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Relationship between phytochemical profiles and phytotoxic proprieties of Tunisian fig leaf cultivars

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ABSTRACT

The fig tree (*Ficus carica* L.) is used as an advantageous rich source of bioactive compounds with high economic values for cosmetic and pharmaceutical industries. The different biologically active compounds from this plant may be influenced by genotypes (varieties), environmental factors and their interaction. This study focused on the phytotoxic activity of two well-known Tunisian fig cultivars, Bidhi and Hemri, and its chemical profiles were compared. The phytotoxicity of leaf extracts from fig cultivars was evaluated on the germination and seedling growth of target species. Results indicated that the petroleum ether extract from both studied fig cultivars was the most toxic, showing drastic inhibition of 75.5% on lettuce root growth, which was more sensitive than radish. In fact, Bidhi leaf petroleum ether extract induced the highest phytotoxic effect on seedling growth of lettuce and radish with an average inhibition of 80.3% and 52.3%, respectively, at the highest concentration. The phytotoxic potential was reflected by terpenes, sterols and fatty acids as major compounds that were detected by Gas Chromatography–Mass spectrometry (GC–MS). A similar phytochemical profile was detected from both cultivars with a significant disparity in terpenoids composition. The β-Sitosterol represented the highest values of 1158.84 and 373.31 μg/g DW for cultivars Bidhi and Hemri, respectively. These disparities of chemical profiles explain the phytotoxic effect of cultivar Bidhi more than Hemri. This work arises valuable bases that can be used in future studies for agricultural purposes.

1. Introduction

The success of modern agricultural practices is due to the discovery and adoption of chemicals for weed and pest control (Vyvyan, 2002; Dayan et al., 2009; Jabran et al., 2015). Recently, the massive use of synthetic agrochemicals to enhance crop productivity causes harm to humans and environment. This potential impact stimulates the discovery of new technologies to control weeds and pests based on natural products. Plants represent a rich source of bioactive chemicals, which were used as a source of active compounds in the formulation of new herbicides and other pesticides (Cantrell et al., 2012; Dayan et al., 2012, 2015). The allelopathy phenomenon has been suggested to be one of the alternative strategies to reduce the massive use of herbicides (Abu-Romman et al., 2010; Salhi et al., 2012; Ladhari et al., 2013). Numerous medicinal plants are reported to possess allelopathic potential, and used to protect the crops against weeds and pathogens (Ladhari et al., 2013, 2014; Salhi et al., 2014). The allelopathic application could be either through the direct utilization of natural allelopathic interactions or by using allelochemicals as natural herbicides (Singh et al., 2009; Ladhari et al., 2014). During the development of sustainable agriculture systems, some plants were found to provide excellent weed control ability in intercropping and/or as soil additives due to their phytotoxic property (Weston, 1996; Semidey, 1999; Caamal-Maldonado et al., 2001). The phytotoxic compounds of plant origin elicit quite effective applications in managing health and improving the productivity of agricultural systems (Khanh et al., 2005; De Albuquerque et al., 2011; Farooq et al., 2013).

The fig (*Ficus carica* L.), Moraceae family, is a subtropical species widely cultivated around the Mediterranean areas since ancient times (Flaishman et al., 2008). In Tunisia, fig trees are grown all over the country, with more than 2,600,000 trees occupying about 34,000 ha. The total annual production is about 28,000 metric tons (MARH, 2015). Figs are mainly consumed as fresh fruits or dried, while a small quantities are used for jam and alcoholic beverage production (Gaaliche et al., 2012a). Tunisian fig cultivars are numerous and well adapted to local agroecological conditions (Gaaliche et al., 2012b; Essid et al., 2015).

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Fig fruits and leaves have been used for human consumption for centuries, and recently their nutritive and pharmacological values have been investigated (Yang et al., 2009; Lazreg et al., 2011; Trad et al., 2014). In fact, there is a high correlation between the consumption and its chemical composition (leaves, latex, bark and roots) in various disorders such as gastrointestinal respiratory, cardiovascular disorders and ulcerative diseases (Canal et al., 2000; Joseph and Raj, 2011). In traditional medicine, the roots of fig are used in treatment of leucoderma and ringworms, while the fruit have antipyretic, purgative, aphrodisiac properties and have shown to be useful in inflammations and paralysis (Ross and Kasum, 2002). *Ficus carica* also possess a notable biological activities, such as antiviral, antibacterial, hypoglycemic, anthelmintic (Wang et al., 2004; Jeong et al., 2005; Solomon et al., 2006), antimicrobial, antifungal (Aref et al., 2010), and nematicidal activities (Liu et al., 2011). The latex of fig fruit has been used in several traditional herbal medicine remedies, most of them aimed to treat skin viral infections (Lazreg et al., 2011). Recently, several studies have been assessed the ability of natural compounds investigated from plant as an alternative strategy to reduce the massive use of agrochemicals in order to improve the productivity (Dayan et al., 2009; Cantrell et al., 2012; Dayan and Duke, 2014). Over the years, the characterization of active compounds from *Ficus carica* is one of the most important areas of research. Most of the studies focus on fig fruits and leaves, from which has been identified flavonoids, phenolic compounds, phytosterols and fatty acids (Gibernau et al., 1997; Oliveira et al., 2009; Vallejo et al., 2012). Nevertheless, there is scarce information about the phytotoxic potential of *Ficus carica* or about its chemical profile related to phytotoxicity. Indeed, knowledge about the ecological interaction between *Ficus carica* and other plants, animals or micro-organisms is limited. The only potent allelopathic interaction in Moraceae species was reported for jackfruit (Kumar et al., 2008) and *Sorocea bonplandii* (Maraschin-Silva and Aqüila, 2006). Thus, in this work we aimed to assess the phytotoxic potential of two Tunisian fig cultivars, Bidhi and Hemri, on two target species, i.e., two typical acceptor plants, sensitive to most allelochemicals: lettuce (*Lactuca sativa* L.) and radish (*Raphanus sativus* L.).

