 600 bp amplified fragment from ITS (ITS1-5.8S-ITS2) region of 5 representative isolates of *Botryosphaeria dothidea* using ITS1 and ITS4 primers on 1% agarose gel: M: DNA marker, 100 bp, lanes 1 to 5: *B. dothidea* isolates, lane 6: negative control.

References

- Michailides, T. J., Teviotdale, B. L. and Weinberger, G. 1999. *Botryosphaeria* Blight of Pistachio: Identification and Control Manual. California Pistachio Industry. Fresno, CA.
- Phillips, A., Rumbos, I. C., Alves, A. and Correia, A. 2005. Morphology and phylogeny of *Botryosphaeria dothidea* causing fruit rot of olives. *Mycopathologia*, 159: 433-439.
- Rice, R. E., Uyemoto, J. K., Ogawa, J. M., and Pemberton, W. M. 1985. New findings on pistachio problems. *California Agriculture* 39: 15-18.
- Slippers, B., Crous, P. W., Denman, S., Coutinho, T. A., Wingfield, B. D. and Wingfield, M. J. 2004. Combined multiple gene genealogies and phenotypic characters differentiate several species previously identified as *Botryosphaeria dothidea*. *Mycologia*, 96: 83-101.
- Úrbez-Torres, J., Leavitt, G., Voegel, T. and Gubler, W. D. 2008. Identification and pathogenicity of *Lasiodiplodia theobromae* and *Diplodia seriata*, the causal agents of bot canker disease of grapevines in Mexico. *Plant Disease*, 92: 519-529.
- White, T. J., Bruns, T., Lee, J. and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Gelfand, M. A., Sninsky, D. H. and White, T. J. (Eds.), *PCR Protocols: a Guide to Methods and Applications*. Academic Press, San Diego, CA, USA, pp. 315-322.

اولین گزارش از *Botryosphaeria dothidea* همراه با بلایت خوشه پسته (*Pistacia vera* L.) در ایران

حمید محمدی^{۱*}، مهدی سرچشمه پور^۲ و ابراهیم مافی^۱

۱- گروه گیاهپزشکی، دانشکده کشاورزی، دانشگاه شهید باهنر کرمان، کرمان، ایران.

۲- گروه خاکشناسی، دانشکده کشاورزی، دانشگاه شهید باهنر کرمان، کرمان، ایران.

* پست الکترونیکی نویسنده مسئول مکاتبه: hmohammadi@uk.ac.ir

دریافت: ۱ اردیبهشت ۱۳۹۳؛ پذیرش: ۲۶ شهریور ۱۳۹۳

چکیده: در طول بهار و تابستان سال ۱۳۹۱ علائمی مشابه با بیماری بلایت خوشه در بعضی از باغ‌های پسته (*Pistacia vera* L.) در استان کرمان مشاهده گردید. در طول این مطالعه ۱۸ جدایه از یک قارچ مربوط به خانواده Botryosphaeriaceae از خوشه‌های آلوده پسته جداسازی گردید. براساس خصوصیات ریخت‌شناختی و مولکولی این جدایه‌ها به‌عنوان *Botryosphaeria dothidea* شناسایی شدند. براساس اطلاعات موجود این اولین گزارش از *B. dothidea* همراه با خوشه‌های پسته در ایران است.

واژگان کلیدی: Botryosphaeriaceae، internal transcribed spacer، استان کرمان، *Pistacia vera*