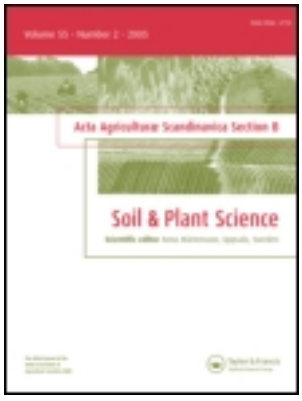

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Phytotoxic potential of sugar beet (Beta vulgaris) and eucalyptus (Eucalyptus camaldulensis) to control purslane (Portulaca oleracea) weed

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ORIGINAL ARTICLE

Phytotoxic potential of sugar beet (*Beta vulgaris*) and eucalyptus (*Eucalyptus camaldulensis*) to control purslane (*Portulaca oleracea*) weed

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Abstract
Two experiments, a Petri dish assay and a pot experiment were carried out to evaluate possible allelopathic effects of water extracts (0, 10 and 20 gL^{-1}) of sugar beet (*Beta vulgaris*) and eucalyptus (*Eucalyptus camaldulensis*) on germination and growth of purslane (*Portulaca oleracea*). Results showed that germination percentage of purslane seeds was not inhibited by concentration of water extract of tested plants. However, seed vigour index and seedling growth strongly influenced by aqueous extracts of tested plants. Maximum inhibitions on seedling growth were recorded when using the higher concentration of the aqueous extract (20 gL^{-1}). It was apparent that sugar beet had a greater inhibitory effect than eucalyptus. Shoot length of purslane seedlings significantly decreased at 10 and 20 gL^{-1} aqueous extract concentration of sugar beet by 65.5 and 92.1%, respectively, compared to the control, while eucalyptus decreased seedling shoot length of target weed at 10 and 20 gL^{-1} extract concentration by 29.8 and 52.9%. Root length was more affected than shoot so that low aqueous extract concentration (10 gL^{-1}) of sugar beet and eucalyptus decreased root length of target weed by 79.8% and 46%, respectively. Foliar sprays with both 10 and 20 gL^{-1} of tested plants extracts significantly decreased leaf area, leaves, stem and root dry weight of purslane weed. Maximum leaf area (1010 cm^2) was obtained from the untreated plants (0% extract), while the lowest value (166.6 cm^2) occurred with 20 gL^{-1} water extract of sugar beet. High aqueous extract concentration (20 gL^{-1}) of sugar beet decreased leaves, stem and root dry weight of purslane plants by 96, 86.7 and 91.9%, respectively, compared to controls.

Keywords: *Allelopathy,* Beta vulgaris*, Eucalyptus, plant growth, weed.*

Introduction

Weeds are unwanted, undesirable and non-economic plants that compete with crops for water, nutrients and sunlight. In addition, some weeds interfere with crop plants through allelochemicals which inhibit crop growth and development (Batish et al., 2005). Weeds are responsible for the decline in crop yield. Losses caused by weeds can be as high as 24% of yield compared with 16.4 and 11.2% for disease and pest, respectively (Hegab et al., 2008). Existing weed control methods are either expensive or hazardous. Heavy use of chemical herbicides in most integrated weed management systems is a major concern since it causes serious threats to the environment, public health and increase cost of crop production. Therefore, alternative strategies against weed must be developed (Batish et al., 2005; Dadkhah, 2012b).

This study considered a very widespread species *Portulaca oleracea* L. (purslane). This plant species is mainly regarded as a drought hardy weed, colonizing bare areas, but also thriving in moist and fertile soils. Overall, purslane is considered as a serious threat to cultivated fields, throughout tropical, subtropical and temperate areas, attaining this status more because of its very widespread importance, than by being amongst the top few weeds in any country. Indeed, purslane was ranked 9th of the world's worst weeds, being recorded in 45 crops in 81 countries. Purslane is a fleshy annual herb, reproducing by seeds, or by stem-fragments rooting when lying on moist soil. The stems are succulent, reddish and

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0.2–0.5 m in length. Flowers are yellow, sessile, self-pollinated. Flowers open on sunny morning and produce numerous (up to 243,000 seed per plant), tiny (0.5 mm diameter) and black seeds (Holm et al., 1977).