2. Materials and methods

2.1. Plant material

The mature leaves of two fig cultivars Bidhi and Hemri were collected from 15-year-old trees grown in a commercial fig orchard (altitude, 18 m; latitude, 35° 55′ N; longitude, 10° 34′ E) located at the Center-east of Tunisia. Samples were washed several times with tap water and dried in hot-air oven at 60 °C for 72 h. Then, they were cut into 1 cm pieces, powdered in blender and sieved through 40 mesh (420 μm) sieve.

2.2. Organic extraction

Sequential extraction was carried out with petroleum ether, ethyl acetate and acetone. One hundred grams of dried leaf powder was extracted successively with organic solvents for 24 h at room temperature. The organic extracts were evaporated to dryness under reduced pressure at 45–50 °C. The residue was weighted and the yields were expressed in percentage of residue on dry weight basis. Residues were stored at 4 °C until use. The yield (%, w/w) from all dried extracts was calculated as:

$$\text{Yield}\ (\%) = (W1 * 100)/W2$$

where W1: weight of the extract after evaporation of solvent, and W2 is the weight of the plant powder.

2.3. Bioassays with organic extracts

The leaf organic extracts obtained from petroleum ether, ethyl acetate and acetone were dissolved in methanol at 3000 and 6000 ppm, to estimate their effect on germination and early growth of target species, i.e., lettuce (*Lactuca sativa* L.) and radish (*Raphanus sativus* L.). These target species were used as a model seeds because of its highly sensitivity to most of allelochemicals. Distilled water and methanol were used as controls. Filter papers placed in Petri dishes were soaked with 5 mL of distilled water, methanol or the organic extracts obtained. Solvents were evaporated for 24 h at 24 °C, then 5 mL distilled water was added and 20 soaked seeds. The plates were placed in a growth chamber for 7 days to germinate with 400 μmol photons m^{-2} s^{-1} photosynthetically active radiation (PAR) at 24/22 °C for 14/10 h light and dark periods respectively. Cumulative germination was determined by counting the number of germinated seeds at 24 h intervals during 6 days. Shoot and root lengths were measured after 7 days of sowing. The total germination (TG) was determined according to Chiapuso et al. (1997):

$$TG = NT * 100/N$$

where, NT: number of germinated seeds for each treatment at the end of the assay and N: total number of seeds used in the assay. This index is the most commonly applied.

The inhibitory/stimulatory percentage was calculated using the equation given by Chung et al. (2001):

$$\text{Inhibition}\ (-)/\text{stimulation}\ (+)\% = (\text{Extract} - \text{control})/\text{control} * 100$$

where the extract was the parameter measured in the presence of fig leaf extracts and control: parameter measured in the presence of distilled water.

2.4. Cytotoxicity test

For germination assays, seeds of *Lactuca sativa* L. were soaked in 5 mL of distilled water on one layer of filter paper in Petri dishes and incubated in germination room [400 μmol photons m^{-2} s^{-1} photosynthetically active radiation (PAR) at 24/22 °C for 14/10 h light and dark periods]. The newly emerged roots (1.50–2.00 cm) were treated with petroleum ether, ethyl acetate and acetone leaf extracts from both cultivars Bidhi and Hemri for 48 h. To avoid toxic effect of solvents, the filter papers were placed in fume hood for 30 min to allow complete solvent evaporation. Subsequently, 5 mL of distilled water were added to each Petri dish. The control group was treated with distilled water. At the end of each exposure period, root tips were cut and subsequently fixed, macerated, stained and squashed as described by Koodkaew et al. (2012), with some modifications. A region approximately 1 cm from the tip was collected and immediately fixed in ethanol/acetic acid (3/1, v/v) for 24 h and transferred to 70% ethanol. The roots were macerated for 25 min with 1 N HCl which used for hydrolysis and maceration. Staining of the chromosome was carried out with acetic carmine for 30 min. One millimeter of the meristematic zone was immersed in a drop of 45% acetic acid on a clean slide and squashed under a cover-slip by thumb pressure. Three slides were prepared for each treatment and the mitotic figures were evaluated randomly in at least 1000 cells per slide using a light microscope. The mitotic index (MI) was calculated as the proportion of dividing cells (M phase) to the total number of cells observed. The frequency of each mitotic phase was calculated as the percentage in relation to the number of cells in mitosis in the treatment.