

Allelopathic activities of celery extract and its fractions against *Corchorus olitorius*, *Echinochloa crusgalli* and *Portulaca oleracea* weeds

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Abstract: Aqueous extract of celery (2.5-20 g l⁻¹, w/v) was evaluated for its phytotoxicity against three weed species, under laboratory and greenhouse conditions. Celery extract had a strong inhibitory effect on germination and seedling growth of *Corchorus olitorius*, *Echinochloa crusgalli* and *Portulaca oleracea* seeds. From dose response curves of tested seeds, LC₅₀ were calculated to be in the range from 6.3 to 8.3 g l⁻¹ for germination percent, from 7.2 to 8.0 g l⁻¹ for shoot length and from 1.7 to 3.6 g l⁻¹ for root length. Completely inhibition of root growth was exhibited *C. olitorius* and *P. oleracea* at 7.5 g l⁻¹ corresponded with 15 g l⁻¹ for *E. crusgalli* seed. Total phenolics in celery extract at 20 g l⁻¹ constituted 201 mg l⁻¹. Ten phenolic acids were identified in extract by HPLC, among of them *p*-coumaric acid and *p*-hydroxybenzoic acid were presented in high amounts. Aqueous extract was partitioning between three solvents, hexane, methylene chloride, ethyl acetate. Generally, water residue after partitioning aqueous extract with the three solvents had the most phytotoxic effect on seedling growth of target seeds. In greenhouse trial, foliar spray of aqueous extract of celery (30, 60 and 90 g l⁻¹) and its fractions did not produce any significant effect on growth of two-weeks-old *C. olitorius*, or *E. crusgalli* or *P. oleracea* weeds.

1. Introduction

Worldwide, weeds caused about 34% yield losses among the major crops. Herbicide application is the most reliable weed control methods. Negative impacts of herbicides on environmental human health and herbicide resistant weeds were considered the two problems faced in weed management with herbicides (Jabran *et al.*, 2015). Thus, manipulating the allelopathy can help to improve weed control in agriculture and increase the acceptance of agricultural products in today's demanding consumer markets (Trezzi *et al.*, 2016).

Allelopathy has been used as the basis for identifying plant species which may contain phytotoxic chemicals. These natural compounds can

offer excellent potential for new herbicidal solutions, or lead compounds for new natural herbicides (Duke *et al.*, 2000; Vyvyan, 2002). The main purposes of research on allelopathy include the application of the allelopathic effects to agricultural production, reduction of the input of chemical pesticides and consequent environmental pollution, and provision of effective methods for the sustainable development of agricultural production and ecological systems (Han *et al.*, 2013; Jabran *et al.*, 2015). Knowledge concerning allelopathy can also be a key component in supporting organic farming, for which weed control is a major problem (Trezzi *et al.*, 2016) Organic farming can involve reduced weed infestation by using plant extracts or intercropping plant species with an allelopathic potential (Bajwa *et al.*, 2015). Plant extracts are the way of using allelochemicals for weed control in agroecosystems, as they have been already used as post-emergence natural herbicides in some countries. In Pakistan, for example, an aqueous extract deriving from sorghum shoots with a 10% concentration was left to ferment for several weeks and was subsequently sprayed post emergence for weed control. This fermented water extract, known as "Sorgaab", reduced weed density and weed dry weight up to 50% in field trials (Cheema and Khaliq, 2000; Cheema *et al.*, 2002).

Celery (*Apium graveolens* L.; Apiaceae) has been cultivated for the last 3000 years, notably in Egypt, and was known in China in the fifth century BC. It has been used as a popular aromatic herb and spice (Chevallier, 1998). Earlier studies of *A. graveolens* led to isolation of some phenolic compounds as phthalides (Tang *et al.*, 1990; Momin and Nair, 2001) and furocoumarins (Garg *et al.*, 1979). These compounds are reported for their insecticidal, nematocidal, antifungal and phytotoxic activities (Kato *et al.*, 1977; Momin and Nair, 2001; Pavela and Vrchotová, 2013). Recently, Sbai *et al.* (2017) reported that the aqueous extract (10-50 g/L) of *A. graveolens* had great inhibitory effect on root growth of germinated seeds of lettuce (in the range between 80% and 90%). They isolated six compounds which included three phthalides [senkyunolide A, (3S)-butylphthalide and sedanolide], two furanocoumarins (bergapten and scopoletin) and one phenyl propanoid (p-hydroxyphenethyltrans ferulate). Senkyunolide A compound was the most toxic on lettuce germination and shoot growth, however, p-hydroxyphenethyl trans-ferulate was the most toxic on root growth.

