

## First report of lemon verbena crown rot caused by *Phytophthora cactorum* in Iran

### Primer informe de pudrición de la corona en cedrón causado por *Phytophthora cactorum* en Irán

Moshrefi Zarandi D<sup>1</sup>, MM Aminae<sup>2</sup>, S Rezaee<sup>3</sup>, A Sharzei<sup>4</sup>

**Abstract.** Lemon verbena (*Lippia citriodora*) is a medicinal plant from the Verbenacea family. Its plantation and medicinal use in Iran has risen greatly in recent years. Crown and root rot symptoms were observed on this plant in some farms and greenhouses of Kerman province during November 2012. The infected plants were taken to the laboratory. Then, samples of infected root tissues were removed and were surface sterilized with 0.5% sodium hypochlorite and cultured onto CMA-PARPH medium. Based on morphological features, the isolated fungus was identified as *Phytophthora cactorum*. Pathogenicity test was performed using inoculated wheat seeds. To our knowledge this is the first report of *Phytophthora cactorum* on lemon verbena in Iran.

**Keywords:** *Lippia citriodora*; *Phytophthora cactorum*; Iran.

**Resumen.** Hierba Luisa (*Lippia citriodora*) es una planta medicinal de la familia verbenacea. Su plantación y uso medicinal en Irán ha aumentado mucho en los últimos años. Se observaron síntomas de pudrición de la corona y de la raíz en esta planta en algunas granjas e invernaderos de la provincia de Kerman en noviembre de 2012. Las plantas infectadas fueron llevadas al laboratorio. A continuación, se extrajeron muestras de tejidos de la raíz infectados, se esterilizaron superficialmente con 0,5% de hipoclorito de sodio y se cultivaron en un medio de CMA-PARPH. Sobre la base de las características morfológicas, el hongo aislado fue identificado como *Phytophthora cactorum*. La prueba de patogenicidad se realizó con semillas de trigo inoculadas. A nuestro entender este es el primer informe de *Phytophthora cactorum* en hierba luisa en Irán.

**Palabras clave:** *Lippia citriodora*; *Phytophthora cactorum*; Iran.

<sup>1</sup> Young Researchers and Elite Club, Science and Research Branch, Islamic Azad University, Tehran, Iran.

<sup>2</sup> Department of Plant Protection, Agricultural and Natural Resources Research Center of Kerman, Iran.

<sup>3</sup> Department of Plant Pathology, College of Agriculture and Natural Resources, Science and Research Branch, Islamic Azad University, Tehran, Iran.

<sup>4</sup> Department of Plant Pathology, Aburaihan Campus, University of Tehran, Tehran, Iran.

Address correspondence to: Delbar Moshrefi Zarandi, Young Researchers and Elite Club, Science and Research Branch, Islamic Azad University, Tehran, Iran. 14778-93855, e-mail: delbar.moshrefi@yahoo.com

Received 14.VII.2014. Accepted 22.I.2015.

