

Allelopathy of Sage and White Wormwood on Purslane Germination and Seedling Growth

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Abstract

A bioassay run was carried out in the incubator to evaluate possible allelopathic effects of water extracts of sage and white wormwood on germination and seedling growth of purslane. Results showed that the type of extract and its concentration (0, 5, 10, 15 and 20%) significantly influenced final germination percentage, germination rate and index, root and shoot length, root/shoot ratio, fresh and dry weight of seedling of purslane. The interaction between these two experimental factors was always significant, producing different results according to the different combination levels. The statistical comparison of means indicated that the maximum germination percentage and germination rate (respectively 68% and 11.4% d⁻¹) were obtained from the untreated control (0% extract), while the lowest values for the same two characters occurred with 15% of sage (respectively 45% and 6.4% d⁻¹) and 20% of white wormwood (38% and 7.5% d⁻¹). Concerning germination indexes, the highest (55) and the lowest (32) values were observed respectively on the control and with 15% of sage extract or 20% of white wormwood extract. The longest (4.5 cm) and the shortest (1.3 cm) root of purslane were obtained with 5% of white wormwood and 20% of sage extracts, respectively. Changes in shoot length with sage and white wormwood extracts were similar to those in root length, even though shoot length was less affected by the concentration of extracts. Root and shoot length changes brought to maximum (22.0) and minimum (8.9) values for the root/shoot length ratio, respectively with 5% of white wormwood and 20% of sage extracts. The maximum fresh (2.111 g) and dry (0.338 g) seedling weight of purslane were obtained from untreated control, producing the same seedling weight with 5% of sage, 5 and 10% of white wormwood extract. While the minimum fresh (0.692 g) and dry (0.111 g) seedling weight were obtained from 15% of white wormwood extract, so that there is no significant differences between this value and the seedling weight produced under 10, 15 and 20% of sage and 20% of white wormwood extract.

Keywords: *Artemisia sieberi*, germination, *Portulaca oleracea*, purslane, sage, *Salvia officinalis*, seedling

Introduction

Allelopathy interactions are primarily based on the ability of certain plant species to produce secondary chemical compounds, that exert some sort of biological effects on other organisms, many of which are still unknown (Waller, 2004). Allelopathic compounds are released into the environment through root exudation, leaching by dews and rains and volatilization from decaying plant tissues (Rice, 1984). In most cases, these compounds inhibit the germination or growth of neighboring plants, although sometimes they may show a stimulating effect (Ebana *et al.*, 1981). Inhibitory effects of allelopathic plants may be exploited within an integrated weed management system to modify crop-weed interactions and lower the need for chemical means of weed control; indeed, yield losses caused by weeds are well documented in many studies and it has been shown that these losses may frequently be high-

er than those caused by pests and diseases (Culter, 1988; Steinsiek *et al.*, 1982).

This present study considered a very widespread species, i.e. *Portulaca oleracea* L. (purslane). Although it is characterized by several ecological types, some of which are occasionally used as vegetables in human dietary (Miyaniishi and Cavers, 1980), this plant species is mainly regarded as a drought hardy weed, colonizing waste places and 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 one country. Indeed, purslane was ranked 9th of the world's worst weeds, being recorded in 45 crops in 81 countries (Holm *et al.*, 1977), it showed a rating of 10 in Southeast Asia, while it was ranked 6th in the Pacific and 49th in Australia (Waterhouse, 1993a,

b). In Southeast Asia, it is particularly important in many upland crops, including vegetables, rice, maize, sorghum, groundnuts and sugarcane.

Purslane, as an important weed (Duke, 1987), is a fleshy annual herb, reproducing by seeds, or by stem-fragments rooting when lying on moist soil. The stems are succulent, often reddish and 0.2 to 0.5 m in length. The leaves are alternate and frequently clustered at the end of branches; flowers are yellow, sessile, self-pollinated and occur either singly or several together in the above mentioned leaf clusters. Flowers open on sunny mornings and produce numerous (up to 243000 per plant), tiny (0.5 mm diameter) and black seeds (Waterhouse, 1994). These latter are spread by wind, water, as contaminants of crop seeds and by birds, surviving passage through the digestive tract. They also survive burial for long periods and germinate best above 30°C, but poorly below 24°C (Waterhouse, 1994).