However, in spite of the wide range of biological

activity of celery extract, but information concerning the herbicidal activity of this extract is rarely available. Therefore, the main objective of this study was to evaluate the herbicidal activity of the aqueous celery extract and its fractions against *Corchorus olitorius*, *Echinochloa crusgalli* and *Portulaca oleracea* weeds, with the goal of developing an effective plant derived herbicide. Moreover, the phenolic acids that considered the main source of all bioactive phenolic substances were identified in celery extract via HPLC.

2. Materials and Methods

Plant material

Plants of celery (*A. graveolens* L. var. dulce) were purchased from a local market in Cairo, Egypt. Identification of celery based on morphological traits that extensive observation of mature plants. The leaves located at the top of the leaf stalks were collected and dried in hot-air oven at 50°C for 72 h, powdered and used for extraction.

Preparation of aqueous extract

Different concentrations (w/v) of extracts were prepared by soaking known weight of dried leaves in known volume from distilled water at room temperature, and shaken for 24 h. The extracts were filtered through a Whatman No. 1 filter paper and kept at 4°C in the dark until use.

Germination bioassay

Mature seeds of *Corchorus olitorius*, *Echinochloa crusgalli* and *Portulaca oleracea* were collected from plants growing in fields of the experimental station of the National Research Centre (Research and Production Station, Nubaria region, Behaira Governorate, Egypt). Uniform healthy seeds were selected. Seeds were surface-sterilized with sodium hypochlorite (0.1%, w/v) for 2 min, washed under running tap water for 5 min followed by distilled water for 2 min, and stored for further use. Twenty seeds were placed in a 9-cm plastic Petri dish lined with a single Whatman No. 1 filter paper, and then 4 mL of each extract concentration (2.5, 5, 7.5, 10, 12.5, 15, 17.5 and 20 g l⁻¹) was added onto filter paper. Distilled water (4 mL) was applied to a Petri dish to serve as a control. Four replicates of Petri dishes of each treatment were placed, in a completely randomized manner, in a growth chamber at 25°C. After 5 days, germination percent, shoot length and root length of germinated seeds were determined.

Extract concentrations required to cause 50% inhibition of different germination criteria (germination percentage, root length and shoot length) were calculated by Probit analysis.

Determination of total phenolics in aqueous extract

Extract of celery at 20 g l⁻¹ was subjected to determine total contents of phenolics using Folin-Ciocalteu reagent (Singleton and Rossi, 1965).

Analysis of phenolic acids in aqueous extract by High-Performance Liquid Chromatography (HPLC)

Phenolic acids in aqueous celery extract at a concentration 20 g l⁻¹ were hydrolyzed with sodium hydroxide (McKeehen *et al.*, 1999) and subjected to HPLC analysis. Approximately 15 ml of aqueous extract was added to 15 ml of 8 N NaOH in a 50 ml Pyrex centrifuge tube, purged with nitrogen, shaken for 2 h in the dark with a shaker and acidified with ice-cold 6 N HCl to reduce pH to 2. Sample was centrifuged at 3000g, and the supernatant was decanted into separatory funnel. The supernatant was extracted with ethyl acetate (3 x 50 ml) with shaking for 10 s, and the mixture was allowed to settle for 5 min between extractions. The phenolic acids rich ethyl acetate fraction was dried by addition of anhydrous sodium sulfate and concentrated using rotary evaporator at 40°C to dryness. The residue was re-solubilized in 2.5 ml of methanol and filtered through a 0.2 µm PTFE filter prior to analysis. HPLC analysis was performed using equipment from Shimadzu (Japan): a Shimadzu LC-2010A liquid chromatograph, a Shimadzu SPDM10A Diode Array Detector and a Shimadzu Class-vp V6.12 SP4 offline processing system. Phenolics were analyzed using a Luna RP-C18 (2) column (250×4.6 mm i.d, 5 µm, Phenomenx). The mobile phase consisted of a mixture of acetate buffer: acetonitrile (9:1, v/v). Acetate buffer was prepared by dissolving 6.35 g sodium acetate in one-liter H₂O and 20 ml acetic acid. The detecting wavelength was 260 nm. Standard phenolic acids (gallic, protocatechuic, *p*-hydroxybenzoic, syringic, ferulic and *p*-coumaric) were purchased from Sigma Aldrich and vanillic, caffeic, salicylic and cinnamic were purchased from Fluka.

