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RESEARCH ARTICLE

Allelopathic Effect of Four Wild Species on Germination and Seedling Growth of *Echinocloa crus-galli* (L.) P. Beauv

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Abstract

Allelopathy is biological method of weed control, lead to reduced labour costs and increased efficiency without any adverse effects on the environment. The present investigation was designed to determine the allelopathic effects of extracts from four different wild plants (*Cakile maritima, Calligonum polygonoides, Senecio glaucus* and *Zygophyllum album*) on germination and seedling growth of *Echinochloa crus-galli*. *Calligonum polygonoides* and *Zygophyllum album* expressed high contents of saponins (17.33 and 18.47 mg/g dry weight, respectively) and phenolic (8.14 and 12.94 mg/g dry weight, respectively). All extracts significantly reduced the germination of *Echinochloa crus-galli* ($P \leq 0.05$) after 4 days of treatment. The extract of *Calligonum polygonoides* was the most potent as it inhibited root growth by about 99.12% at 75 g/L and 98.10% at 50 g/L. Also, all extracts of the tested wild plants significantly reduced the shoot growth of *Echinochloa crus-galli* in concentration-dependent manner. The phytochemical analysis revealed that *Senecio glaucus* attained high contents of phenolics (23.52mg/g dry weight), tannins (17.61mg/g dry weight), flavonoids (11.51 mg/g dry weight) and alkaloids (8.09 mg/g dry weight).

INTRODUCTION

Allelopathy is described as any effect: direct or indirect, stimulatory or inhibitory, mediated by exuding chemicals into the environment by a given plant or microorganism (Rice, 1984; Brooks, 2008). These chemicals, termed as secondary metabolites, allelochemicals, natural products or phytogrowth-inhibitors, are a major factor in regulating the structure of plant communities (Smith and Martin, 1994; Aliotta et al., 2006).

There is increasing evidence that allelochemicals or natural plant products derived from higher plants/microbes can be ideal agrochemicals (Mattner, 2006; Soyler et al., 2012). Allelopathy interactions are based primarily on the production of secondary chemicals by higher plants that produce a wide array of biochemical compounds that create biological changes (Reigosa and Pedrol, 2002; Hierro and Callaway, 2003; Heidarzade et al., 2010).

The world's agricultural and forestry production, as well as the ecological dimensions in relation to population, calls for global changes to be brought about by allelopathy (Kohli et al., 2001; Macías, 2004). The widespread use of herbicides has resulted in the increasing incidence of weeds resistance to them and in environmental pollution and associated health problems (Macias et al., 1998; Nikneshan et al., 2011; Delye et al., 2013). Insects, weeds, disease and rodents destroy 30% of the world's food supply. In developing countries, the crop losses may be even higher (Kohli et al., 2001), hence, they must be controlled.

Like elsewhere in Egypt, weeds cause major yield losses in crops of cultivated lands, weeds are undesirable plants and interfere with the growth of cultivated plants (Anonymous, 1994; Sodaeizadeh and Hosseini, 2012).

Echinochloa crus-galli (barnyardgarss) is the most serious weeds in the world (Holm et al., 1991; Aliotta et al., 2006).

In Egypt barnyardgarss is one of the most successful yield-limiting weeds in the rice fields which its control mostly relies on herbicides. Increasing use of herbicides might lead to enhance environmental pollution and human exposure to toxic materials (Zimdahl, 2004). Several workers have shown that allelopathy plays an important part in weed and weed interaction (Newman and Rovira, 1975; Tajuddin et al., 2002; Abd El-Gawad, 2014) and weed crop interaction (Colton and Einhellig, 1980; Olofsdotter et al., 1999; Naderi and Bijanzadeh, 2012).

The goal of this study was to determine the possible allelopathic effects of commonly distributed wild species in the Deltaic Mediterranean coast of the country (*Cakile maritima* Scop., *Calligonum polygonoides* L., *Senecio glaucus* L. and *Zygophyllum album* L.) on germination and growth of *Echinochloa crus-galli*.

Salient features of the wild plants used

1- *Cakile maritima* Scop.

C. maritima (Sea rocket, family Brassicaceae) is an annual succulent herb, stem ascending with highly branched, 15-60 cm and facultative halophyte, widely distributed along Mediterranean seashores and Nile Delta (around the lakes), with high tolerance to salty and dry environment (Täckholm, 1974; Boulos, 1999).