A number of plants have allelopathic effects on other plant species (Azania et al., 2003; Asghari & Tewari, 2007; Dadkhah & Asaadi, 2010; Dadkhah, 2012a). Dadkhah (2012a) reported that growth traits of *Cirsium arvense* weed significantly decreased when sprayed with aqueous extract of *Ephedra major*. Allelopthy associated with plants due to the presence of allelochemicals such as monoterpences, phenolic and volatile compounds in their foliage (El-Rokiek & Eid, 2009). Therefore, this study aimed to evaluate the effect of foliar aqueous extract of *Eucalyptus camaldulensis* and *Beta vulgaris* on germination, seedling growth and plant growth parameters of a widespread weed species *P. oleracea* which cause major constraints to crop production in Iran.

Materials and methods

Aqueous extracts preparation

Aerial parts of sugar beet (*B. vulgaris* L. cv) and seeds of purslane (*P. oleracea*) were collected from farm land of Shirvan Agricultural College (North Khorasan Province, Iran) and foliar sections of *E. camaldulensis* was gathered from north area of Iran (Golestan Province). Foliage parts were washed with distilled water and dried at shade room temperature. Dried tissues were ground into fine powder (using an electric mill until homogeneity was achieved). Twenty grams of ground tissue were placed in a 2–l Erlenmeyer flask and 1000-ml deionized water was added to it. The flask was covered with aluminum foil to protect them from photodecomposition and placed on a rotary shaker ($\sim$250 revolutions per minute) for 24 hours. The mixture was filtered through Whatman No.1 filter paper using vacuum pump. The pH and electrical conductivity of extract were determined using a digital pH meter and conductivity meter. These filtrates were considered as stock solution. A series of solutions including the stock solution (extract of 20 g dry weight per litre of water), concentration dilution of 10 gL^{-1} (was developed from the stock solution) and deionized distilled water (control) were used for germination tests. The extra solutions were kept in $-18^{\circ}C$ for later use.

Petri dish bioassays

A Petri dish assay based on completely randomized design with five replications was carried out for screening the effect of different concentrations of aqueous extracts of *B. vulgaris* and *E. camaldulensis* on germination and seedling growth of purslane (*P. oleracea*). Weed seeds were per-sterilized with 2% sodium hypochlorite for 5 minutes and washed with distilled water. Thirty seeds (pre-tested seeds with more than 95% germination) were evenly distributed on two layers of Whatman No.1 filter papers in each 9-cm disposable Petri dish. 5 ml of each of a dilute series was added to each Petri dish covered with a lid. Germination was carried out in germinator at average maximum and minimum temperatures 25 ± 2 and $16 \pm 2^{\circ}C$ for 14 days. Seeds were considered germinated radicle had emerged 1 mm from the seed coat. Germination percentage, germination rate and seed vigor index were calculated (Agraval, 2005) for each Petri dish. Root and shoot length of seedlings were recorded 10 days after germination.

Foliar spray bioassay

Pot experiment was conducted based on completely randomized design with five replications under greenhouse condition. The minimum temperature in the greenhouses was 20 ± 2 and the maximum 35 ± 2. Plastic pots of 15-cm diameter and 25-cm depth were filled with sandy loam soil. Fifteen-day-old purslane (*P. oleracea*) seedlings carefully transplanted to pots (two seedlings in each pot). Aqueous extracts (20 gL^{-1} and 10 gL^{-1}) of foliar dry matter of *B. vulgaris* and *E. camaldulensis* were prepared as described earlier. The extracts were sprayed on pot grown purslane plants after 7 days of transplantation. Plants were sprayed three times (three days interval). Plants in control treatment were sprayed with distilled water. Plants were harvested after 5 weeks and data regarding leaf area, leaves, root and shoot dry biomass were determined. Fresh tissues were dried in an oven at $75^{\circ}C$ for 24 h. The plant leaf area was measured by leaf area meter (Delta-T Devices Ltd, UK).

