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Allelopathic Potential of *Chrysanthoglossum trifurcatum* (Desf.) Extracts on Seed Germination and Seedling Growth of *Phalaris canariensis L*

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Abstract

Allelopathic effects of roots, stems, leaves and flowers extracts of Tunisian *Chrysanthoglossum trifurcatum* (Desf.) on seed germination, seedling growth and dry weight of *Phalaris canariensis L*. were studied. The results also indicate that the all extracts inhibited the germination and the shoot and root growth of P. canariensis seedlings. The inhibition percentage increases with increasing extract concentration. At 4 mg ml-1, the ethyl acetate flowers extract reduced the germination of the canary grass seeds, the shoot and root growth by 100.0%. The observed allelopathic activity of the extracts was attributed to the presence of the allelopathic phytochemicals in *C. trifurcatum* organs. Thus, the *C. trifurcatum* extracts may be used as a natural herbicide.

Keywords: *C Trifurcatum*; Seed Germination; Seedling Growth; Allelopathic

Abbreviations: PE: Petroleum Ether, EA: Ethyl acetate, M: Methanol; SD: Standard Deviation.

Introduction

Chrysanthemum spp. are known to be a rich source of secondary compounds with a various biological activities. In fact, phenolic acids identified by gas chromatography from flowers, leaves and roots of *C. morifolium* have been implicated as phytotoxic and responsible at least in part for the allelopathic activity of this species [1]. Additional, the aqueous extract of *C. coronarium* showed strong and selective allelopathic effect against weeds [2,3]. However, with regard to allelopathic potential of plant secondary metabolites, it is now generally recognized that simple phenols and phenolic acids are responsible for growth inhibition of competing plants [4]. The *Chrysanthemum* genus belongs to the Asteraceae family which is very common in Mediterranean basin countries [5,6]. In Tunisia we counted 13 *Chrysanthemum* species [7]. *Chrysanthoglossum trifurcatum* (Desf.) Wilox, et al. (synonym of *Chrysanthemum trifurcatum* Desf. var. macrocephalum (Viv.) Beg [8]. Is an herbal plant bearing small yellow flowers. This plant is widely distributed in Tunisia regions and the plant parts are used for treating constipation, intestinal transit problems, and post delivery pains [9].

It has reported that the methanolic extract of Tunisian

C. trifurcatum could stimulate duodenal smooth muscle contractions through muscarinic receptors [10]. The same authors reported that the petroleum ether, ethyl acetate, methanol, and hot water extracts of *C. trifurcatum* exhibited activity against Gram-positive and Gram-negative bacteria and 4 yeasts [11,12]. On the other hand, the same extracts have a lower effect against HSV type-1. More, methanolic extracts of stems and flowers from *C. trifurcatum* exhibited an important antioxidant activity and petroleum ether extracts of leaves and roots and aerial parts oil showed a potent α-glucosidase inhibitory activity [13]. Other researchers have reported that the most important phytochemicals of *C. trifurcatum* methanolic extracts of stems, leaves and flowers were detected and identified as flavonoids. The analysis revealed two predominant subclasses of flavonols and flavones [14]. On the other hand, researchers have identified flavonoids and phenolic acid in the butanolic fraction of Algerian *C. trifurcatum* which had a scavenging activity of the stable DPPH radical [15].

To our knowledge, works concerning the allelopathic potential of various organic extracts of *C. trifurcatum* growing in Tunisia are not yet available. Therefore, the present study was intended to investigate the allelopathic effects of roots, stems, leaves and flowers extracts of Tunisian *C. trifurcatum* on seed germination, seedling growth and dry weight of *Phalaris canariensis* L.

Experimental

Plant material

Chrysanthoglossum trifurcatum (Desf.) was the synonyme of *Chrysanthemum trifurcatum* Desf. var. *macrocephalum* (Viv.) Beg. By the new classification of Le Floc'h, et al. (2010) [8]. Plant material was collected in Mars 2015 (the period for flower collection) from the area of Monastir in the centre of Tunisia. The plant material was botanically identified by Pr.Skhiri Fethia (High institute of biotechnology, University of Monastir, Tunisia) and according to the morphological description presented in Tunisian Flora [7]. After separating the different plant parts (roots, stems, leaves and flowers), the fresh material was air-dried in a shady place at room temperature for 10 days, ground into a powder using a Wiley mill, and stored until use.