Though it does not compete well with other weeds, it is often successful because it establishes rapidly after soil disturbance and may flower and set its seeds before being out competed by taller plants. The succulent leaves and stems are rich in oxalates and nitrates and have been implicated in livestock deaths (Miyanishi and Cavers, 1980).

Two plant genera with potential allelopathic effects were considered in this study, i.e. *Salvia* and *Artemisia*. Concerning the first one, fifty-eight species of *Salvia* (sage; family of *Lamiaceae*) are found in Iran, seventeen of which are endemic (Mozaffarian, 1996). Several of these species have been reported to produce secondary metabolites with medicinal usage (Hitokoto *et al.*, 1980). Among these, *Salvia officinalis* L. is very widespread; it is a semi-woody shrub reaching a height of 60 cm and originates from the Mediterranean regions of North Africa, Spain and the Balkans. It has been grown as a medicinal and culinary herb for thousands of years and it can now be found everywhere in gardens (D'Antunono *et al.*, 2002). A comparison of the composition of the Iranian sage oils during the different developmental stages revealed that 1,8-cineole (15.3-22%), α -thujone (9.1-25.1%) and β -pinene (7.1-16.4%) were the principal components (Mirjalili *et al.*, 2006).

Artemisia (family of *Asteraceae*) is the largest and most widely distributed genus among the approximately 60 belonging to the tribe of *Anthemideae*. This genus comprises a variable number of species, ranging from 200 to over 400, which are predominantly distributed in the northern regions of the world, within the 0-50 cm precipitation area (Tan *et al.*, 1998). Thirty-four species of *Artemisia* are reported in Iran and some of them are endemic. In Iranian folk medicine, some species of *Artemisia* are used for their various medicinal properties and local people used aerial parts of these plants for their antiviral and spasmolytic effects (Ramezani *et al.*, 2004).

Within this genus, *Artemisia sieberi* Bess. (white wormwood) is widely distributed in desert areas of Iran (Mozaffarian, 1988). The main components of essential

oil of *Artemisia sieberi* from Iran were found to be camphor (44-49.3%), 1,8-cineole (11.1-19%), bornyl acetate (5.8%) and camphene (5%) (Weyerstahl *et al.*, 1993). In other studies, thirty-one compounds were identified, including β -thujone (19.8%), camphor (19.5%), α -thujone (10.6%), verbenole (9.7%), ρ -mentha-1,5-dien-8-ol (6.4%) and 1,8-cineole (5.7%) (Ghorbani-Ghouzhdhi *et al.*, 2008). Considering *A. sieberi* from the Kerman province of Iran, the main components of essential oil were (a) in non-grazed sites were: 1,8-cineol (29.9%), myrcene (14.1%); (b) in moderate grazed site: myrcene (15.9%), 1,8 cineol (15.1%), Eudesm-7(11)-en-4-ol (11.1%); (c) in heavy grazed site: 4-tepinyl acetate (23.3%), davanone (21.9%), *p*-cymene (19%) (Bagheri *et al.*, 2007).

Recently, the interest in the application of essential oils to control plants and post harvest pathogens has increased and their potential role in food preservation has been exploited (Lanciotti *et al.*, 2004; Vazquez *et al.*, 2001). However, *S. officinalis* and *A. sieberi* can also play an allelopathic role, because their components might exert an inhibitory effect on seed germination and seedling growth of *P. oleracea*, even though no data is available in literature with this respect.

The aim of this study was to evaluate the allelopathic potential of water extracts obtained from *S. officinalis* and *A. sieberi*, with respect to the above mentioned weed species (*P. oleracea*).

Materials and methods

Aerial parts of *S. officinalis* L. and *A. sieberi* were collected from the Urmia region (Iran), were air dried at room temperature (20-25°C) and were ground by an electric grinder. Water extracts were prepared at concentrations of 0, 5, 10, 15 and 20%. To prepare 20, 15, 10, and 5% of extract 40, 30, 20, and 10 g dry matter of both species soaked in 30°C distilled water during 48 hours, so that it produced 200 ml extract, respectively.

Seeds of *Portulaca oleracea* were collected at the Research Farm of the University of Urmia (1320 m above sea level, 37°32' N, 45°5' E), Iran. They were sterilised with sodium hypochloride (10%) and washed by distilled water. For each treatment, one hundred seeds were randomly placed in four Petri dishes with 9 cm diameter, lined with two Whatman discs with filter paper No. 1 at the bottom. Three ml of deionized water were added to each dish at the beginning of the experiment, by using a pipette. Considering water extract type (*S. officinalis* and *A. sieberi*) and concentration (0, 5, 10, 15 and 20%), the experiment was organised according to a two - factor factorial, randomised complete block design with four replicates.