2- *Calligonum polygonoides* L.

C. polygonoides subsp. *comosum* (Arta plant, family Polygonaceae) is a small shrub with woody branches, usually 4 to 10 feet high (Boulos, 1999). It commonly grows on dry sandy soils and on sand dunes. It is very hardy and being capable of growing under adverse conditions of soil and moisture. It produces root suckers and is easily propagated by cutting and layering (Tadevosyan, 2001).

3- *Senecio glaucus* L.

S. glaucus (Almorrar, family Asteraceae) is an annual herb, native to many regions of Northern Africa and Northern Asia. In Egypt, it distributed in the Egyptian deserts and the Mediterranean coastal strip (Boulos, 2002)

4- *Zygophyllum album* L.

Z. album (Rotreit, family Zygophyllaceae) is a perennial low shrub with many branched stems 25-50 cm. *Zygophyllum* is found in many coastal and inland saline sandy soils of the world, widely distributed along Mediterranean seashores, Sinai, Oases and Nile Delta (around the lakes), with high tolerance to salty and dry environment (Täckholm, 1974; Boulos, 2000; Bellstedt et al., 2008).

MATERIALS AND METHODS

The plants were collected from Deltaic Mediterranean coast of Egypt at a vegetative stage. The plant tissues were clipped by hand 1 cm above the soil, washed with distilled water and left to dry in room temperature in shaded place for several day till complete dryness. The dried samples were ground to pass a 1 mm screen and then stored in a refrigerator. Seeds of *Echinochloa crus-galli* were collected from the different localities of study area, sterilized by 0.3% sodium hypochlorite, rinsed by distilled water and shade dried again on the filter paper in the laboratory at room temperature for 7 days (Uremis et al., 2005).

Preparation of methanolic extract

For bioassay tests, stock methanolic extract (10% w/v) were diluted with distilled water to obtain concentrations of 15, 25, 50 and 75% (v/v) test extracts. All osmotic concentrations of bioassay solutions were less than 0.1 Mpa and hence not considered a factor affecting germination (Hegazy, 1997). The solutions were filtered thought a double layers of muslin cloth followed by a Whatman No.1 filter paper, the pH values were adjusted to 7 with 1M HCl, these were kept in refrigerator at 4 ºC until further use (Rice, 1972).

Germination bioassays

Two layers of Whatman No. 1 filter paper were placed in 90 mm diameter glass petri dishes. In each petri 25 seeds were placed and 10 mm of each plant extract added in a concentration of 15, 25, 50 and 75% (v/v). A check treatment was assigned with distilled water and let at room temperature. Starting from the first day after experiment set on, germinated seeds were counted and removed daily. A seed with 0.5 cm of radical was considered germinated. Experiment designed was RCB (Randomized Complete Block) with three replicate and experiment repeated twice and the percentage of germination was calculated.

Growth bioassays

The seeds of *Echinochloa crus-galli* were germinated on filter paper in the dark at room temperature for 2 days. Fifteen germinated seeds were transferred to petri dishes which were filled with 25 g quartz sand and 10 ml of shoot powder extract added in concentrations of 15, 25, 50 and 75% (v/v). In addition, a check added to experiment without any powder treatment. Experiment designed was RCB with three replicate and experiment repeated twice. The shoot and root lengths of seedlings were measured on 14 day after treatment (DAT).

Statistical analysis

All the data were subjected to ANOVA and the mean values were separated on the basis of Least Significant Difference (LSD) at 0.05 probability level using the COSTAT 6.3 program.

Phytochemical analysis

Cakile maritima, *Calligonum polygonoides*, *Zygophyllum album* and *Senecio glaucus* were collected and prepared as previously mentioned. The amount of phenol was determined spectrophotometrically (Sadasivam and Manickam, 2008), tannins was determined using the method of Van Buren and Robinson (1969) and saponin content was estimated according to Obdoni and Ochuko (2001). Flavonoid content was determined according to Bohm and Kocipai-Abyazan (1994), while alkaloid was determined according to Harborne (1973).