Phenolic contents of aqueous extract of test plants determined by Folin–Ciocalteau phenol according to the method outlined by Jindal and Singh (1975).

Statistical analysis

These two experiments were carried out base on completely randomized design. Percentage of data were transformed using arcsine prior to statistical analysis. The data for all characters were analysed using the analysis of variance procedure of Statistical Analysis System (SAS) software, version 6.12. Means were compared by Duncan's multiple range tests at the 0.01 probability level for all comparisons.

Table I. Effect of different concentrations of foliar water extracts (0, 10 and 20 g shoot dry weight per litre) of *Eucalyptus camaldulensis* and *Beta vulgaris* on germination percentage, rate of germination (seed germinated per day), seed vigour index, shoot length (cm) and root length (cm) of purslane seedlings.

	Sugar beet			Eucalyptus		
Traits	Control (0)	10 g	20 g	Control (0)	10 g	20 g
Germination percentage	88.6±6	85.5±8.5	82.5±6.7	90±5.4	91.8±3.1	90.1±4.1
Rate of germination	8.5±0.5	7.0±0.5	7.1±0.9	7.8±0.8	6.8±0.63	6.5±0.6
Seed vigour index	51.7±13	14.5±2.9	3.8±4.5	48±9	25.1±7	16.3±4.3
Shoot length	3.2±0.3	1.2±0.25	0.3±0.1	3.2±0.26	2.1±0.35	1.1±0.4
Root length	2.7±0.6	0.5±0.1	0.11±0.02	2.4±0.38	1.2±0.25	0.85±0.1

Note: Each number is mean±S.D of five replications.

Results and discussion

Germination percentage of target weed seeds (*P. oleracea*) was not affected by different concentrations of aqueous extracts from foliage tissues of sugar beet and eucalyptus (Table I).

Seeds germination rate of purslane were less affected by foliar aqueous extract of tested plants. There was no significant differences in seed germination rate between low (10 gL^{-1}) and high (20 gL^{-1}) concentrations of aqueous extract of both tested plants. Highest aqueous extract (20 gL^{-1}) concentrations of sugar beet and eucalyptus decreased germination rate of purslane by 21 and 19.8%, respectively, compared to control plants (Table I). Although germination percentage and germination rate of purslane were not much affected by aqueous extract of tested plants, seed vigour index strongly decreased (Table I).

Growth of young seedlings of purslane was severely reduced especially at the higher aqueous extract concentration (20 g L^{-1}). Root length of purslane seedlings decreased by 79.8% and 92.5% at low (10 gL^{-1}) and high (20 gL^{-1}) concentrations of aqueous extract of sugar beet, respectively (Table I). However, root length of purslane seedlings decreased by 46 and 64% at the same aqueous extract concentrations of eucalyptus, respectively, compared to controls. Shoot length of purslane seedlings also decreased by 65.5 and 92.1% at low (10 gL^{-1}) and high (20 gL^{-1}) concentrations of aqueous extract of sugar beet compared to control (Table I) while the same aqueous extract concentrations of eucalyptus decreased shoot length of seedlings by 29.8% and 52.9%, respectively, compared to control plants (Table I). Therefore, changes on shoot length were on average smaller that those in root length. This is in agreement with Ahn and Chung (2000), who found that the length and dry weight of roots of *Echinochloa crusgalli* were more affected by hull extract than the shoots. An et al. (2001), on evaluation of Vulpia (*Vulpia myuros*) allelochemicals, also found all phenolic compounds caused greater inhibition on root elongation than the shoot length. The strong inhibitory effects of extract on seedling roots might have been caused by the fact that roots were in direct contact with the extract and subsequently chemicals.