Germination assays were conducted in an incubator at constant temperature of 25°C in darkness, for six days. Germinated seeds from individual Petri dishes were counted and removed every day at an interval of 24 hours. A seed was considered as germinated when radicle protruded.

sion was more than 2 mm length. The final germination percentage, average germination rate (n. per day) and germination index were measured for each Petri dish as follows (Ellis and Roberts, 1981; Maguire, 1962):

$$\text{Germination Rate} = \Sigma X_n / Y_n$$

$$\text{Germination Index} = \Sigma (X_n / Y_n)$$

in which, X_n was percentage of germinated seeds at Y_n days.

Fifteen germinated seedlings of *P. oleracea* from each Petri dish were grown in light during day and darkness during night under treatment conditions (different concentrations of extracts) until cotyledonal leaves fully opened and then root length, shoot length, seedling fresh and dry weight were measured.

Statistical analysis: All data were submitted to ANOVA. A graphical inspection of residuals showed that the basic assumptions for linear models were not severely violated and that stabilizing transformations were not needed for any of the measured variables. As regression models could not be successfully fitted into this dataset, means were separated by using a protected Least Significant Difference (LSD), at $p = 0.05$. All the analyses were performed by using the statistical package MSTATC software.

Results and discussion

Results of analyses showed that the effects of extract type (sage and white wormwood), concentration of extract (0, 5, 10, 15 and 20%) and their interaction on final percentage germination, germination rate and index, root and shoot length, root/shoot length ratio, seedling fresh and dry weight were significant (Tab. 1).

Mean comparisons indicated that the maximum germination percent (68%) was obtained on the control (0% extract) treatment. Application of 5% sage and white wormwood extracts produced final germination percentages of 56 and 61%, respectively. At higher concentrations, final germination percentages decreased progressively, down to minimum values of 45 and 38%, respectively at 15% sage and 20% of white wormwood (Fig. 1-I).

The germination rate of *P. oleracea* under different concentrations of both sage and white wormwood extracts showed the same trend as final germination percentage (Fig. 1-II), i.e. the maximum and minimum germination rates were obtained respectively on the control treatment (11.4% d^{-1}) and with either 15% of sage (6.4% d^{-1}) or 20% of white wormwood (7.5% d^{-1}) extracts.

Considering the germination index, the highest value was observed in the control treatment (55.1) that was not significantly different from that observed with extracts at concentrations of 5 and 10% of sage and 5, 10 and 15% of white wormwood. On the other hand, the lowest germination index (31.7) was obtained with 15% of sage that was not significantly different from that obtained with 20% of sage and white wormwood (Fig. 1-III).