RESULTS

Phytochemical analysis

The phytochemical analysis of *Cakile maritima*, *Calligonum polygonoides*, *Zygophyllum album* and *Senecio glaucus* are shown in Table 1. *Senecio glaucus* attained high contents of phenolics (23.52mg/g dry weight), tannins (17.61mg/g dry weight), flavonoids (11.51 mg/g dry weight) and alkaloids (8.09 mg/g dry weight). *Cakile maritima* contained low contents of active constituents except saponins (43.36 mg/g dry weight), which was highly appreciable. On the other hand, *Calligonum polygonoides* and *Zygophyllum album* contained high contents of saponins (17.33 and 18.47 mg/g dry weight) and phenolic (8.14 and 12.94 mg/g dry weight).

Table 1. Active constituents in mg/g dry weight for the selected plant species.

Plant species	Tannins	Saponins	Flavonoids	Alkaloids	Phenolics
	(mg/g dry weight)				
Calligonum polygonoides	7.58 ±0.08	17.33 ±0.05	6.86 ±0.10	4.26 ±0.04	8.14 ±0.04
Cakile maritima	6.15 ±0.08	43.36 ±0.39	5.49 ±0.07	3.30 ±0.05	6.53 ±0.06
Senecio glaucus	17.61 ±0.23	22.42 ±0.17	11.51 ±0.27	8.09 ±0.24	23.52 ±0.15
Zygophyllum album	8.35 ±0.06	18.47 ±0.03	7.32 ±0.05	5.45 ±0.01	12.94 ±0.06

Effect of different extracts on *Echinochloa crus-galli* germination

The allelopathic effect of *Cakile maritima*, *Calligonum polygonoides*, *Zygophyllum album* and *Senecio glaucus* extracts on the germination percentage of *Echinochloa crus-galli* after four days after treatment (DAT) are shown in Table 2. After 4 days of treatment, all extracts significantly reduced the germination of *Echinochloa crus-galli* ($P \leq 0.05$). *Zygophyllum album* recorded high germination inhibition percentage of 81.59% at 75 g/L concentration and this was reduced into 67.34%, 44.89% and 24.47% for seed of *Echinochloa crus-galli* germination under methanol extracts 50g/L, 25g/L and 15g/L, respectively. The extracts of *Cakile maritima* attained the lowest effective at different concentrations, while the other extracts expressed its allelopathic potential (Table 2).

Effect of different extracts on root growth of *Echinochloa crus-galli* seedlings

The allelopathic activities of methanol extracts from tested wild plant on root growth of *Echinochloa crus-galli* seedlings showed inhibition at both low and high concentrations (Table 3). The extract of *Calligonum polygonoides* was the most effective as it inhibited root growth by about 99.12% at 75 g/L and 98.10% at 50 g/L, followed by *Zygophyllum album*, which extract inhibited root growth by about 98.87% at 75 g/L and 95.74% at 50 g/L and then *Senecio glaucus*, which extract inhibited root growth by about 93.10% at 50 g/L and 94.24% at 75 g/L. *Cakile maritima* was the least effective as it inhibited root growth by about 92.67% at 75 g/L and 90.0% at 50 g/L.

Table 2. Effect of different plant extracts on the germination inhibition percentage (mean value ± standard error) of the *Echinochloa crus-galli* after 4 DAT.

Plant species	Concentration (g/L)*			
	15	**25**	**50**	**75**
Cakile maritima	19.53[c] ±0.58	34.16[c] ±0.52	43.92[c] ±1.20	48.23[c] ±0.33
Calligonum polygonoides	16.67[c] ±0.30	29.17[d] ±0.24	47.92[b] ±0.20	60.42[b] ±0.30
Senecio glaucus	30.22[a] ±0.57	39.52[b] ±0.88	44.17[bc] ±0.58	65.11[b] ±0.57
Zygophyllum album	24.47[b] ±0.33	44.89[a] ±0.58	67.34[a] ±0.33	81.59[a] ±0.25
$LSD_{0.05}$	3.61	4.30	4.28	5.21

*: mean value ± standard error. Different superscript letters indicate values significantly lower than the respective control ($P \leq 0.05$).

Table 3. Effect of different plant extracts on the root growth inhibition percentage (mean value ± standard error) of the *Echinochloa crus-galli* after 14 DAT.