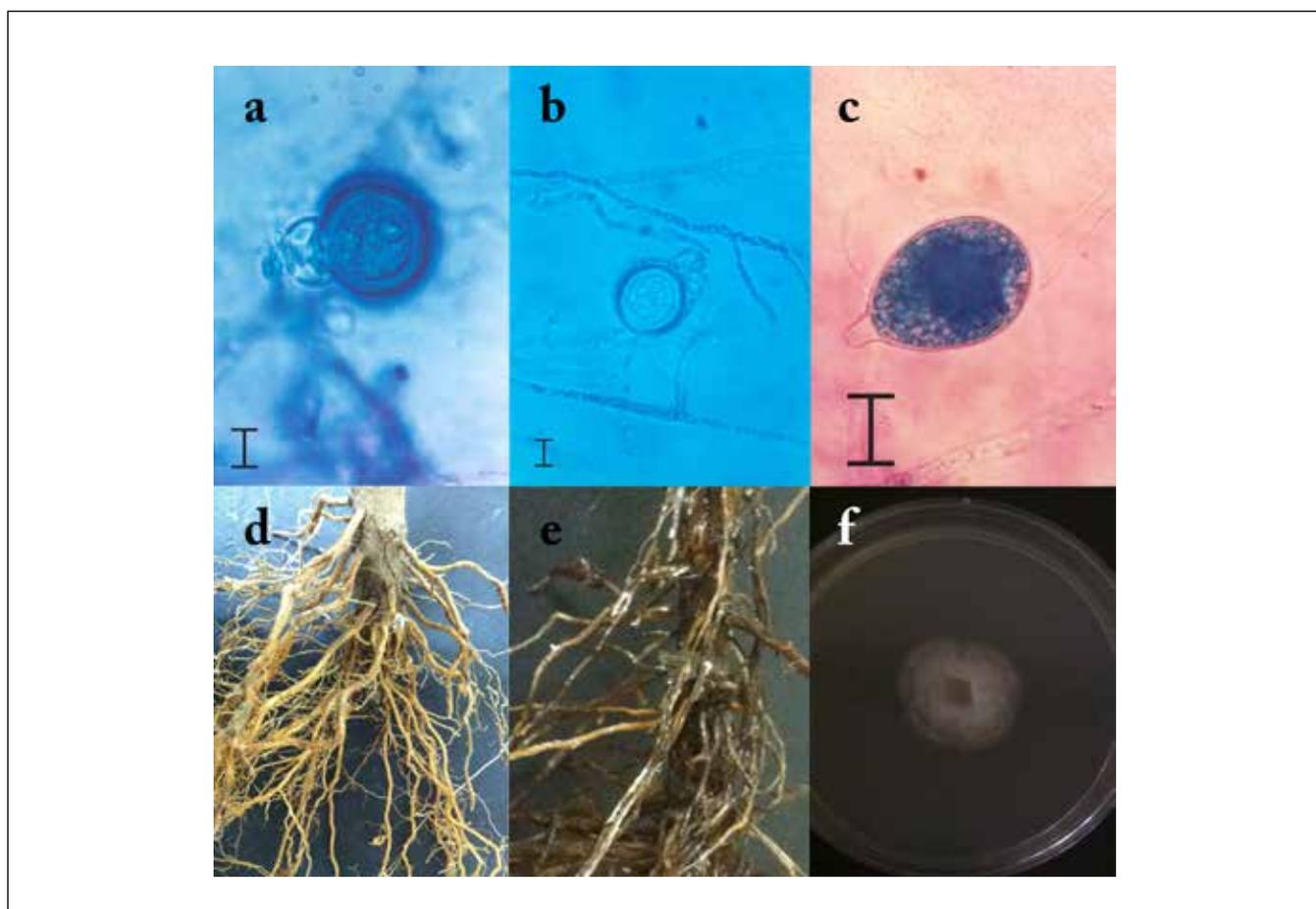
## INTRODUCTION

Lemon verbena (*Lippa citriodora*) is a woody shrub from the Verbenacea family, native to Argentina and Chile, which has shiny lanceolate aromatic leaves with a lemony taste. Its leaves and flowers are used for culinary purposes in teas, desserts, fruit salads and jams, for making perfumes and cosmetics, and as herbal medicines to cure colds, fevers, dyspepsia and diarrhea.

Due to the medicinal benefits, its cultivation has increased greatly during recent years in Iran. In our visits to some lemon verbena farms and greenhouses in suburbs of Kerman (the capital of Kerman province) in November 2012, severe crown and root rot symptoms were observed which in some cases led to plant death. Of about one hundred and forty lemon verbena plants examined, more than 45% of the plants showed symptoms.