Foliar sprays with both 10 and 20 gL^{-1} aqueous extracts of tested plants significantly reduced leaf area of purslane plants (Figure 1). Maximum

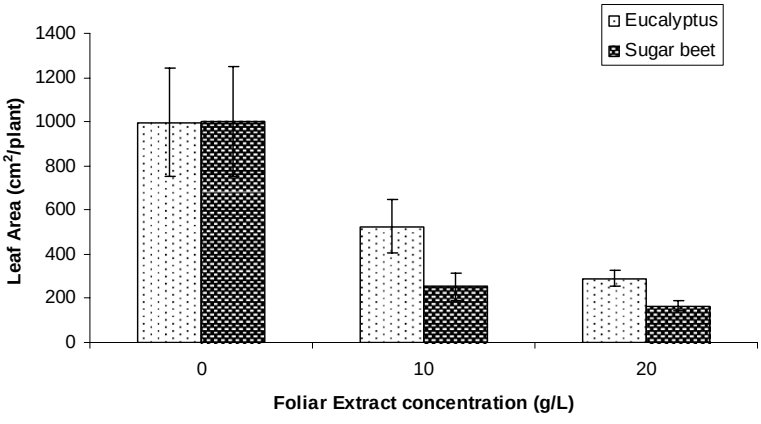

Figure 1. Mean Leaf area (cm^2) of purslane (*Pourtulaca oleracea*) plants at five weeks after spraying with various concentrations of aqueous extracts of eucalyptus (*Eucalyptus camaldulensis*) and Sugar beet (*Beta vulgaris*). Each reported value represents the means±S.D of five replications.

leaf area (1010 cm^2) was obtained from the untreated plants (0% extract), while the lowest value (166.6 cm^2) occurred with 20 gL^{-1} water extract of sugar beet. Leaf area of purslane plants reduced by 74.78 and 85.4% at low (10 gL^{-1}) and high (20 gL^{-1}) concentrations of aqueous extract of sugar beet, respectively. However, leaf area of purslane plants decreased by 47.4 and 70.9% at the same aqueous extract concentrations of eucalyptus, respectively, compared to controls (Figure 1). Foliar sprays with extracts also reduced leaves, shoot and root biomass of purslane (Figure 2).

In the study, response indices revealed that the inhibition of growth parameters of seedlings was more pronounced than that of seed germination. In other words, the seedling growth of the target weed more suppressed than the germination. Ben-Hammounda et al. (1995) found aqueous leaf extracts of several species have suppressed seedling growth in target plants more than seed germination.

The inhibitory effect of sugar beet and eucalyptus on germination rate and seedling growth of target weed may be related to the presence of allelochemicals including phenolic contents and volatile