Plant species	Concentration (g/L)*			
	15	25	50	75
Cakile maritima	10.83[c] ±0.23	83.33[c] ±0.17	90.00[b] ±0.10	92.67[b] ±0.03
Calligonum polygonoides	18.74[b] ±0.33	88.75[b] ±0.06	98.10[a] ±0.0	99.12[a] ±0.0
Senecio glaucus	26.21[a] ±0.19	86.21[b] ±0.03	93.10[ab] ±0.03	94.24[b] ±0.10
Zygophyllum album	25.53[a] ±0.29	93.62[a] ±0.01	95.74[a] ±0.00	98.87[a] ±0.00
$LSD_{0.05}$	2.5	4.29	5.12	3.6

*: mean value ± standard error. Different superscript letters indicate values significantly lower than the respective control ($P \leq 0.05$).

Effect of different extracts on shoot growth of *Echinochloa crus-galli* seedlings

The allelopathic effect of wild plants extracts on the shoot growth inhibition percentage of *Echinochloa crus-galli* are showed in Table 4. At higher concentrations 75 g/L, *Calligonum polygonoides* and *Zygophyllum album* extracts were the most effective extract as it inhibited shoot growth of seedlings by about 84.61% and 67.10%, respectively (Table 4). *Senecio glaucus* extract was inhibited seedling shoot growth by about 57.67% at 50 g/L and 56.0% at 75 g/L, while *Cakile maritima* expressed less effect as it inhibited shoot growth by about 46.67% at 75 g/L and 32.59% at 50 g/L. Generally, all extracts of the tested wild plants significantly reduced the shoot growth of *Echinochloa crus-galli* at concentration dependent manner.

Table 4. Effect of different plant extracts on the shoot growth inhibition percentage (mean value ± standard error) of the *Echinochloa crus-galli* after 14 DAT.

Plant species	Concentration (g/L)*			
	15	25	50	75
Cakile maritima	8.89[c] ±0.49	14.81[c] ±0.17	32.59[c] ±0.23	46.67[c] ±0.10
Calligonum polygonoides	12.91[b] ±0.29	36.78[a] ±0.18	50.97[b] ±0.03	67.10[b] ±0.10
Senecio glaucus	26.67[a] ±0.24	35.33[a] ±0.27	57.67[a] ±0.44	56.00[b] ±0.15
Zygophyllum album	15.38[b] ±0.33	21.53[b] ±0.21	52.30[b] ±0.07	84.61[a] ±0.17
$LSD_{0.05}$	3.17	3.57	4.82	9.91

*: mean value ± standard error. Different superscript letters indicate values significantly lower than the respective control ($P \leq 0.05$).

DISCUSSION

The choice of measurement parameter for the demonstration of allelopathy must be considered. In several bioassays, seed germination and seedling development is measured after the exposure to alleged allelochemicals because seed and seedlings development is generally considered to be the most susceptible stages (Inderjit and Olofsdotter 1998).

All extracts of the tested wild plants significantly reduced the germination of *Echinochloa crus-galli* at different concentrations (15, 25, 50, and 75 g/L). The inhibition was concentration dependent (Otusanya et al., 2007; Kayode and Ayeni, 2009)

In the present study, the extract of *Zygophyllum album* (75 g/L) was the most potent inhibitor of seed germination of *Echinochloa crus-galli* (81.59% inhibition), root growth (98.87% inhibition), and shoot growth (84.61% inhibition). The probable reason for its potent allelopathic activity may be due to the presence of many bioactive secondary metabolites in this plant, particularly phenolics (12.94 mg/g dry weight) and saponins (18.47 mg/g dry weight), as shown from the present results. In addition, previous investigation of *Z. album* revealed that, the plant contains Zygophylin, β- sitosterol- β-D- glucopyrano- side, tannin, lactones (Hassanean et al. 1993; Hani et al. 1995); phenolic and flavonoid contents, antioxidant (Debouba et al. 2012). Hence the allolopathic potential of *Zygophyllum album* could be attributed to these bioactive compounds. This is similar to the findings of Al-Malki (2006) in which the *Z. coccineum* reduce germination and growth of some crop plant (*Zea mays* and *Cucumis sativus*). Also, Nesrine et al. (2012) studied the allelopathic effect of *Z. album* control of *Bromus tectorum*. According to these results, the allelopathic potential of *Zygophyllum* species is agree with our investigation in present study.