Preparation of organic extracts

The powdered material of the different parts (flowers, leaves, stems and roots) of *C. Trifurcatum* (100 g) was successively extracted at ambient temperature using 3 solvents with increasing polarity: petroleum ether (PE), ethyl acetate (EA), and methanol (M). The 12 organic extracts were filtered, evaporated to dryness in a vacuum at 40°C with a rotary evaporator and stored at 4 °C until use.

Allelopathic assay

The inhibitory potential of the *C. trifurcatum* extracts obtained from flowers, leaves, stems and roots on the seed germination, the shoot and root lengths and the seedling dry weight of *Phalaris canariensis* L. seeds was investigated. Different concentrations of extracts (0.5, 1, 2 and 4 mg ml^{-1}) were prepared and dispersed in sterile Petri dishes (9 cm diameter) lined with double-sterile filter paper (whatmanNo.2). *Phalaris canariensis* L. seeds were sterilized for 20 min in 1% NaClO before use.

Three replicates were prepared for each concentration of the sample (20 seeds/Petri dish). Dishes prepared without extract were used as a negative control. Then, 4 ml of distilled water was added to each Petri dishe, those were sealed with Parafilm to prevent water loss and stored in the dark at 25◦C for 7 days. Seeds that did not germinate were considered to have a radical length of 0 mm. After 7 days, the germination percentage was determined. Then, the seedlings of *P. canariensis* L. were collected, the shoots and roots lengths were measured, and the fresh and dry weights per Petri dish were determined to evaluate the allelopathic activity of the extracts. The inhibitory or stimulatory effects were calculated using the following equation, with slight modifications from Chung et al. (2001) [16]: Inhibition (−)/stimulation(+) % = ((EXe − Ce)/Ce) × 100; where EXe (extract effect) is the parameter measured in the presence of *C. trifurcatum* extracts and Ce (control effect) the parameter measured in the presence of distilled water.

Statistical analysis

All results were reported as means ± standard deviation (SD) from three separate observations. The data were subjected to ANOVA, and Duncan's multiple range test was used to compare means. Statistical analyses were performed with the SPSS statistical software program (SPSS v.13). p values <0.05 were regarded as significant.

Results

Effect on seed germination

The effects of the roots, stems, leaves and flowers extracts of *C. trifurcatum* on the seed germination are summarized in Table 1. The allelopathic influence of extracts on *Phalaris canariensis* L. germination varied according to the extraction solvent and plant parts. Allelopathic effect was observed at the lowest concentration tested, 0.5 mg ml^{-1}. Indeed, the percentage inhibition of seed germination varied between 38.4±2.51% (of roots methanol extract at 0.5 mg ml^{-1}) and 86.7±0.5% (of flowers ethyl acetate extract at 0.5 mg ml^{-1}) at the seventh day of germination.

The *C. trifurcatum* extracts at different concentrations

showed very high phytotoxic effects against *P. canariensis*, with an inhibition of seed germination of 100% at 4 mg ml^{-1} (of flowers ethyl acetate extract). Therefore, petroleum ether, ethyl acetate and methanol extracts marked germination inhibition that was concentration-dependent. From the results shown in Table 1, it is evident that the recovery of inhibitory effect was dependent on the solvent used and its polarity (for all organs). For flowers, ethyl acetate extract gave the total inhibition (-100%) of the seed germination. At the dose of 4 mg ml^{-1}, highest inhibition of the seed germination (-56.7 to -100%) was detected for all the extracts.