Greenhouse bioassay

Another experiment was performed to study the effect of the aqueous celery extract on the growth of two-weeks-old *C. olitorius*, *E. crusgalli* and *P. oleracea* weeds grown under controlled conditions in an

experimental greenhouse (25±3°C, 12 h photoperiod). Aqueous extracts at four concentrations (0, 30, 60 and 90 g l⁻¹) were prepared as previously mentioned and used in a greenhouse bioassay. Plants were raised from the collected seeds in 12-cm diameter earthenware pots. The pots were filled with 750 g soil (sand:peat moss = 3:1, w/w) and 20 seeds of each plant species were sown per pot. Two weeks after emergence, plants were sprayed with plant extracts at 0, 30, 60 and 90 g l⁻¹ concentrations. Extract solutions were applied to shoots of tested weeds using a Epoca sprayer (Italy). The solution was sprayed evenly over the entire surface of the plant, including the adaxial and abaxial surface of leaves. A total of 48 pots were maintained (i.e. 4 extract concentrations × 3 weeds × 4 replicates) in a completely randomized design. Seven days after extract spraying, the plants were examined for visible injury levels and the percent of chlorotic and necrotic areas were recorded.

Fractionation of extract

One hundred milliliter of high extract concentration that used either in germination trial (20 g l⁻¹, w/v) or in greenhouse trial (90 g l⁻¹, w/v) was subsequently partitioned with organic solvents with increasing polarity. The extract was partitioned three times with 200 mL aliquots of each solvent: hexane, methylene chloride (MeCl₂) and ethyl acetate (EtOAc) using separatory funnel. Solvents were dried by addition of anhydrous sodium sulfate, filtered and evaporated using Buchi Rotary Evaporator at 40°C to dryness. The residues of hexane, MeCl₂ and EtOAc fractions were dissolved in 100 mL of DMSO-water solution (0.1%, v/v). These three fractions and water residue (H₂O fraction) were subjected to bioassay as previously mentioned.

Statistical analysis

All data was subjected to ANOVA analysis using costat software to evaluate the effect of extract rates upon germination and growth parameters of tested weeds. After ANOVA, the parameters that were statistically significant (*A. graveolens* extracts with P≤0.05), were subjected to Pro-bit analysis using LdP line. Pro-bit curves were derived by plotting the extract concentration (on the x-axis) and inhibition % (on the y-axis). With the curves obtained, LC₅₀ value was calculated for each parameter. This value represents the extract concentration at which 50% inhibi-

tion in different germination parameter occurs.

3. Results

Effect of celery extract on germination percentage of seeds

As shown in Table 1, aqueous extract of celery had a strong inhibitory effect on germination percentage of the three tested seeds and the inhibition increased with concentration. Increasing extract concentration above 5 g l⁻¹ produced gradual decrease in germination percentage of *C. olitorius*. The highest reduction effect was produced by extract at highest concentrations, 17.5 and 20 g l⁻¹ (89% and 93%, respectively). Also, germination percentage of *E. crusgalli* and *P. oleracea* was reduced by the extract at all tested concentrations. The reduction percent-

Table 1 - Effect of aqueous celery extract on germination percentage of three weed species

Concentration (g l ⁻¹)	Germination %		
	<i>C. olitorius</i>	<i>E. crusgalli</i>	<i>P. oleracea</i>
Control	100±0 a	100±0 a	100±0 a
2.5	95±5 a	63±7 b	91±9 b
5	93±2 a	67±4 b	84±4 b
7.5	49±5 b	52±2 c	73±3 c
10	41±4 c	43±5 c	74±7 c
12.5	23±3 d	39±7 c	60±2 d
15	11±5 e	25±4 d	40±2 e
17.5	11±5 e	14±5 d	37±2 e
20	7±2 e	4±1 e	27±3 f

Values are given as means of three replicates ± standard error. Means with the same letters in a column are not significantly different at P<0.05.

age varied from 37% and 9%, respectively at the lowest concentration to 96% and 73%, respectively at the highest ones.

Effect of celery extract on Seedling growth of seeds

Aqueous extract of celery exhibited a great inhibitory effect on shoot length of the three germinated seeds (Table 2). The extract improved the shoot growth of *E. crusgalli* and *P. oleracea* at the lowest concentrations. The progressive increase in extract concentration followed by progressive reduction in shoot length of all tested seeds. More than 90% reduction in shoot length of three germinated seeds was obtained by soaking seeds in celery extract at 17.5 and 20 g l⁻¹.

An inhibition of root growth was observed in presence of all extract concentrations and roots more sensitive than shoots (Table 2). Root length of three target seeds varied in their response to different extract concentrations and *E. crusgalli* was less effective one. Soaking seeds in celery extract at the lowest concentration produced great reduction in root elongation of all treated seeds ranged between 36% and 71%, relative to control. Using extract at 7.5 g l⁻¹ and above completely inhibited root growth of both *P. oleracea* and *C. olitorius*, whereas complete inhibition of *E. crusgalli* roots was obtained at 15 g l⁻¹.

