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Effect of treated wastewater on growth and secondary metabolites production of two *Eucalyptus* species

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ABSTRACT

The use of Treated Waste Water (TWW) as an alternative to limit water use in agriculture practices in the context of global warming is of particular importance, especially in countries where water resources are limited. In this study, we assessed the effect of TWW irrigation on two *Eucalyptus* species *(E. camaldulensis* and *E. occidentalis*) in their early stages of growth (up to one year after seedling and transplantation) in pot experiment and compared to Well-Water (WW) irrigation. Changes in plant growth traits/ characteristics including plant height, stem diameter, number of leaves and Leaf Mass per Area (LMA) were analysed as well as the modification of root and leaves secondary metabolic profiles and total phenolic contents. Soil physico-chemical parameters were also measured before and after the experiment.

Our results showed that both species do not have the same response to TWW irrigation: while most growth recorded parameters were most affected in *E. camaldulensis* after one year of growth with TWW, *E. occidentalis* had its leaf number significantly increased with TWW after seven months. Leaves were lighter and smaller than the ones obtained with WW irrigation after four months but the reduction of weight and area was proportional in such a way that LMA was not affected and this effect was transitory. Such effect was not observed with *E. camaldulensis*.

Concerning root and leaf secondary metabolite profiles, changes in both plant part extract compositions were observed according to irrigation type, though these were weak compared to plant species identity. Common metabolic patterns following TWW irrigation could be showed in both species like for example the increase of quercetin glucosinnapate in leaf extracts or the decrease of two peduncalagin isomers detected as major compounds in the same extracts. Though some differences were also observed, particularly, *E. occidentalis* had more compounds decreased in its roots than *E. camaldulensis* when irrigated with TWW while in leaves the tendency was inverted. Total phenolic content was decreased in both species and for both plant parts, but this diminution was more pronounced in *E. occidentalis* roots. We also noticed modifications in soil parameters after TWW exposure and some were dependant on the species of *Eucalyptus* like for example soil organic matter content which increased with TWW in the case of *E. occidentalis*, whereas it decreased for *E. camaldulensis*. All these results let us hypothesize that *E. occidentalis* possess better adaptation capabilities to TWW irrigation than *E. camaldulensis* and that these might be related to changes in root metabolism.

Abbreviations: COD, chemical oxygen demand; EC, *Eucalyptus camaldulensis*; EO, *Eucalyptus occidentalis*; GAE, gallic acid equivalents; HRMS, high resolution mass spectrometry; ICP-MS, inductively coupled plasma mass spectrometry; LMA, leaf mass per area; MS^2, tandem mass spectrometry; PCA, principal component analysis; RT, retention time; SM, suspended matter; SOM, soil organic matter; TWW, treated waste water; UV/Vis, ultra-violet/visible light; WW, well-water

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1. Introduction

The issue of water shortage inside arid and semiarid regions such as Africa, South Asia, Southern Europe and the Middle East is one of the greatest challenges in today's world. The challenge involves the exploitation of water used for drinking, agriculture and the preservation of limited freshwater ecosystems (Gatica and Cytryn, 2013). As a result, treated waste water (TWW) can replace fresh water and satisfy different agricultural demands such as medicinal plants and industrial crops cultivation.

Previous studies have shown that TWW irrigation has got many advantages in agriculture associated with soil structure and fertility (Pedrero and Alarcón, 2009). It can improve growth productivity of some species (Bedbabis et al., 2010), improve air quality and increase local timber production (Farahat and Linderholm, 2013). Also, TWW irrigation increases soil organic matter (Jueschke et al., 2008; Mañas et al., 2009) as well as the concentrations of different nutrients involved in plant growth such as nitrogen, phosphorus, iron, manganese, potassium, calcium, magnesium and others (Gatica and Cytryn, 2013). As a result, the use of TWW for irrigation can have significant effects on soil quality, increased salinity and decreased soil pH (Gatica and Cytryn, 2013).

Despite the benefits of TWW irrigation, this operation is associated with biological risk factors: many studies have found a high number of total coliforms and fecal coliforms in crops irrigated with TWW while others have detected bacterial pathogens such as *Salmonella*. *Streptococcus*, *Clostridium*, *Shigella* and *Vibrio* spp. (Mañas et al., 2009; Samie et al., 2009). Several non-biological risk factors can result from the direct application of TWW such as phytotoxicity due to the high content of salts and the contamination with metals such as cadmium (Cd), nickel (Ni) and chromium (Cr), which may not be safe for medicinal use and thus represents potential risks for human beings or livestock (Bedbabis et al., 2010; Farahat and Linderholm, 2013).

World Health Organization (WHO) has prepared in collaboration with Food and agriculture organization (FAO) and United Nations Environment Program (UNEP) guidelines for safe use of wastewater especially in the agriculture sector (Yasmeen et al., 2014). The use of recycled wastewater has been adopted in Tunisia since 1960 (Klay et al., 2010). TWW quality is confirmed at the secondary level using biological processes (Bedbabis et al., 2010).