Concentration (mg/ml)		0.5	1	2	4
Flowers	PE	-56.4 ± 2.08^{b}	-65.0 ± 2·64^{b}	-66.67 ± 3.5^{b}	-68.4 ± 0·57^{b}
	EA	-86.7 ± 0.5^{d}	-91.7 ± 0.57^{d}	-93.34 ± 0.4^{d}	-100.0^{e}
	M	-45.0 ± 3.0^{b}	-53.4 ± 3.16^{a}	-55.0 ± 2.64^{a}	-56.7 ± 0.57^{a}
Leaves	PE	-63.4 ± 3.21^{b}	-64.9 ± 0.57^{b}	-70.0 ± 2.64^{c}	-80.0 ± 1.73^{c}
	EA	-76.67 ± 1.1^{c}	-83.4 ± 1.53^{d}	-86.67 ± 1.5^{d}	-91.7 ± 0.57^{d}
	M	-58.4 ± 2.08^{b}	-58.5 ± 0.57^{b}	-65.0 ± 2.6^{b}	-68.4 ± 0.57^{b}
Stems	PE	-65.0 ± 2.0^{b}	-66.67 ± 1.5^{b}	-71.7 ± 2.08^{c}	-72.9 ± 2.57^{c}
	EA	-70.0 ± 2.64^{c}	-76.67 ± 0.5^{c}	-85.0 ± 1.0^{d}	-90.0 ± 1.0^{d}
	M	-84.9 ± 1.08^{d}	-89.8 ± 0.57^{d}	-90.6 ± 1.1^{d}	-91.7 ± 0.56^{d}
Roots	PE	-67.1 ± 1.0^{b}	-68.4 ± 1.53^{b}	-78.4 ± 1.53^{c}	-80.0 ± 1.0^{c}
	EA	-61.7 ± 1.52^{b}	-71.7 ± 1.15^{c}	-73.4 ± 0.57^{c}	-75.0 ± 2.0^{c}
	M	-38.4 ± 2.51^{a}	-48.4 ± 3.05^{a}	-53.4 ± 3.21^{a}	-65.0 ± 1.57^{a}

Means in each column followed by different letters are significantly different ($p < 0.05$).

Table 1: Inhibitory effect of different extracts from *C. trifurcatum* on seed germination of *Phalaris canariensis* compared to control (%).

Effect on seedling growth

The shoot and root growth sensibility to the various extracts of *C. trifurcatum* are summarized in Table 2 and 3. The allelopathic influence on *Phalaris canariensis* L. seedling growth varied with the plant part and solvent. Methanol, ethyl acetate and petroleum ether extracts showed a seedling growth inhibition which was dependent on the concentration. The flowers ethyl acetate extract marked the total inhibition (-100%) of the shoot and root lengths of *P. canariensis*. The inhibition of the root growth varied from 24.12±0·73 (roots methanol extract at 0.5 mg ml^{-1}) to 100% (flowers ethyl acetate extract at 4 mg ml^{-1}) (Table 3) and that of the shoot varied from 27.0±0·67 to 100%. At the dose of 4 mg ml^{-1}, highest inhibition of the root (-53.36 to -100%) and shoot elongation (-60.54 to -100%) was detected for all the extracts.

Concentration (mg/ml)		0.5	1	2	4
Flowers	PE	-64.11 ± 1.28^{c}	-71.44 ± 0.27^{c}	-76.10 ± 0.61^{c}	-83.46 ± 0.53^{c}
	EA	-95.71 **± 0.09**^{d}	-98.15 **± 0.1**^{d}	-98.32 **± 0.09**^{d}	-100.0^{e}
	M	-31.14 ± 1.8^{a}	-46.28 ± 1.42^{b}	-54.97 ± 1.2^{b}	-75.42 ± 0.16^{b}
Leaves	PE	-76.51 ± 0.38^{c}	-85.63 ± 0.16^{d}	-77.0 ± 0.56^{c}	-89.17 ± 0.39^{c}
	EA	-81.8 ± 0.09^{c}	-87.9 ± 0.39^{d}	-90.1 ± 0.51^{d}	-96.9 ± 0.12^{d}
	M	-48.0 ± 0.65^{b}	-57.54 ± 1.01^{b}	-60.6 ± 0.10^{b}	-67.41 ± 0.87^{a}
Stems	PE	-81.7 ± 0.09^{c}	-87.94 ± 0.23^{d}	-89.39 ± 0.06^{d}	-90.45 ± 0.21^{c}
	EA	-78.54 ± 0.45^{c}	-83.74 ± 0.26^{d}	-94.63 ± 0.14^{d}	-95.94 ± 0.16^{d}
	M	-81.98 ± 0.5^{c}	-82.92 ± 0.25^{d}	-91.54 ± 0.10^{d}	-96.99 ± 0.15^{d}

Roots	PE	-80.69 ± 0.37^{c}	-81.15 ± 0.38^{d}	-90.77 ± 0.32^{d}	-97.33 ± 0.07^{d}
	EA	-78.6 ± 0.45^{c}	-80.83 ±0.18^{cd}	-81.39 ± 0.09^{c}	-82.18 ± 0.16^{c}
	M	-27.0 ± 0.67^{a}	-29.54 ± 1.32^{a}	-36.58 ± 1.04^{a}	-60.54 ± 1.34^{a}

Means in each column followed by different letters are significantly different ($p < 0.05$).

Table 2: Inhibitory effect of different extracts from *C. trifurcatum* on shoot length of *Phalaris canariensis* seedlings compared to control (%).