Extract concentrations required to cause 50% inhibition (LC50)

Inhibition percent and levels of LC₅₀ of all germination parameters of tested weeds were calculated and dose-response curves were illustrated in figure 1. The three tested weeds varied in their inhibition percentages as affected with celery extract. Among tested seeds, germination percent of *P. oleracea* was con-

Table 2 - Effect of aqueous celery extract on shoot and root lengths of three weed species

Rate (g l ⁻¹)	Shoot length (cm)			Root length (cm)		
	<i>C. olitorius</i>	<i>E. crusgalli</i>	<i>P. oleracea</i>	<i>C. olitorius</i>	<i>E. crusgalli</i>	<i>P. oleracea</i>
Control	2.56±0.14 a	3.35±0.35 a	2.17±0.13 b	2.41±0.08 a	1.83 ±0.26 a	1.95±0.23 a
2.5	2.74±0.25 a	2.91±0.34 b	2.50±0.23 a	0.81±0.17 b	1.17 ±0.12 b	0.57±0.07 b
5	2.21±0.19 b	2.43±0.19 c	2.19±0.60 b	0.51±0.11 c	0.65 ±0.20 c	0.29±0.06 c
7.5	1.38±0.40 c	1.73±0.33 d	0.58±0.40 c	0.00 d	0.51 ±0.17 c	0.00 d
10	0.76±0.28 d	0.79±0.20 e	0.60±0.06 c	0.00 d	0.25 ±0.06 d	0.00 d
12.5	0.36±0.06 e	0.77±0.20 e	0.56±0.31 c	0.00 d	0.22 ±0.01 d	0.00 d
15	0.10±0.02 e	0.53±0.31 ef	0.15±0.08 d	0.00 d	0.00 e	0.00 d
17.5	0.05±0.03 e	0.27±0.14 ef	0.14±0.14 d	0.00 d	0.00 e	0.00 d
20	0.00 f	0.06±0.03 f	0.11±0.06 d	0.00 d	0.00 e	0.00 d

Values are given as means of three replicates ± standard error. Means with the same letters in a column are not significantly different at P<0.05.

sidered a less sensitive to celery extract. Since, value of LC_{50} of *P. oleracea* constituted 13.4 g l^{-1} corresponded with 8.3 and 6.3 g l^{-1} for *C. olerarius* and *E. crusgalli*, respectively. Whereas, inhibition percent of shoot length did not produce a great variation among tested seeds and LC_{50} calculated to be 7.8 , 7.2 and 8 g l^{-1} for *C. olerarius*, *E. crusgalli* and *P. oleracea* seeds, respectively. As shown in figure 1, root length exhibited the maximum inhibition effect as affected by celery extract when compared with germination percent and shoot length. Moreover, root length of test-

ed seeds varied in their inhibition percent as affected with celery extract. Depending on LC_{50} levels, root length of *P. oleracea* (1.65 g l^{-1}) was considered the most sensitive, followed by *C. olerarius* (1.8 g l^{-1}) and *E. crusgalli* (3.6 g l^{-1}).

Total phenolics and phenolic acids content in aqueous extract

In this study, we determine total phenolics for celery extract at a concentration 20 g l^{-1} and found that the extract contained high amounts of phenolics con-

