



## Comparative Allelopathic Potential of Ten Field Weeds against Seed Germination of three Economic Plants

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**ABSTRACT:** The present study was carried out to evaluate the allelopathic potential of ten common weeds against three crop plants. All weeds extracts, even those more diluted, completely prevented seeds of *Eruca sativa* from germination. The high extract strength of *Ammi majus* and *Desmostachya bipinnata* prevented seeds of *Triticum aestivum* and *Vicia faba* from germination. The germination percentage, seed vigor index, coefficient of velocity and seedling length of *T. aestivum* and *V. faba* differentially inhibited by the extracts of weeds. The rate of elongation of hypocotyl and epicotyl of *T. aestivum* inhibited by all weeds, while the low extract strength of six weeds stimulated the rate of elongation in *V. faba* sprouts. All estimated germination and elongation parameters of receiving plants negatively correlated by total phenolics, flavonoids and alkaloids in donor weeds. Terpenoids were less influence and weakly correlated with germination parameters, so it suggested to be stimulatory. The magnitude of allelopathic effect, inhibitor or stimulator, was primarily depends on the donor plant and its content of secondary metabolites and secondarily on the target species as indicated by  $\eta^2$ . The weeds exerting negative allelopathy can be categorized into competitive weeds which inhibit cell division and elongation or phytotoxic weeds that germination-preventing.

**Keywords:** Allelopathy, Elongation rate, *Eruca sativa*, Germination, Phytochemicals, *Triticum aestivum*, *Vicia faba*, Weeds.

### INTRODUCTION

Allelopathy is defined as the direct or indirect inhibitory or stimulatory effects of one plant on another plant through the production of bioactive chemical compounds called allelochemicals or allelopathins, which escape or released into the environment (Saxena *et al.*, 2016). This phenomenon includes interference between weed-weed, weed-crop or crop-crop (Batish *et al.*, 2007, Chon *et al.*, 2003, Lehoczy *et al.*, 2011). Release of allelochemicals is held as a major factor in regulating the structure of plant communities in both natural and agroecosystem (Gawronska and Golisz, 2006, Smith and Martin, 1994). These chemicals are mostly secondary metabolites of plants such as phenolic compounds, flavonoids, alkaloids and terpenes that present in all parts of different plants and released into the environment by different mechanisms including volatilization, root exudation and decomposition of residues. As reached to neighbors, the allelochemicals affect their growth, behavior and reproduction and hence on composition of plant communities and agricultural development (Ding *et al.*, 2016, Geimadil *et al.*, 2015, Usuah *et al.*, 2013). The overall allelopathic effect, either stimulatory or inhibitory,

depends upon the concentration and types of secondary metabolites produced (Bhowmik and Inderjit, 2003).

Allelopathy is important resource for weed management in agricultural grounds that could give perfect possibilities for environmentally healthy, integrated crop production (Cheng and Cheng, 2015, Ghafarbi *et al.*, 2012). However, selective allelochemicals from extracts of allelopathic plants can be used as bioherbicide because they are easy to decompose (biodegradable) and safer than synthetic herbicide and very useful for the environment (He *et al.*, 2012, Islam, 2016). Seed germination is the most sensitive process to bioactive chemical compounds, so seed emergence has been preferred in allelopathic studies (Aliotta *et al.*, 2006, Bhadoria, 2011, Naseem *et al.*, 2009). The weed is any plant that is objectionable or interferes with the activities of humans and cause invisible damage until the crop is harvested qualitatively and quantitatively (Ankita and Chabbi, 2012, Booth *et al.*, 2003).

The lack of crop yields due to weeds is huge (Appleby *et al.*, 2000). Therefore, the phenomenon of allelopathy could be one of the possible alternatives for achieving sustainable weed management (Abu-Romman *et al.*, 2010, Salhi *et al.*, 2011, Salhi *et al.*, 2012).

Currently, there is a trend towards the searching for new natural plant products to discover and develop new bioherbicides friendly to the environment (Algendaby and Salama, 2016). The seed is a sensitive plant organ to such bioactive chemical compounds that affects its hypocotyl and epicotyl emergence (Bhadoria, 2011, Naseem *et al.*, 2009). The allelopathic effects on seed germination are related to the types and concentrations of allelochemicals, species of recipient plants and environmental conditions (Yang *et al.*, 2005). However, still some questions unresolved: 1- Are all economic plants equally affected by an allelopathic donor species? 2- Regardless mineral utilization, are all agroecosystem weeds harmful to crops? 3- Are there some weeds that benefit the crop plant at any stage of its life cycle, and what about using such weeds in accelerating germination or enhancement growth or crop status at a definite stage? The main objective of the present study was to evaluate the allelopathic effects of extracts of ten common weeds on the germination stage of three crop plants.