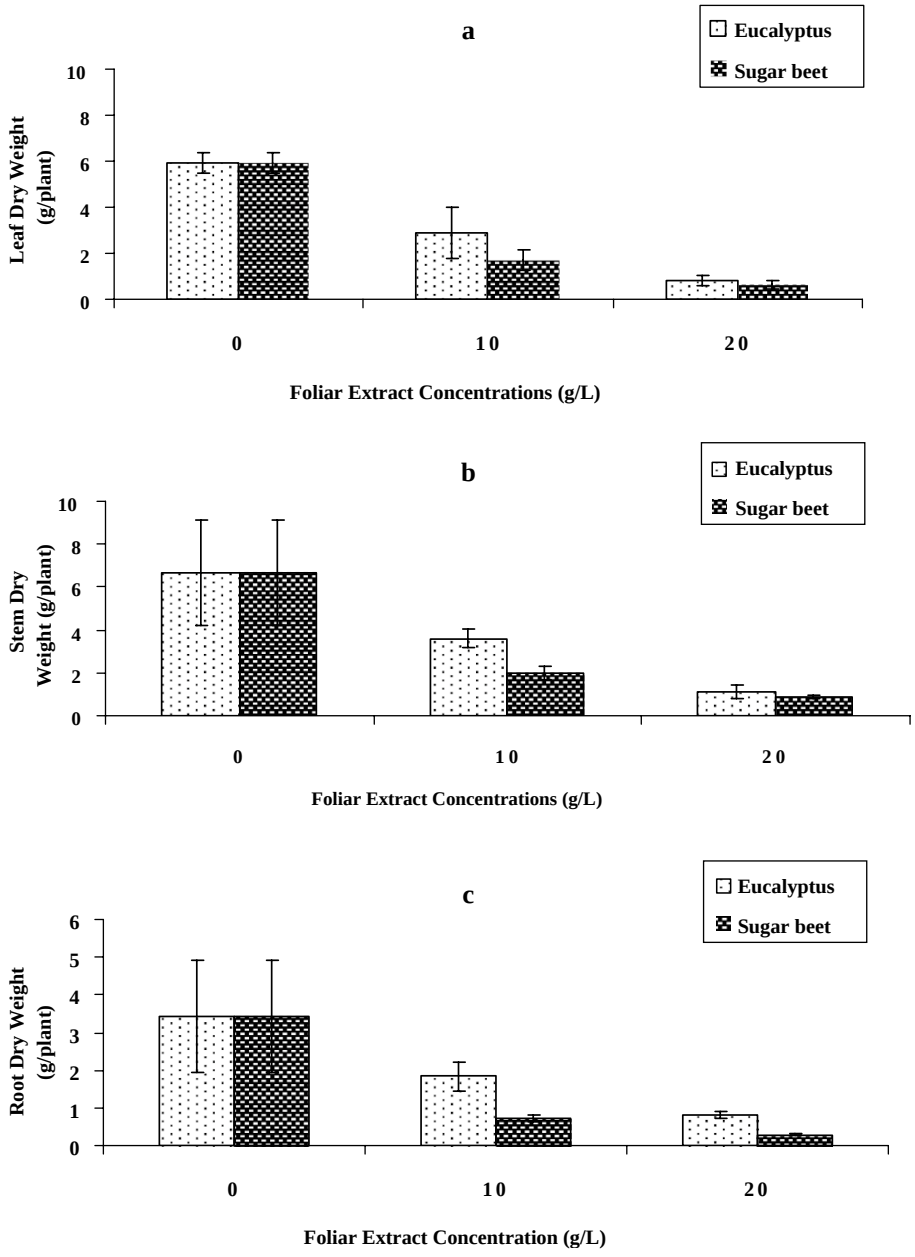

Figure 2. Mean Leaf dry weight (a), mean stem dry weight (b) and mean root dry weight (c) of purslane plants at five weeks after spraying with various concentrations of aqueous extracts of eucalyptus (*Eucalyptus camaldulensis*) and Sugar beet (*Beta vulgaris*). Each reported value represents the means $\pm$ SD of five replications.

compounds in their foliage. Furthermore, the toxicity might be due to synergistic effect rather than single one (Blum, 1996). Phenolic acids have been shown to be toxic to germination and plant growth processes (Enhilelling, 1995). El-Rokiek and Eid (2009) reported that the inhibitory effects of eucalyptus on weeds correlated with accumulation internal contents of total phenols, compared to their respective controls.