Senecio glaucus extract at the same concentrations followed *Z. coccineum* in its allelopathic potential as it inhibited the seed germination of *Echinochloa crus-galli* (65.11% inhibition), root growth (94.24% inhibition) and shoot growth (56.0% inhibition). This result could be attributed to the presence of phenolics (23.52 mg/g dry weight), saponinns (22.42 mg/g dry weight), flavonoids (11.51 mg/g dry weight) and alkaloids (8.09 mg/g dry weight), as reveled by the phytochemical analysis in the present investigation. The leaves and flowers of *S. glaucus* contain many volatile oils (El-Shazly 2002; De Pooter et al. 2006). Also, *Senecio* spp. contains glycosides, steroids, terpenes and chalocones (El-Shazly 2002; Ajiboye et al. 2013; Aparna et al. 2013; Hussain et al. 2013). Hence the allelopathic potential of *S. glaucus* could be attributed to these bioactive compounds.

Qasem and Hill (1989) studied the possible role of allelopathy between tomato, *Senecio vulguris* and *Chenopodium album*. Allelopathic potential of vegetative and flowering ragwort (*S. Jocobaea* L.) against associated pasture species was studied by Ahmed and Wardle (1994). The results were in agreement with the effect of *S. glaucus* in the present investigation.

Calligonum polygonoides extract (at concentrations 75 g/L) inhibited seed germination of *Echinochloa crus-galli* by about 60.42%, while it inhibited the root and shoot growth by about 99.12% and 67.10%, respectively. The phytochemical analysis of *Calligonum polygonoides* in the present study showed that it rich in tannins (7.58 mg/g dry weight), saponins (17.33 mg/g dry weight), flavonoids (6.86 mg/g dry weight) and phenolics (8.14 mg/g dry weight). In addition, previous investigation of genus *Calligonum* revealed that the presence of new lipoxygenase (Yawer et al. 2007); flavonoids, alkaloids, proteins, tanins, steroids, phenols and terpenoids (Samejo et al. 2011); oil (Abdel et al. 2011) and essential oil (Samejo et al. 2013).

Most instances of allelopathic inhibition are the result of the simultaneous action of several compounds, and often these include compounds whose chemistry is divergent (Einhellig 2002). The visible evidence in bioassays is that many phenolics, quinones, sesquiterpene lactones, alkaloids and others alter root morphology. Although, the cell membrane is an early interface with allelochemicals, relatively little attention has been given to membrane-related effects and their molecular targets (Abd El-Gawad 2014). Hence the allelopathic potential of *C. polygonoides* could be attributed to these bioactive compounds. The allelopathic potential of *C. polygonoides* was investigated on germination and seedling growth of *Abelmoschus esculentus* and *Helianthus annus* (Sherwani et al. 2013). The result is in agreement with the effect of *C. polygonoides* in the present study.

On the other hand, the extract of *Cakile maritima* (75 g/L) was the potent inhibitor of seed germination of *Echinochloa crus-galli* (48.23% inhibition), root growth (92.67% inhibition) and shoot growth (46.67% inhibition). The phytochemical analysis of *Cakile maritima* in the present study showed that it rich in saponins (43.36 mg/g dry weight), tannins (7.58 mg/g dry weight), flavonoids (5.49 mg/g dry weight) and phenolic compounds (6.53 mg/g dry weight). Saponins may be phytotoxic through their effects on membrane lipids or by effects on specific enzymes (Galindo et al. 2004). *Cakile* spp. contains alkaloids, tannins, flavonoids, phenols (El-Halawany 1988); vitamin (Debez et al. 2004); aromatic source (Heneidy and Bidak 2004) and antioxidant (Ksouri et al. 2007). Hence these bioactive compounds were responsible for many biological activities of *Cakile maritima*.

Generally, allelochemicals inhibit seed germination by blocking hydrolysis of nutrients reserve and cell division (Irshad and Cheema 2004), and cause significant reductions in the growth of plumule and radical of various crops (Ogbe et al., 1994).

In conclusion, the wild plants (*C. maritima*, *C. polygonoides*, *S. glaucus* and *Z. album*) are of wide ecological amplitude; each attains a relatively high value of abundance in at least two habitat types in Deltaic Mediterranean coast of Egypt (El-Amier et al., 2014). The present results revealed that higher concentrations of methanol extracts of tested species could be used for control of *Echinochloa crus-galli*. Root is more sensitive to extract than those plumule, thus farmers are advised to treat the young seedlings of *E. crus-galli* in the field at first two weeks of germination. There is the need for further studies to be carried out on identifying the inhibiting allelochemicals in the tested species.

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