Concentration (mg ml^{-1})		**0.5**	**1**	**2**	**4**
Flowers	PE	-71.82 ± 0.9^{c}	-79.98 ± 0.29^{b}	-81.22 ± 0.6^{c}	-83.74 ± 0.6^{c}
	EA	**-96.8 ± 0.0^{8}e**	**-98.45 ± 0.01^{d}**	**-98.17 ± 0.05^{d}**	**-100.0^{de}**
	M	-40.26±1.66^{b}	-49.41 ± 1.44^{a}	-56.07 ± 1.14^{a}	-80.36 ± 0.42^{c}
Leaves	PE	-78.0 ± 0.67^{c}	-80.33 ± 0.12^{b}	-81.26 ± 0.5^{c}	-88.23 ± 0.47^{c}
	EA	-85.58±0.17^{d}	-87.42 ± 0.12^{c}	-90.9 ± 0.35^{d}	-91.84 ± 0.52^{d}
	M	-71.33±1.16^{c}	-75.54 ± 0.37^{b}	-51.79 ± 0.74^{a}	-53.36 ± 1.35^{a}
Stems	PE	-82.04±0.07^{d}	-86.22 ± 0.33^{c}	-90.76 ± 0.01^{d}	-91.95 ± 0.07^{d}
	EA	-83.15±0.48^{d}	-85.15 ± 0.27^{c}	-93.2 ± 0.14^{d}	-96.4 ± 0.18^{d}
	M	-74.2 ± 0.5^{c}	-77.0 ± 0.2^{b}	-78.37 ± 0.51^{b}	-91.68 ± 0.28^{d}
Roots	PE	-94.16±0.15^{e}	-89.93 ± 0.34^{c}	-95.71 ± 0.22^{d}	-99.3 ± 0.04^{de}
	EA	-82.21±0.54^{d}	-89.66 ± 0.51^{c}	-93.13 ± 0.05^{d}	-93.79 ± 0.04^{d}
	M	-24.12±0.73^{a}	-35.94 ± 1.24^{a}	-71.66 ± 1.06^{b}	-71.7 ± 1.14^{b}

Means in each column followed by different letters are significantly different ($p < 0.05$).

Table 3: Inhibitory effect of different extracts from C. trifurcatum on root length of Phalaris canariensis seedlings compared to control (%).

Effect on biomass

The effects of *C. trifurcatum* extracts on seedling dry weight are given in figure 1. Ethyl acetate extract of flowers showed the total inhibition (-100%) of the seedling dry weight of *P. canariensis*. The biomass production was inhibited in the presence of different extracts (Figure 1), and the dry weight of the seedlings treated with 4 mg ml^{-1} was highly reduced, it varied from 63.29 (stems petroleum ether extract) to 0.0% (flowers ethyl acetate extract).

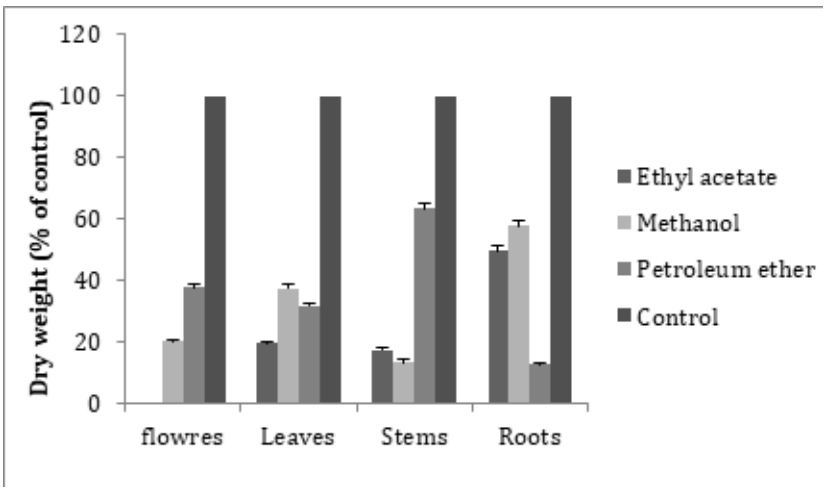

Figure 1: Effect of different organic extracts (4 mg ml^{-1}) of *C. trifurcatum* flowers, leaves, stems and roots on dry weight (% of control) of test plant (*P. canariensis*).