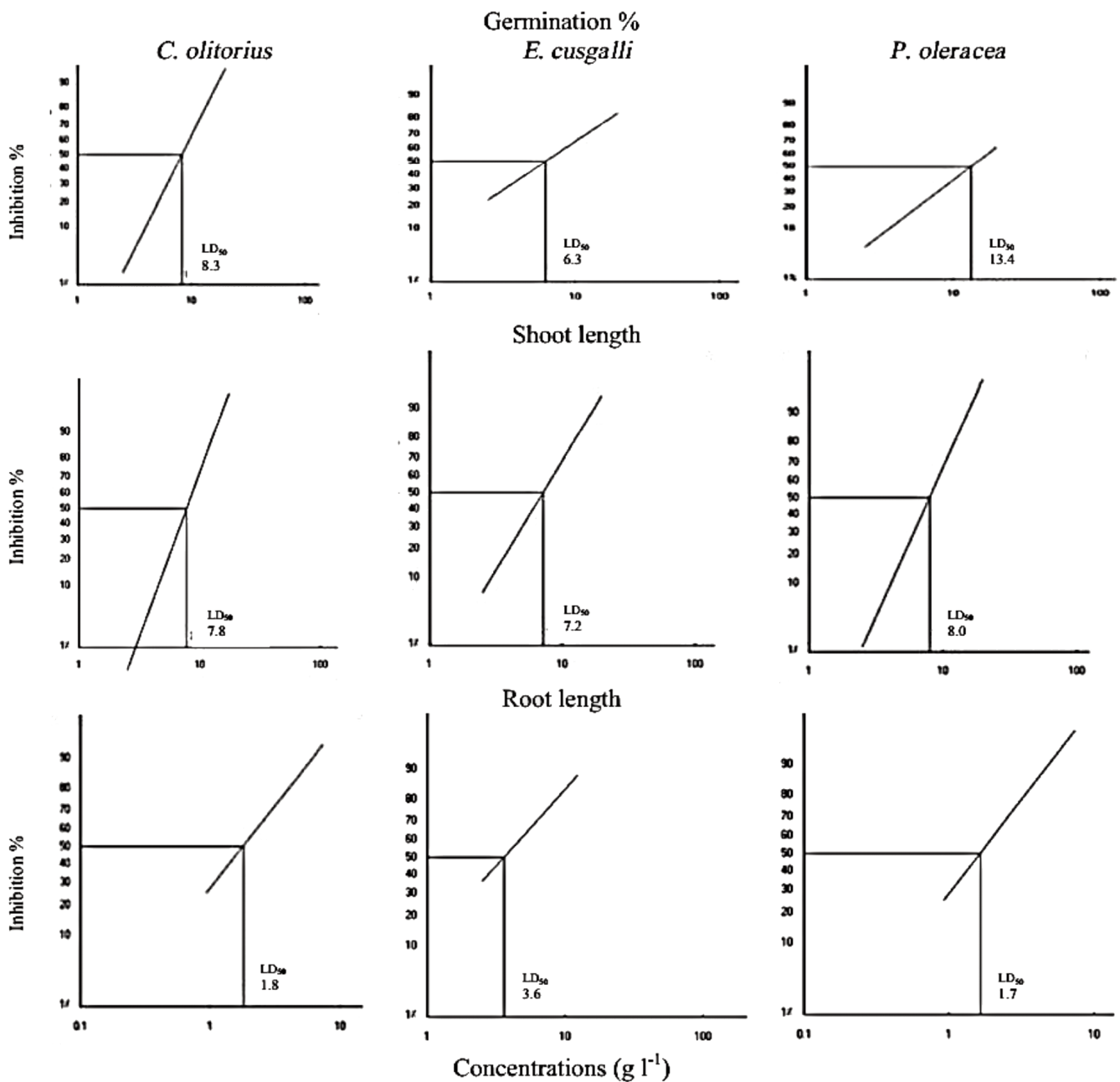


Fig. 1 - Dose-response curves showing the effect of celery extract (inhibition % and LC_{50}) on percent germination and seedling growth of three tested seeds.

stituted 201 mg l⁻¹, as gallic acid. Using HPLC, ten phenolic acids were detected in this aqueous extract, of which six compounds consisted of benzoic acid derivatives and four of cinnamic acid derivatives (Table 3). Among the identified phenolic acids, *p*-hydroxybenzoic acid, *p*-coumaric acid and ferulic acid were presented in high amounts (18.72, 30.32 and 11.12 mg l⁻¹, respectively). Except the minor quantity of cinnamic acid, moderate concentrations from other phenolic acids were determined in celery sample.

Table 3 - Phenolic acids in aqueous extract of celery at concentration 20 g l⁻¹

Acid derivatives	Concentration (g l ⁻¹)
Benzoic acid derivatives	
<i>p</i> -Hydroxybenzoic acid	18.72
Gallic acid	8.56
Vanillic acid	8.8
Syringic acid	6.4
Protocatechuic acid	9.28
Salicylic acid	7.84
Cinnamic acid derivatives	
Cinnamic acid	0.64
Caffeic acid	9.52
<i>p</i> -Coumaric acid	3.032
Ferulic acid	11.12

Effect of fractions on germination of weeds

As shown in Table 4, fractions obtained from the aqueous extract of celery varied in their effects on germination criteria of target seeds. As for germination percentage, the significant reduction effect was recorded only for MeCl₂ fraction on *P. oleracea* (15%) as well as for H₂O residue on both *C. olitorius* (30%) and *P. oleracea* (36%). But, all examined fractions did not produce any significant effect on germination

percentage of *E. crusgalli* seeds. Whereas, H₂O fraction induced a great reduction on shoot elongation of *C. olitorius*, *E. crusgalli* and *P. oleracea* seeds, reached 64%, 47% and 74%, respectively relative to control. With less extent, hexane and MeCl₂ fractions reduced shoot length of *E. crusgalli*. With few exceptions, all fractions reduced root elongation of target seeds. Water fraction was the most toxic, inducing an inhibition of 99%, 94% and 50% in root length of *P. oleracea*, *C. olitorius* and *E. crusgalli*, respectively. Whereas, the other fractions, hexane, MeCl₂ and EtOAc fractions reduced root length of *E. crusgalli* and *P. oleracea* between 17% and 43%, relative to control.