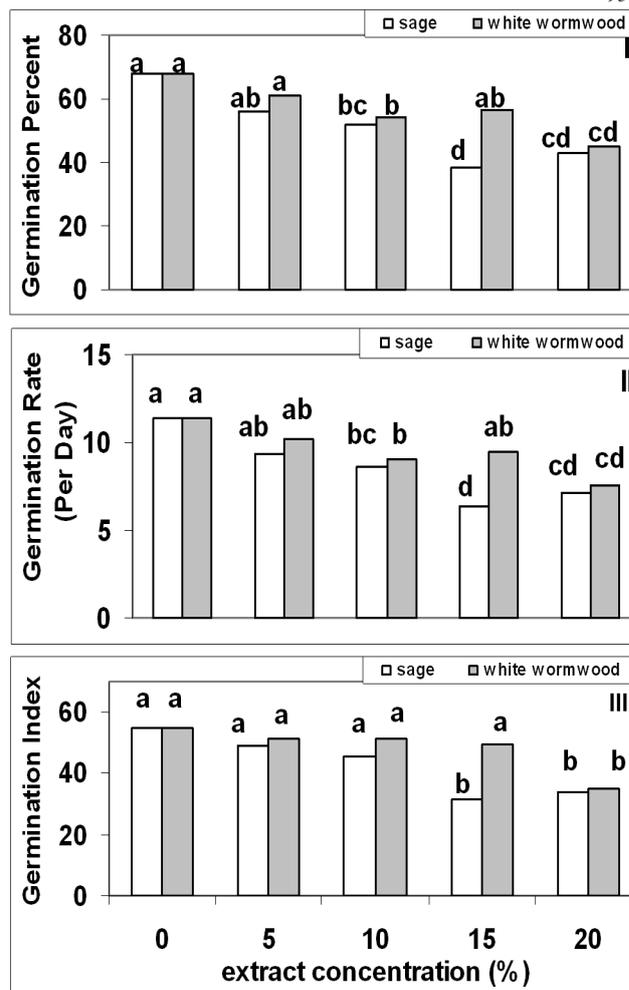


Fig. 1. Means comparisons of interaction effects between extract origin (sage and white wormwood) and concentration on germination percent (I), rate (II) and index (III) of *Portulaca oleracea* seeds. The same letters show non significant differences

The highest root length of *P. oleracea* (4.5 cm) was obtained from extracts at 5% concentration of white wormwood that was significantly higher than the value obtained from control treatment. Increasing the concentration of white wormwood led to shorter purslane roots and such an effect was on average higher with sage extracts., so that the shortest root (1.3 cm) was obtained with 20% of sage extract (Fig. 2-I).

Changes in shoot length were on average smaller than those in root length, and significant differences were only observed between plants treated with 5% white wormwood or sage and plants treated with 10% sage (Fig. 2-II).

Trends in root and shoot lengths led to maximum (22.0) and minimum (8.9) root/shoot length ratios with 5% of white wormwood and 20% of sage, respectively. Variations in root/shoot length ratio followed closely variations in root length, thus this latter variable appeared to affect the above ratio more than shoot length (Fig. 2-III).

The maximum fresh (2.1 g) and dry (0.39 g) weight of purslane seedlings were obtained from the control treat-

Tab. 1. Analysis of variance (MS) of sage and white wormwood water extracts effect on germination and seedling growth of purslane

| Source of Variation | df | Germination | | | Length | | | Seedling | |
|---------------------|----|---------------------|---------------------|---------------------|---------------------|----------------------|---------------------|---------------------|---------------------|
| | | Percent | Rate | Index | Root | Shoot | Root/Shoot | Fresh weight | Dry weight |
| Replication | 3 | 0.004 ^{ns} | 0.004 ^{ns} | 0.001 ^{ns} | 0.500 ^{**} | 0.0003 ^{ns} | 0.009 ^{ns} | 0.001 ^{ns} | 0.001 ^{ns} |
| Extract (A) | 1 | 0.025 ^{**} | 0.025 ^{**} | 0.030 ^{**} | 3.469 ^{**} | 0.002 ^{ns} | 0.029 [*] | 0.014 [*] | 0.010 ^{**} |
| Concentration (B) | 4 | 0.047 ^{**} | 0.047 ^{**} | 0.056 ^{**} | 5.493 ^{**} | 0.002 ^{ns} | 0.104 ^{**} | 0.088 ^{**} | 0.066 ^{**} |
| A×B | 4 | 0.009 ^{**} | 0.009 ^{**} | 0.013 ^{**} | 1.633 ^{**} | 0.004 ^{**} | 0.024 [*] | 0.029 ^{**} | 0.021 ^{**} |
| Error | 27 | 0.002 | 0.002 | 0.002 | 0.095 | 0.001 | 0.006 | 0.002 | 0.001 |
| C.V. (%) | | 2.53 | 4.61 | 2.41 | 11.01 | 17.45 | 6.53 | 12.29 | 16.58 |

ns, *, **: non-significant and significant at P<0.05 and P<0.01, respectively; df: Degrees of Freedom