Discussion

Our results demonstrated that the compounds isolated from the Tunisian *C. trifurcatum* tissues with various solvents delayed the seed germination and the growth of *P. canariensis* seedlings. This high inhibitory activity can be mainly due to toxic compounds present in all plant parts especially in ethyl acetate extract from flowers. Indeed, the reduction in seed germination and shoot and root lengths may be attributed to the reduced rate of cell division and cell elongation due to the presence of the allelochemicals [17].

To the best of our knowledge, this is the first report on the phytotoxic effect of *C. trifurcatum* organic extracts. Nevertheless, the phytotoxic activity of *Chrysanthemum* species has been reported. The phytotoxic activity of *C. morifolium* has been mentioned by Kil and Lee (1987) [18]. These authors have shown that the *C. morifolium* extract inhibited the germination of six flowering plants. They also attributed the observed effect to the occurrence of phenolic acids namely salicylic, p-hydroxybenzoic, vanillic, gentisic, protocatechuic, syringique, gallic, ferulic and cafeic acids.

In the same species, Beninger, et al. (2003) [19] have shown that the phytotoxic activity was due, at least partially to the flavonoid eriodyctiol. Two years later, Beninger and Hall (2005) [20] have successfully isolated the luteolin-7-O-β-glucuronide from *C. morifolium* leaves and confirmed its allelopathic activity. Hosni, et al. (2013) [2] reported that the aqueous extracts of *C. coronarium* suppress the germination and reduce the seedling growth of the *Sinapis arvensis* and *Phalaris canariensis*. The phytotoxic effect was could be partly ascribed to tricin. Nevertheless, Gonzáles, et al. (1995) [21] studied the allelopathic effect of *Acacia melanoxylon* on the germination and growth of *Lactuca sativa* and found that the negative effect of the plant extract was highly associated with their high content on quercetin- 3-O-rutinoside and luteolin. Previous studies have reported that luteolin and its glycosylated derivative (luteolin7-O-β-glucuronide) reduced the growth of *Lemna gibba* plants [20].

Scognamiglio, et al. (2012) [22] investigated the phytotoxic effects of cafeic acid and its derivatives (chlorogenic acid; neochlorogenic acid; 3,5-dicafeoylquinic acid; 3,4-dicafeoylquinic acid and its methyl ester and 4,5-dicafeoylquinic acid) on seed germination and seedling growth of two weeds *Dactylis hispanica* and *Aegilops geniculata*. They found that these components potentially inhibit the germination and reduce the growth of the test weeds. Tahri, et al. (2016) [14] reported that methanolic extracts of aerial organs of *C. trifurcatum* were rich on flavonoids. Nevertheless, the main subclass of compounds was flavonols.

The flower extract was rich in flavonoid components and among the identified compounds, they are seven polyphenols as pelargonium chloride, which is the only anthocyanin, two flavanones (eriodictyol and phloridzil), two flavonols (kaempferol-3-Oglucoside and quercitrin) and two flavones (luteolin-7-O-glucoside and luteolin-6-C-glucoside). Leaves and flowers extracts of *C. trifurcatum* contained six flavones (luteolin, luteolin-6-C-glucoside, luteolin-4'-O-glucoside, apigenin-6-C-glucoside, apigenin-8-C-glucoside, tamarixetin), 3 flavonols (quercetin, quercetin-3-O-rutinoside, kaempferol-3-O-rutinoside) and one phenolic acid (caffeic acid) in common.

The comparison of stem and flower methanolic extracts revealed five common compounds: chlorogenic acid, ferulic acid, quercetin3-O-glucoside, kaempferol and quercitrin [14]. These results showed that *C. trifurcatum* stems, leaves and flowers contain important bioactive compounds. Based on the aforementioned data and our findings, it can be assumed that the phytotoxic activity of the organic extracts of C. *trifurcatum* is principally ascribed to phenolic compounds. Furthermore, the overall results provide promising baseline information about the suspected allelochemicals of *C. trifurcatum* and for the potential use of these extracts as bioherbicides.

Conclusion

To the best of our knowledge, this is the first study reporting the allelopathic activity of organic extracts of different *C. trifurcatum* parts from Tunisia. Our results clearly showed that the *C. trifurcatum* extracts contained phytotoxic compounds that inhibited the development of canary grass seedlings, a target species used as a model in this study. Further applied studies are required, but the present results indicated an allelopathic potential for this species, that could be explored in controlling weeds, as a replacement or in addition to synthetic herbicides.

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