Accumulation of phenols is often characteristic of stress conditions (El-Rokiek, 2002, 2007). Some researchers reported that allelochemicals like salt and drought stresses exhibited inhibitory effects on physiological processes that translate to growth (Jefferson & Pennacchio, 2003). The effects of allelopathy on germination and plant growth may occur through a variety of mechanisms including reduced mitotic activity in roots and shoots, suppressed hormone activity, reduced rate of nutrient uptake, inhibited photosynthesis and respiration, inhibited protein formation, decreased permeability of cell membranes and/or inhibition of enzyme action (Rice, 1984). Leaf area reduction can be closely linked to slower leaf production and development of smaller leaves. It was reported that at stress condition leaf area decreases due to a combination of a decrease in cell number and in cell size (De-Herralde et al., 1998). A possible reason for dry matter reduction could be the greater reduction in uptake and utilization of mineral nutrients by plant under allelochemical stress condition. Hegab et al. (2008) found that higher concentration of allelochemical-induced inhibitory effect on amylase activity in wheat seedlings. They also reported the application of allelochemicals at high concentrations decreased protein content of wheat seedlings. The nature of inhibitory effect of allelochemical to seed germination and plant growth could be attributed to inhibit water absorption which is a precursor to physiological processes (Oyun, 2006). Although some researchers attributed more inhibitory effect of plant extract to higher concentration of allelochemicals such as phenolic compounds in extract, our data did not confirm that because foliar aqueous extract of sugar beet had lower concentration of phenolic content rather than foliar aqueous extract of eucalyptus (Figure 3) while sugar beet aqueous extract had more inhibitory effect than eucalyptus. The correlation coefficient ($R^2 = 0.0557$) of total concentration of phenolic contents of extracts with percentage reduction of total plant dry weight was very low (not shown). This indicates that higher concentration of phenolic content can not be a strong reason for more inhibitory on plant growth. Therefore, more inhibitory effect of *B. vulgaris* extract might be due to existence some more active phenolic compounds in its aqueous extract. Hegab et al. (2008) identified and quantified eight phenolic compounds such as shikimic acid, camphor, hydroxybenzoic, p-coumaric and vanillic acids as well as trace amounts of coumarin and protocatechuic acids in water extract of *B. vulgaris* var. cical. These phenolic acids were reported to play an important role in allelopathic interactions and their biological activities on growth of some crop plants and weeds. Chung et al. (2002) demonstrated that p-hydroxybenzoic, p-coumric acids were the most active compounds in rice hull extracts which have inhibitory effect on the growth of barnyardgrass (*Echinochola crus-galli*) seedlings. An et al. (2001) on the basis of evaluation of the biological activity of identified allelochemical from *V. myuros* found that individual compounds were not equally inhibitory to tested plants; allelochemicals present in large quantities possessed low activity, while those present in small quantities possessed a strong inhibitory activity.

The study concludes that foliar aqueous extracts of sugar beet and eucalyptus have significant herbicidal effects on seedling and plant growth of

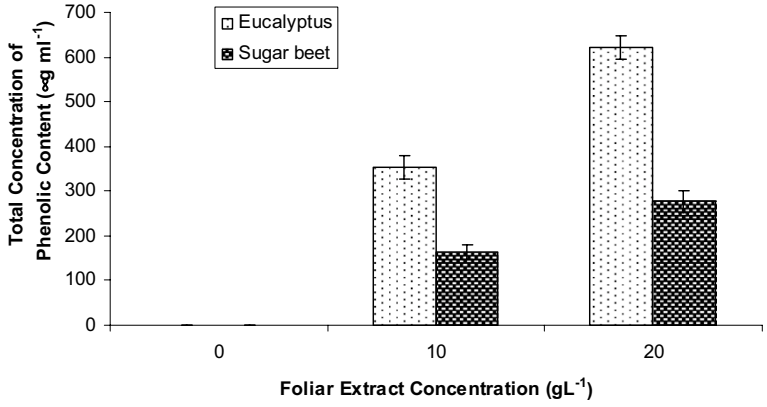

Figure 3. Total concentrations of phenolic content of eucalyptus and sugar beet aqueous extract concentrations. Each reported value represents the means ± SD of five replications.

purslane. More inhibitory effect of sugar beet might be due to existence some more active allelochemicals in aqueous extract. Therefore, further studies are required to identify and isolate the most effective allelochemical from this crop and develop natural-product based herbicides to control one of the world's most aggressive weeds.

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