Effect of celery extract and its fraction on growth of weeds

Data presented Table 5 revealed that spraying aqueous celery extract and its fractions did not produce any significant effect on growth of two-weeks old of either *C. olitorius*, or *E. crusgalli* or *P. oleracea* weeds.

Table 5 - Effect of aqueous celery extract and its fractions on the growth of two-weeks-old of three weeds

Treatments	Injuries (% of control)		
	<i>C. olitorius</i>	<i>E. crusgalli</i>	<i>P. oleracea</i>
30 g l ⁻¹	2±0 a	0±0 a	1±0 a
60 g l ⁻¹	3±1 a	2±5 a	3±2 a
90 g l ⁻¹	3±2 a	3±1 a	3±1 a
Hexane fraction	2±1 a	3±2 a	4±2 a
MeCl ₂ fraction	4±2 a	1±0 a	1±0 a
EtOAc fraction	3±1 a	2±1 a	3±1 a
H ₂ O residue	4±1 a	1±0 a	1±0 a

Values are given as means of three replicates± standard error. Means with the same letters in a column are not significantly different at P<0.05.

Table 4 - Effect of fractions obtained from aqueous extract of celery on germination of three weeds (% of control)

Criteria	Weed species	Fractions				
		Control	Hexane	MeCl ₂	EtOAc	H ₂ O
Germination (%)	<i>C. olitorius</i>	100 a	104 a	96 a	102 a	70 b
	<i>E. crusgalli</i>	100 a	98 a	104 a	102 a	95 a
	<i>P. oleracea</i>	100 a	107 a	85 b	109 a	64 c
Shoot length	<i>C. olitorius</i>	100 a	97 a	102 a	102 a	36 b
	<i>E. crusgalli</i>	100 a	86 b	83 b	95 a	53 c
	<i>P. oleracea</i>	100 a	95 a	100 a	87 a	26 b
Root length	<i>C. olitorius</i>	100 a	101 a	74 b	103 a	6 c
	<i>E. crusgalli</i>	100 a	57 c	83 b	79 b	50 c
	<i>P. oleracea</i>	100 a	61 b	76 b	63 b	1 c

Means with the same letters in a raw are not significantly different at P<0.05.

4. Discussions and Conclusions

Aqueous extract of celery was evaluated for its phytotoxicity against three plant species typically present as weeds in summer crops, under laboratory and greenhouse conditions. The extract displayed a great inhibition on germination percentage and seedling growth of target seeds. In line of these results, Sbai *et al.* (2017) reported that lettuce germination was completely inhibited by celery extract at concentration above 20 g l⁻¹. They extracted and identified the allelochemicals compounds that responsible of toxicity namely phthalides, among of them senkyunolide A was the most toxic in lettuce germination. Current study revealed that the inhibitory effect of celery extract on germination percent varied between tested seeds, and *P. oleracea* possessed the least sensitivity. Variation between different plant species in their sensitivity to plant extracts was previously observed by many investigators (Al-Humid and El-Mergawi, 2014; Han *et al.*, 2008). This study showed that root growth was the most sensitive to extract than shoot growth. Since, LC₅₀ of shoot length of three target seeds ranged between 7.2 g l⁻¹ and 8 g l⁻¹ whereas, values of LC₅₀ for root growth are calculated to be in the range of 1.7 g l⁻¹ and 3.6 g l⁻¹. These results are in agreement with Sbai *et al.* (2017), who reported that celery extract had more pronounced effects on roots of lettuce, rather than shoots. Generally, in germination bioassay, water extract of allelopathic plants have more pronounced effect on root rather than shoot growth (Inderjit and Dakshini, 1995; Muhammad *et al.*, 2011). This may be attributed to the fact that roots are the first to absorb the phytotoxic compounds (Turk and Tawaha, 2002).

In order to identify and distribute the chemical groups of toxic allelochemical constituents, celery aqueous extract was partitioning between three organic solvents varied in their polarities. Generally, the most phytotoxic compounds were represented water residue (H₂O fraction). It can be suggested that the high toxicity compounds in celery extract may be related to presence of more polar compounds. These results are in general agreement with the results obtained by Sbai *et al.* (2017). Who observed that extraction of celery with non-polar solvents, petroleum ether or chloroform had no significant effect on lettuce germination, contrarily to the high toxicity effect of methanol extract (polar solvent). The high toxicity of celery extracts may be attributed to the present water soluble compounds as saponins, glyco-

sides, hormones or enzyme which could affect growth directly or by altering the mobilization of storage compounds during germination (Chaves and Escudero, 1997; El-Khatib, 1997).

