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## First report of *Botrytis cinerea* on *Lilium polyphyllum*, a critically endangered herb in Uttarakhand, India

Primera cita de *Botrytis cinerea* sobre *Lilium polyphyllum*, especie en peligro de extinción en Uttarakhand, India

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**Abstract.** *Lilium polyphyllum* is a critically endangered herb in the wilds of Uttarakhand, India. Gray mold appeared as a new disease on this species from July to August (2008-2009) causing death of inflorescences and shoots. *Botrytis cinerea* was consistently isolated on PDA, from infected inflorescences. Pathogenicity was established by inoculation of potted plants of *L. polyphyllum* in a glasshouse. *Botrytis cinerea* was recorded as a gray mold pathogen on *L. polyphyllum* for the first time in India, causing flower and shoot blight. This disease may seriously affect reproductive stages of this endangered host. To our knowledge, *B. cinerea* had not been reported previously on *L. polyphyllum*.

**Keywords:** *Lilium polyphyllum*; Gray mold; *Botrytis cinerea*; First report; India.

**Resumen.** *Lilium polyphyllum* es una planta herbácea que se encuentra en estado de peligro en ambientes naturales de Uttarakhand, India. La enfermedad conocida como moho gris fue detectada sobre esta especie desde Julio a Agosto (2008-2009) causando muerte de inflorescencias y varas. *Botrytis cinerea* fue aislado consistentemente de inflorescencias. Su patogenicidad fue confirmada mediante inoculación de plantas de *L. polyphyllum* cultivadas en macetas en un invernáculo. Como resultado, se cita a *Botrytis cinerea* por primera vez como causante de moho gris sobre *Lilium polyphyllum* en India, cuyos síntomas son tizón de flores y varas. Esta enfermedad puede afectar seriamente a este hospedante en riesgo durante su estado reproductivo. A nuestro entender, no existe otro antecedente de *B. cinerea* sobre este hospedante.

**Palabras clave:** *Lilium polyphyllum*; Moho gris; *Botrytis cinerea*; Primera cita; India.

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## INTRODUCTION

The genus *Lilium* L. (Liliaceae) comprises approximately 100 species distributed throughout cold and temperate regions (Siljak-Yakovlev et al., 2003), 11 of which have been recorded in India (Hooker, 1990). *Lilium polyphyllum* D. Don ex Royle is a perennial, bulbous herb with a wide range of medicinal and ornamental uses (Dhyani et al., 2010). These plants are 30-90 cm high, with alternate leaves, and racemes of pendulous flowers of a creamy white color speckled with pink (Fig. 1a). It is distributed from North-West Himalaya to Westward of Afghanistan (Hooker, 1990; Gaur, 1999) in China, Tibet, Nepal, Pakistan, the Hunza Valley (which borders on Russia, Afghanistan, China, India) and Wanga Valley (in the North-Western Himalaya) (Dhyani, 2009). In India, it is found in the states of Jammu and Kashmir, Himachal Pradesh and Uttarakhand, at 1800-3600 m.a.s.l. The bulb is used in traditional and modern medicine (Warrier et al., 1997; Dhyani et al., 2010). This species was categorized as critically endangered due to specific habitat requirements and fast decline caused by human intervention for medicinal, ornamental and fodder uses (Ved et al., 2003). In addition, forest fires and pests are also threatening its survival (Dhyani, 2007).

A new disease was observed on *L. polyphyllum* plants in the temperate region of Garhwal Himalaya, Uttarakhand (Dhanaulti; 30° 25' N, 78° 19' E, 2200 m.a.s.l.) in 2008 and 2009. Petals showed circular necrotic spots that enlarged until the whole flowers became blighted and covered with a grey mold (Fig. 1b). Flower infections occasionally spread towards the base of the plants, leading to premature drying and death of entire shoots. The disease appeared during May after the onset of the Monsoon. Incidence was 35-45% during the flowering period (June to middle of July) and became 50-60% (end of July to first week of August) for both years, respectively.

