Letter to the Editor

Lack of *Cryptococcus gattii* from *Eucalyptus* in Ahvaz

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Dear Sir

The basidiomycetous yeast genus *Cryptococcus* contains two medically important pathogens, *Cryptococcus neoformans* and *C. gattii* [1-3]. *C. neoformans* is one of the common pathogens in acquired immunodeficiency syndrome (AIDS), whereas the most cases of diseases due to *C. gattii* happened in the healthy individuals [2, 4]. *C. gattii* has a tendency to affect the respiratory and nervous systems of the humans and domestic animals such as, dogs, cats, and horses [5]. *C. gattii* is more geographically restricted than *C. neoformans* and is largely confined to tropical and subtropical regions. Several reports show that *C. gattii* was isolated from *Eucalyptus* trees (*Eucalyptus tereticornis*, *E. citriodora* and *E. camaldulensis*) in Australia [4, 6, 7].

These two species are divided into five serotypes including: serotype A (*C. neoformans* var. *grubii*), serotype D (*C. neoformans*), serotype A/D (*C. gattii*) and serotypes B and C (formerly *C. neoformans* var. *gattii*) [1, 3]. *C. gattii* has two mating types, a and α [2] and four genotypes designated VGI to VGIV [8]. VGI of C. *gattii* has a worldwide distribution and is the most customary genotype in Australia. VGII is restricted to the Northern Territory, VGIII reported from Colombia, India and the United States, while VGIV is common in Africa and Central America [4, 9, 10]. The aim of the present study was to evaluate the isolation of *C. gattii* from Eucalyptus trees in Ahvaz, a capital city of Khuzestan province in south western Iran.

Khuzestan province is located in the southwestern part of Iran with subtropical climatic conditions (Figure 1). June-August temperatures arise more than 52°C. In the present study, a total of 156 samples of flowers (20), fruits (33), leaves (41) and barks (31) of *Eucalyptus* trees and also soil underneath *Eucalyptus* trees (31), were collected over a period six months (October-March). Samples were collected from *Eucalyptus* trees and soils in public gardens and natural reserves in various parts of Ahvaz. In addition, a part of the samples was also collected from the gardens of Ahvaz Jundishapur University of Medical Sciences and Shahid Chamran University of Ahvaz campus. All samples (with exception soils) were cut into small pieces and then 5-10 g of each sample was mixed with 25 ml of sterile distilled water that contained 0.05 mg/ml chloramphenicol (Merck, Germany) in sterile flasks. The flasks were vigorously shaken for few minutes, and then settled for 30 minutes. 0.2 ml of each supernatant sample was inoculated on the Niger seed agar plates and incubated at 30°C for two weeks [11]. Plates were observed daily and all brown and moist colonies suspected to *Cryptococcus* species, sub-cultured on Sabouraud dextrose agar (Merck, Germany) and incubated at 30°C. Then, all isolates were tested for urease production, growth at 37°C and the presence of capsule around yeasts using India ink preparation.

In the present study, several saprophytic fungi including, *Candida* species and black yeasts were isolated from samples using Niger seed plates. Although, several isolates were grown as mucoid and brown colonies on selected medium, their urease activity, growth at 37 °C and presence of capsule were negative. As a result, we could not isolate any *C. gattii* from

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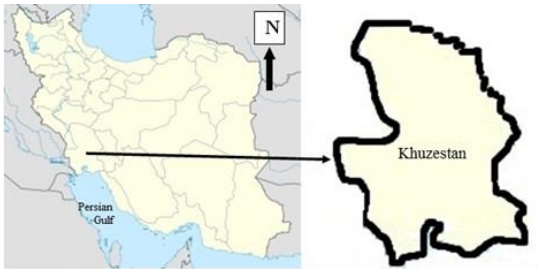

Figure 1. Descriptive map of Khuzestan province and its location in Iran

Eucalyptus trees and soils in Ahvaz.

C. gattii is less virulent than *C. neoformans* and *C. neoformans* var. *grubii*. Thus, most reported cases of disease were among immunocompromised patients. According to Datta et al., [3] *C. gattii* has been increased as a human and animal pathogen in the Pacific Northwest region. Several reports show that most cases of *cryptococcosis* due to *C. gattii* were reported from tropical areas [12].

The successful isolation of *C. gattii* from *E. camaldulensis* in Punjab represents the first Indian isolation [6]. However, Australian *Eucalyptus* trees are the source of *C. gattii*. Bineshian and Zaini were the only isolated 2 cases of *C. gattii* from the 600 samples from *E. camaldulensis* in Northern Iran [11]. Although this study shows that Iranian *Eucalyptus* trees can be a source of *C. gattii*, we cannot isolate any *C. gattii* from *Eucalyptus* trees in the Southwest of Iran. Direct sunlight sterilizes the sites contaminated by *Cryptococcus*, in the summer months. With expert studies done in this field, in this research temperature differences in flowering time can be the reason of the unsuccessful isolation of this organism in the environment. In addition, the high temperature in this area probably inhibits the growing of *C. gattii* on trees.

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Authors' contributions

A.ZM. and M.Z. designed and managed the research. Z.S. collected the samples, cultured and identified them in the medical mycology laboratory. Z.S. analyzed the data, wrote the draft manuscript and A.ZM. edited the final manuscript.

Conflicts of interest

The authors state no conflict of interest.

Financial Disclosure

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