## MATERIALS AND METHODS

### A. Collection and preparation of plant materials

Aerial shoots of weeds [four herbs: *Ammi majus* L. (Apiaceae), *Oxalis corniculata* L. (Oxalidaceae), *Plantago lagopus* L. (Plantaginaceae) and *Urtica urens* L. (Urticaceae); and six grasses (Poaceae): *Cynodon dactylon* (L.) Pers., *Desmostachya bipinnata* (L.) Stapf., *Dichanthium annulatum* (Forssk.) Stapf., *Echinochloa colona* (L.) Link, *Phragmites australis* (Cav.) Trin. ex. Steud. and *Sorghum virgatum* (Hack.) Stapf.] as donor plants were collected from cultivated fields and reclaimed desert around Assiut city, Egypt (27°11' 27.6'' N, 31° 10' 17.1'' E) during the winter season, 2016. All weeds were collected in the vegetative stage. The shoots were air-dried in the shade, powdered and stored, in desiccator, in dark condition at room temperature in plastic bags until use.

A definite weight, 150 or 300 g, of dry shoots from each weed were placed in conical flask (two or one liter capacity) containing 900 or 450 ml of methanol for 48 h at room temperature. Methanol was used in this study because it is considered as a good solvent for many plant secondary metabolites (Chung *et al.*, 2005, Zhao and Hall, 2008). After that, infusions were filtered through filter paper, and methanol was evaporated at 65°C by using rotary evaporator. The residual plant extract was then dissolved in distilled water and completed to 150 or 120 ml to obtain extracts 1.0 or 2.5 g DWml<sup>-1</sup>. The obtained extracts were kept in a refrigerator at 4°C.

### B. Germination of target plants

The caryopses of *Triticum aestivum* L. (wheat), and seeds of *Vicia faba* L. (faba beans) and *Eruca sativa* L. (arugula) as target plants were introduced from the Agriculture Research Centre Giza, Cairo. Seeds of each plant were surface sterilized by 0.5% sodium hypochlorite for 2 mins, thoroughly washed in sterilized water, and then placed on sterile filter papers in petri dishes contain 4 ml of donor plant extract. The seeds were then covered by another moistened filter paper. For control, the seeds were germinated by sterilized distilled water. Ten seeds were placed in each of three petri dishes, as replicates, and incubated in dark under normal laboratory conditions with day and night temperatures 20 ± 2 °C and 10 ± 2 °C, respectively.

After the 3<sup>rd</sup> day, germination was daily monitored and the seed was considered germinated when the radical protrudes through the seed coat by 1 mm (International Seed Testing Association, 1999). Seeds germinated in each day were labelled at the back of petri dishes. At 8<sup>th</sup> day, the total number of germinated seeds, and the lengths of epicotyl, hypocotyl and seedlings were estimated. To investigate the potential allelopathic effect of extracts of donor species on germination of seeds of target plants, at the end of the experiment, the following parameters were calculated:

1) Germination percentage (GP) = (Number of germinated seeds/Total number of seeds) × 100

2) Seed vigor index (SVI) was calculated using the formula of (Abdul-Baki and Anderson 1973): SVI = (SI × GP)/100

Where SI is the average seedling length (cm) and GP is the germination percentage.

3) The sprouting or germination index (GI) and the coefficient of velocity (CV) were calculated according to (Scott *et al.*, 1984) as following:  $GI = \frac{\sum T_i N_i}{S}$ ;  $CV = 100 \left[ \frac{\sum N_i}{\sum N_i T_i} \right]$

Where T<sub>i</sub> is the number of days after sowing, N<sub>i</sub> is the number of seeds germinated on day i, and S is the total number of seeds planted.

4) Relative hypocotyl, epicotyl or seedling length = mean length of treatment/mean length of control × 100.

5) Percentage of inhibition or stimulation =  $100 \times \left( \frac{L_t - L_c}{L_c} \right)$ ; where L<sub>t</sub> is the length of hypocotyl, epicotyl or seedling of treated target plant, L<sub>c</sub> is the corresponding length of control. However, the negative values indicate to inhibition (or phytotoxicity), while positive values indicate to stimulation percentage.

6) Actual elongation rate (E, mm day<sup>-1</sup>)  $E = \left( \frac{\sum L_g}{D_m - D_g} \right) / N_{dg}$ .

Where L<sub>g</sub> is the average length of hypocotyl or epicotyl of seeds emergent in day "g" (mm); D<sub>m</sub> and D<sub>g</sub> are the days of measurement (in this study D<sub>m</sub> = 8) and emergence, respectively [(D<sub>m</sub> - D<sub>g</sub>) ≥ 1]; N<sub>dg</sub> is the number of days in which emergence of seeds occurred.

### C. Phytochemical analysis

**Total phenolics:** Content of total phenolics in the donor weeds was determined according to Sampietro *et al.*, (2009). The reaction mixture was prepared by mixing 0.01 ml of 5% extract, 0.2 ml of Folin-Ciocalteu's reagent and 0.4 ml 0.5N NaOH. After incubation for 15 min at room temperature, the absorbance was determined using a Unico UV-2100 spectrophotometer at 750 nm. Gallic acid was used for obtaining the standard curve, and the concentration of total phenolics was expressed in mg GAEq g<sup>-1</sup> DW.