Here we have used two species of *Eucalyptus* as a plant model. *Eucalyptus* (Myrtaceae family) is a native genus from Australia and comprises about 700 species (Cheng et al., 2009) highly adapted to many environments constraints such as severe saline and drought conditions by maintaining high transpiration rates and characterized by genetic variability and economic value (Fine et al., 2013). Since 1957, 117 species of the genus *Eucalyptus* have been introduced and placed from the north to the south of Tunisia into 30 arboretums (Ben Jemâa et al., 2012). *Eucalyptus* is used in folk medicine for a variety of medical conditions. A number of studies have analysed the phytochemical composition of *Eucalyptus* extracts and are presented in (Table S1) (Boulekbache-Makhlouf et al., 2010). *Eucalyptus* is a good source of bioactive compounds and secondary metabolites such as triterpenic acids and phenolic compounds with important added-value (Santos et al., 2013). *Eucalyptus camaldulensis* and *E. occidentalis* are very common in the arid south Tunisia especially in the region of Gabes (introduced by INRGREF). They have a growth adaptation in the arid region: they show rapid growth, tolerance to salinity, tolerance to drought and intense flowering (Saadaoui et al., 2018). For example, the relative tolerance of *E. camaldulensis* to salinity was studied because this species has a large range of economic use as fodder, fuelwood and furniture (Rawat and Banerjee, 1998). *E. camaldulensis* is also known for containing bioactive products that demonstrated antibacterial (Cimanga et al., 2002), antifungal (Su et al., 2006), analgesic, anti-inflammatory (Silva et al., 2003), antioxidative and antiradical activities (Siramon et al., 2013). *Eucalyptus occidentalis* is an Australian tree species that has been largely employed for forestation in arid areas of the Mediterranean basin, as they are well resistant to salinity, drought and provide high quality wood and fodder (Jeddi et al., 2009). On the other hand, few studies have been published concerning its pharmacological properties. This species is known for containing kaempferol derivatives that showed apoptosis induction via caspase activation (Benyahia et al., 2004).

Being devoid of motility and immune system, plants have elaborated a huge variety of secondary metabolites that serve as alternative defence strategies in the protection from pests and diseases and also serve as tools to overcome abiotic stress constraints, adapt to the changing environment and survive (Ibrahim et al., 2012; Edreva et al., 2008). More specifically, phenolic compounds are well-known free-radical scavengers that protect cells from oxidative and UV stresses, but they are also involved in many biotic interactions from symbiosis establishment and attraction of pollinating organisms, to allelopathic interactions with other plants and organisms (Bennett and Wallsgrove, 1994; Cheynier et al., 2013). They are also involved in plant protection to metal contamination since plants respond to this stress via several mechanisms including the modulation of secondary metabolism in plant tissues, and notably phenolic compounds (Singh et al., 2016; Michalet et al., 2017; Pham et al., 2017). Furthermore, Senior et al. (2016) have shown that in *Eucalyptus*, phenolics dominated root defences (among other components belonging to primary or secondary metabolism), and that these compounds display a significant phylogenetic signal, allowing the authors to conclude that "more closely related species have more similar root chemistry, which may influence their susceptibility to belowground enemies and soil organic matter accrual".

Thus, the objectives of this study are (i) to investigate the impact of TWW irrigation, on soil properties and growth traits of *E. camaldulensis* and *E. occidentalis* in comparison with well-water (WW), and (ii) to compare the secondary metabolite profiles of root and leaf extracts of *Eucalyptus* grown on different conditions, in order to generally assess the effect of TWW irrigation on *Eucalyptus* performance traits.

2. Materials and methods

2.1. Plant material and treatment conditions

Plantation of *E. camaldulensis* and *E. occidentalis* were carried out in a greenhouse at the regional station of the National Institute of Research on Rural Engineering Water and Forest (INRGREF) - Gabes (Tunisia) from May 2. 2014 to September 28. 2015. The seeds of the two *Eucalyptus* species studied (*E. camaldulensis* and *E. occidentalis*) were obtained and identified by the seed department of the National Institute of Research in Rural Engineering, Waters and Forests (Pr. Khouja Mohamed Larbi; INRGREF - Tunisia). They were harvested from two arboreta in Zrig and Hicha (Gabès - Tunisia): the first planted with *E. occidentalis* which was introduced from Australia in 1959 and the second planted with *E. camaldulensis* introduced in 1998 from Pakistan. Voucher specimens are conserved at the seed department of INRGREF under the number 15/101 and 16/035 for *E. occidentalis* and *E. camaldulensis* respectively. Seeds were sown in 100 pots (30 × 60 cm) containing experimental soil (3/4 sand and 1/4 manure).

For *E. camaldulensis*, the sowing of 50 pots was done in May-2014, after germination, only one plant was kept in each pot. During the first three months of cultivation, plants were daily irrigated with WW. After this acclimation period, the experiment started in August-2014 with plant height (28.28 ± 1.23 cm) and stem diameter (3.87 ± 0.13 mm). For *E. occidentalis*, the sowing of 50 pots was done in September 2014, after 2 months of acclimation, the experiment started in November 2014 with plant height (12.55 ± 0.75 cm) and stem diameter (1.20 ± 0.06 mm). Two water types were used: WW and TWW which was obtained from the wastewater treatment station of Chatt- Essalem (Gabes). The experimental design was a totally randomized block; each block consisted of 4 individuals, including two control plants (irrigated