For the greatest inhibition effect of aqueous celery extract, we analyzed phenolics and phenolic acids in the extract at a concentration 20 g l⁻¹. The results indicated the presence of high amount of phenolic compounds (201 mg l⁻¹ as gallic acid) may explain the greatest effect of celery extract. In line of these results, Jung *et al.* (2011) found high levels of phenolic in celery (51.09 mg g⁻¹ dw, as gallic acid). Phenolic acids are the precursor of all phenolic constituents in plants as well as the bioactive constituents in celery extract. Identification and determination the concentration of the phenolic acids in the aqueous extract was conducted by using HPLC. Ten phenolic acids were identified in celery extract, among of them *p*-hydroxybenzoic acid, *p*-coumaric acid and ferulic acid were presented in relatively high amounts. In line of these results, Yang *et al.* (2010) identified caffeic acid, *p*-coumaric acid, and ferulic acid in celery extract. Presence of high concentration of total phenolic and phenolic acids in areal parts of celery was previously reported by many investigators (Yang *et al.*, 2010; Sbai *et al.*, 2017).

In greenhouse trial, we evaluated the effects of foliar spray of aqueous extract of celery and its fractions, hexane, MeCl₂, EtOAc and H₂O-residue on growth of two-weeks-old *C. olitorius*, or *E. crusgalli* or *P. oleracea* weeds. Celery extract at 30, 60 and 90 g l⁻¹ as well as the obtained fractions did not produce any significant effect on growth of three examined weeds. In general, the growth of two-weeks-old weeds tended to be less sensitive to the test fractions than the weed germination process. These results are in agreement of those obtained by Inderjit and Weston (2000), they found that greenhouse bioassays do not adequately predict the responses observed in laboratory bioassay. Hence, weed germination might be the most sensitive index with which to judge allelopathy of plant extracts and its fractions under natural conditions (Corrêa *et al.*, 2008).

In conclusion, in this study aqueous extract of celery was evaluated for its phytotoxicity against three weed species under laboratory and greenhouse conditions. The extract displayed a great inhibition on germination percentage and seedling growth of target seeds. Root growth of *C. olitorius* and *P. oleracea* was completely inhibited by extract at 7.5 g l⁻¹, corresponded with 15 g l⁻¹ for *E. crusgalli*. Celery aqueous extract was fractionated by using three less polar sol-

vents; however, water extract displayed the strongest inhibition effect on germination of three target seeds. Celery extract had a relatively high amount of phenolics and phenolic acids. High concentrations of water celery extract (30, 60, 90 g l⁻¹) and the obtained less polar fractions did not produce any significant effect on growth of two weeks-old of tested weeds. Water extract of celery may be a useful source for the future development of pre-emergence bio-herbicide.