## MATERIALS AND METHODS

The infected plants were transferred to the laboratory. First, the plants were gently washed with tap water for 30 minutes. Then, samples of infected root tissues were removed and were surface sterilized with 0.5% sodium hypochlorite for 3 min and cultured onto CMA-PARPH medium (Jeffers & Martin, 1986) and incubated at 25 °C for five days. Pathogenicity test was performed by inoculated wheat seeds. First, we sterilized the soil by placing it in an autoclave at 120 °C for 15 minutes and let it rest in a sterile environment for 7-10 days before use. We also sterilized compost in the same way as mentioned above and mixed it with the sterilized soil at a ratio of 1/3 sterilized compost and 2/3 sterilized soil. Then, three lemon verbena plants were potted and cultured in the sterilized, compound soil together with inoculated wheat seeds (1 g per 50 g compound soil were placed



**Fig. 1.** (a and b) Oogonia and Antheridia of *Phytophthora cactorum* (bar = 10  $\mu$ m), (c) Sporangia of *Phytophthora cactorum* (bar = 10  $\mu$ m), (d) Crown and root rot symptoms on lemon verbena caused by *Phytophthora cactorum* after three weeks of incubation (healthy to the left; infected to the right), (e) Colony of *Phytophthora cactorum* after five days.

**Fig. 1.** (a y b) Oogonia y anteridios de *Phytophthora cactorum* (barra = 10  $\mu$ m), (c) Esporangios de *Phytophthora cactorum* (barra = 10  $\mu$ m), (d) Síntomas de pudrición de las raíces y coronas en cedrón causados por *Phytophthora cactorum* después de 3 semanas de incubación (raíces sanas a la izquierda; infectadas a la derecha), (e) Colonia de *Phytophthora cactorum* después de 5 días.

2-8 cm deep into the soil surrounding the plant roots (Singleton et al., 1992). One control plant was also used for which non-inoculated wheat seeds were used. The plants were incubated in a greenhouse at 25-28 °C for three weeks.

---

## RESULTS AND DISCUSSION

---

White colonies with aerial mycelium were observed on the infected root tissues cultured on the CMA-PARPH medium (Fig. 1e). Sporangia [24.5-42 µm × 17.5-35.7 µm (33.9 µm × 24.6 µm)] were abundant, ellipsoid to ovoid, caducous and papillate (Fig. 1c). Isolates were homothallic and oogonia [24.2-33.7 µm (29.2 µm)] were hyaline and thin-walled, and oospores [24.9-32.1 µm (27.1 µm)] were plerotic, and antheridia [10.3-15 µm (13.4 µm)] paragynous (Fig. 1a, b). Measurements were performed for fifty sporangia, oogonia, oospores and antheridia.

After three weeks of incubation, all three inoculated plants showed symptoms of crown and root rot (Fig. 1d), while the control plant remained completely healthy. Based on the morphological characters, the fungus was identified as *Phytophthora cactorum* (Ershad, 1992; Waterhouse, 1963; Stamps et al., 1990). The fulfillment of Koch's postulates lets us state that *Phytophthora cactorum* is the causal agent of crown and root rot and leaf necrosis of lemon verbena. This fungus has been previously reported on *Photinia fraseri* (Vettraino et al., 2006), and *Fagus sylvatica* (Vettraino et al., 2008) in Italy. To our knowledge this is the first report of *Phytophthora cactorum* on lemon verbena in Iran.

---

## REFERENCES

---

- Ershad, D. (1992). *Phytophthora* species in Iran (Isolation, Purification, Identification). Agriculture Research Organization. 215 p.
- Jeffers, S.N. & S.B. Martin (1968). Comparison of two media selective for *Phytophthora* and *Pythium* spp. *Plant Disease* 70: 1038-1043.
- Singleton, L.L., J.D. Mihall & C.M. Rush (1992). *Methods for research on soilborn Phytopathogenic Fungi*, Aps press. 265 p.
- Stamps, D.J., G.M. Waterhouse, F.J. Newhook & G.S. Hall (1990). Revised tabular key to the species of *Phytophthora*. Commonwealth Mycological Society, Kew, UK. Mycol Pap 162.
- Vettraino, A.M., L. Antonacci, L. Flamini, P. Nipoti, E. Rossini, M. Righi & A. Vannini (2006). First report of crown rot of *Photinia fraseri* caused by *Phytophthora cactorum*. *Plant Pathology* 55: 573.
- Vettraino, A.M., T. Jung & A. Vannini (2008). First report of *Phytophthora cactorum* associated with beech decline in Italy. *Plant Disease* 92: 1708.
- Waterhouse, G.M. (1963). Key to the species of *Phytophthora* de Bary. *Mycological Papers* 92: 1-22.