## MATERIALS AND METHODS

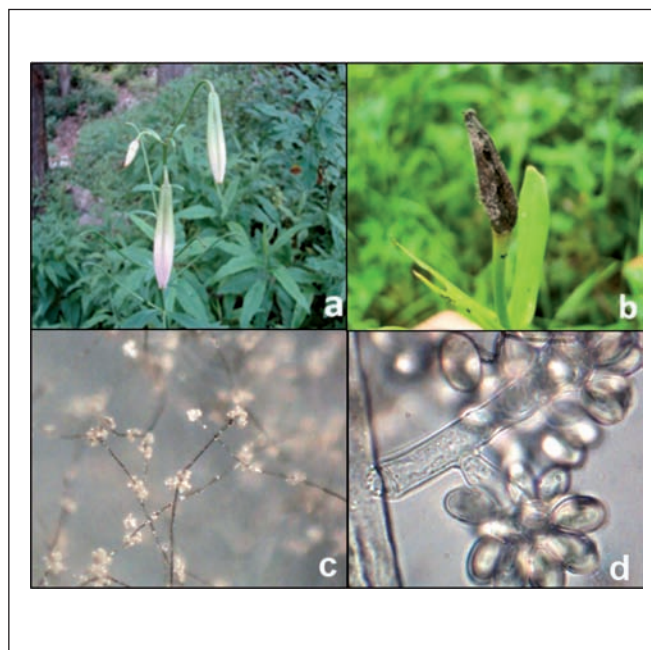
For pathogen isolation, small pieces from diseased flowers were sterilized by immersion in 0.01% mercuric chloride for 1 min, rinsed with sterile distilled water three times and cultivated on potato dextrose agar (PDA). The pathogenicity of the obtained fungal isolate was evaluated by inoculating healthy *L. polyphyllum* plants. Inoculum was prepared from 10-day old PDA cultures by superficially scraping conidia from the surface of the colonies in a 0.1 M MgSO<sub>4</sub> solution. Inflorescences of ten healthy potted plants (average two flowers/plant) were sprayed with a conidial suspension (1 × 10<sup>7</sup> conidia/mL). Flowering plants treated with 0.1 M MgSO<sub>4</sub> served as controls. Both inoculated and control plants were kept in a humidity chamber for 48-72 h and then transferred to a glasshouse bench and monitored for symptom development. The inoculated fungus was re-isolated from symptom-

atic flowers by surface disinfection and growth on PDA as previously described. The cultural and morphological characteristics of the isolate were registered to identify the pathogen to the species level.

## RESULTS AND DISCUSSION

Pinpoint water-soaked lesions developed on flowers and flower buds 72 h after inoculation. Lesions turned necrotic, increased in size, and often coalesced to form patches within 7 to 10 days. Shoots became blighted and died in 12-14 days. All the infected organs were covered with gray mold within 16-18 days. Control plants did not show any symptom of the disease. Re-isolation from the inoculated plants on PDA consistently yielded the inoculated fungus.

Colonies were gray. Septate hyphae (8 to 16 µm wide) bore smooth, botryous, ovoid, colourless conidia (11 to 15 × 8 to 11 µm) (Fig. 1c-d). The isolate produced black sclerotia. These characteristics agree with the descriptions of *Botrytis cinerea* (Ellis & Waller, 1974, Chaube & Pundhir, 2005). The fulfillment of Koch's postulates lets us state that *Botrytis cinerea* is the causal agent of flower and shoot blight of *Lilium polyphyllum*. There is no report of the occurrence of *B. cinerea* or other fungal disease on *L. polyphyllum* from India and other regions (Farr et al., 2011). To our knowledge, this is the first record of gray mold on *L. polyphyllum*.



**Fig. 1.** Gray mold of *Lilium polyphyllum* caused by *Botrytis cinerea*, a. Healthy flower buds; b. Infected flower bud; c. *Botrytis cinerea* grey mold; d. Detail of conidiophores and botryose conidia.

**Fig. 1.** Moho gris causado por *Botrytis cinerea* sobre *Lilium polyphyllum*, a. Inflorescencia sana; b. Pimpollo infectado; c. Conidioforos y conidios de *B. cinerea*; d. Detalle de conidióforo y conidios.

In the fields, disease symptoms were mostly visible on the inflorescences. The pathogen was able to develop downward towards the base of the plants in all cases, under laboratory conditions. Although *B. cinerea* has been reported to damage shoots even of lignified plants (Rivera & Wright, 2002; Vasquez et al., 2007), flower tissues were always the most susceptible to infection. According to Staats (2007) gray mold disease symptoms produced by *Botrytis* species on bulb crops are known as blight, fire or bulb rot. In this work, no lesions were detected on bulbs either during surveys or after inoculations.

*Botrytis* species are important pathogens of numerous ornamentals, vegetables, orchard crops and stored agricultural products (Jarvis, 1977; Elad et al., 2004). Many *Lilium* species are susceptible to *Botrytis* infection (Cotton, 1933; Maclean, 1951; Alippi, 1969; Hou & Chen, 2003). Among 22 *Botrytis* species, ten have been reported to host on bulbous plants and damage their leaves, stems, bulbs, flowers, and rhizomes (Hennebert, 1973; Yohalem et al., 2003; Staats, 2007). *Botrytis cinerea* is a broad host range necrotrophic pathogen, responsible for great economic loss and its control is difficult (Faretra & Pallastro, 1991; Lamondia & Douglas, 1997).

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