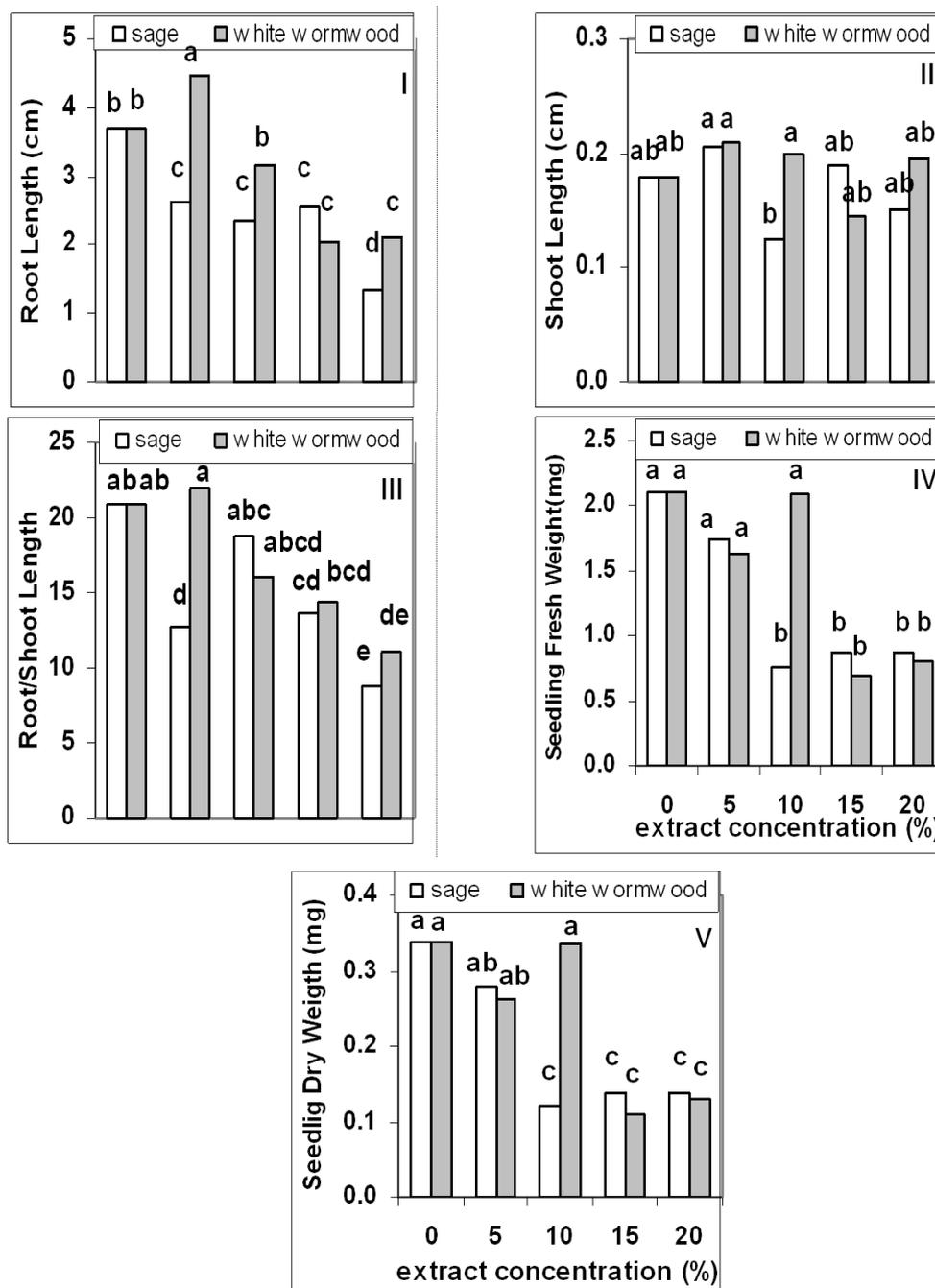


Fig. 2. Means comparisons of interaction effects between extract origin (sage and white wormwood) and concentration on root length (I), shoot length (II), root/shoot length (III), seedling fresh weight (VI) and seedling dry weight (V) of *Portulaca oleracea*. The same letters show non significant differences

ment, while the weight of seedlings obtained with 5% of sage and 5 and 10% of white wormwood were not significantly affected. On the contrary, the minimum fresh (0.69 mg) and dry (0.11 mg) seedling weight were obtained with 15% and 20% of white wormwood and 10, 15 and 20% of sage, with no significant differences among these extract types and concentrations (Fig. 2-IV and Fig. 2-V).

In conclusion, results show that the effect of extract type and concentration strongly interacted and led to different effects according to the different combination of these two experimental factors. Seed germination of purslane was either inhibited or slowed down by the application of little amounts of sage and white wormwood extracts and these effects were greater with sage than with white wormwood. On the contrary, seedling growth of purslane was slightly stimulated by the lowest concentration of sage (5%), while it was inhibited by higher concentration values. Also in this case, the effect of sage appeared to be stronger than that of white wormwood.

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