References

- AL-HUMAIM A., EL-MERGAWI R., 2014 - *Herbicidal activities of seven native plants on the germination and growth of Phalaris minor, Echinochloa crusgalli, Portulaca oleracea and Lactuca sativa*. - J. Agric. Sci. Tech. A, 4: 843-852.
- BAJWA A.A., MAHAJAN G., CHAUHAN B.S., 2015 - *Nonconventional weed management strategies for modern agriculture*. - Weed Sci., 63(4): 723-747.
- CHAVES N., ESCUDERO J.C., 1997- *Allelopathic effect of Cistus ladanifer on seed germination*. - Functional Ecology, 11: 432-440.
- CHEEMA Z.A., KHALIQ A., 2000 - *Use of sorghum allelopathic properties to control weeds in irrigated wheat in a semi-arid region of Punjab*. - Agric. Ecosyst. Environ., 79: 105-112.
- CHEEMA Z.A., KHALIQ A., ALI K., 2002 - *Efficacy of sorghum for weed control in wheat grown at different fertility levels*. - Pak. J. Weed Sci. Res., 8: 33-38.
- CHEVALLIER A., 1998 - *The encyclopedia of medicinal plants*. - DK Publishing Inc., New York, USA, pp. 61.
- CORRÊA L.R., SOARES G.L.G., FETT-NETO A.G., 2008 - *Allelopathic potential of Psychotria leiocarpa, a dominant understory species of subtropical forests*. - South African J. Bot., 74(4): 583-590.
- DUKE S.O., DAYAN F.E., ROMAGNI J.G., RIMANDO A.M., 2000 - *Natural products as sources of herbicides: current status and future trends*. - Weed Res., 40(1): 99-111.
- EL-KHATIB A.A., 1997 - *Does allelopathy involve in the association pattern of Trifolium resupinatum*. - Biologia Plantarum, 40: 425-431.
- GARG S.K., GUPTA S.R., SHARMA, N.D., 1979 - *Coumarins from Apium graveolens seed*. - Phytochemistry, 18: 1580-1581.
- HAN C., PAN K., WU N., WANG J., LI W., 2008 - *Allelopathic effect of ginger on seed germination and seedling growth of soybean and chive*. - Scientia Hort., 116(3): 330-336.
- HAN X., CHENG Z.H., MENG H.W., YANG X.L., AHMAD I., 2013 - *Allelopathic effect of decomposed garlic (Allium Sativum L.) stalk on lettuce (Lactuca sativa Var. Crispa L.)*. - Pak. J. Bot., 45: 225-233.
- INDERJIT M., DAKSHINI K.M., 1995 - *Allelopathic potential of an annual weed, Polygonum sp. peliense, in crops in India*. - Plant and Soil, 173: 251-257.
- INDERJIT M., WESTON L.A., 2000 - *Are laboratory bioassay for allelopathy suitable for prediction of field response*. - J. Chem. Ecol., 26: 2111-2118.
- JABRAN K., MAHAJAN G., SARDANA V., CHAUHAN B. S., 2015 - *Allelopathy for weed control in agricultural systems*. - Crop Prot., 72: 57-65.
- JUNG W.S., CHUNG I.M., KIM S.H., KIM M.Y., AHMAD A., PRAVEEN N., 2011 - *In vitro antioxidant activity, total phenolics and flavonoids from celery (Apium graveolens) leaves*. - J. Medicinal Plants, 5(32): 7022-7030.
- KATO T., KOBAYASHI M., SASAKI N., KITAHARA Y., TAKAHASHI N., 1977 - *The coumarin heraclenol as a growth inhibitor in parsley seeds*. - Phytochemistry, 17: 158-159.
- McKEEHEEN J.D., BUSCH R.H., FULCHER R.G., 1999 - *Evaluation of wheat (Triticum aestivum L.) phenolic acids during grain development and their contribution to fusarium resistance*. - J. Agric. Food Chem., 47(4): 1476-1482.
- MOMIN R.A., NAIR M.G., 2001 - *Mosquitocidal, nematocidal and antifungal compounds from Apium graveolens L. seeds*. - J. Agric. Food Chem., 49: 142-145.
- MUHAMMAD A.K., UMM K., MUHAMMAD I.K., RAHAMDAD K., SHER A.K., 2011 - *Screening the allelopathic potential of various weeds*. - Pakistan J. Weed Sci. Res., 17: 73-81.
- PAVELA R., VRCHOTOVÁ N., 2013 - *Insecticidal effect of furanocoumarins from fruits of Angelica archangelica L. against larvae Spodoptera littoralis Boisd.* - Indus. Crops Products, 43: 33-39.
- SBAI H., ZRIBI I., DELLAGRECA M., HAOUALA R., 2017 - *Bio-guided fractionation and isolation of phytotoxic compounds from Apium graveolens L. aerial parts (Apiaceae)*. - South African J. Botany, 108: 423-430.
- SINGLETON V.L., ROSSI J.A., 1965 - *Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents*. - Amer. J. Enol. Vitic., 16: 144-158.
- TANG J., ZHANG Y., HARTMAN T.G., ROSEN R.T., HO C.T., 1990 - *Free and glycosidically bound volatile compounds in fresh celery (Apium graveolens L.)*. - J. Agric. Food Chem., 38: 1937-1940.
- TREZZI M.M., VIDAL R.A., BALBINOT J.R., VON HERTWIG BITTENCOURT H., DA SILVA SOUZA FILHO A.P., 2016 - *Allelopathy: Driving mechanisms governing its activity in agriculture*. - J. Plant Interactions, 11(1): 53-60.
- TURK M.A., TAWAHA A.M., 2002 - *Inhibitory effects of aqueous extracts of black mustard on germination and growth of lentil*. - Pakistan J. Agron., 1(1): 28-30.
- VYVYAN J.R., 2002 - *Allelochemicals as leads for new herbicides and agrochemicals*. - Tetrahedron, 58: 1631-1646.
- YANG Y., SANG W., ZHOU M., REN G., 2010 - *Phenolic composition and antioxidant activities of 11 celery cultivars*. - J. Food Sci., 75(1): 9-13.