**Flavonoids:** Flavonoids in the plant extracts was determined spectrophotometrically according to Sampietro *et al.*, (2009) using 0.1 ml of 5% donor extracts and 1.9 ml of ethanol and 0.1 ml of 10% Aluminium chloride solution dissolved in ethanol and 0.1 ml potassium acetate. The absorbance was determined using a spectrophotometer at 415 nm after incubation for a half hour at room temperature. Quercetin was used for obtaining the standard curve.

**Alkaloids:** Donor alkaloids were determined by Dragendorff's reagent using the method of Sreevidya and Mehrotra (2003). In this experiment; 2 ml of Dragendorff's reagent was added to 0.5 ml plant extract (pH was maintained at 2-2.5 with diluted HCl). The formed precipitate then centrifuged. The filtrate was discarded and the residue was then treated with 2 ml disodium sulfide solution. Brownish black precipitate was then centrifuged. The residue was dissolved in 2 ml concentrated nitric acid. This solution was diluted to 10 ml with distilled water then 1 ml was then pipetted out with 5 mL Thiourea solution. The absorbance was measured at 435 nm. The same procedure was repeated for the standard solution of Bismuth nitrate for obtaining a standard curve.

**Total terpenoid:** Content of terpenoids in donor weeds was determined according to the method of Dai *et al.*, (1999). Plant powder (0.1 g) was homogenized in 4 ml dichloromethane, and after 5 mins, the fibers removed by filtration. To 1 ml of the supernatant extract, 0.4ml of 0.02% (w/v) vanillin was added and the mixture was left at room temperature for 2 min. Then, 200 µl of concentrated sulfuric acid was added and the mixture was stirred for 10 seconds after each addition. After that, 2 ml methanol was added to convert the two-layer mixture into a homogeneous solution that instantly developed a blue-green color. The solution was left at room temperature for 5 mins before measuring its absorbance at 577 nm. A standard curve was prepared using linalool, and the total terpenoid content was calculated and expressed as mg Linalool Eq g<sup>-1</sup> DW.

### D. Statistical analysis

Data were subjected to statistical analysis using SPSS package (version 21). One-Way ANOVA, followed by a proper Post Hoc multiple comparisons between means; so LSD or Duncan multiple range test were employed and the differences between means deemed to be significant at  $p < 0.05$ . Correlation analyses were carried between secondary metabolites of donor weeds and some germination parameters of target plants. Factorial ANOVA was carried to achieve the effect of extract strength, donor and target plants and their interaction on different germination parameters and  $\eta^2$  ( $\eta^2 = SS_{\text{between}} / SS_{\text{total}}$ ) was calculated.

## RESULTS

### A. Allelopathic activity of weeds extracts on germination of crop plants

Shoot extracts (1 or 2.5 g DW ml<sup>-1</sup>) of the 10 donor weeds were completely suppressed and prevented seed of *Eruca sativa* from germination, despite the control germination was 100%. This was encouraging to examine a series of more diluted extracts on this plant, but none of seeds also germinated. The results in Table 1 show that both strengths of weed extracts significantly reduced the germination of wheat caryopses and faba bean seeds. Generally, when seeds of target plants treated with 1 g DW ml<sup>-1</sup> extracts, the GP, SVI and SGI were significantly higher than that treated with 2.5 g DW ml<sup>-1</sup> extracts. There was a drastic reduction in germination of seeds with increasing the extract strength of donor plants. The GP of both receiver plants didn't affect, or in one of them reduced by less than 20%, by low extract strength of many weeds, e.g. *A. majus*, *O. corniculata*, *U. urens*, *C. dactylon*, *D. annulatum*, *P. australis* and *S. virgatum*. The high extract concentration of *A. majus* and *D. bipinnata* completely prevented seed germination of wheat and faba bean. Also, extract 2.5 g DW ml<sup>-1</sup> of *P. lagopus* completely prevented wheat caryopses from germination, but it enhanced faba bean seed germination to be double of 1 g DW ml<sup>-1</sup>. Germination of faba bean seeds was not affected by both extracts of *U. urens* or weekly affected by *D. annulatum* and *S. virgatum* (Table 1). Extracts of *E. colona* reduced the seed germination of both target plants by 70-100%. The SVI in both target plants treated with low extract strength of most donor weeds was significantly higher than that in plants treated with high strength even when the GP was the same. Also, the SVI of *T. aestivum* unchanged significantly by extracts of *P. lagopus* or *E. colona* and SVI of *V. faba* unchanged significantly by extracts of *D. annulatum* or *E. colona*.

**Table 1: Allelopathic effect of two aqueous shoot extracts, 1g DW ml<sup>-1</sup> (C1) and 2.5g DW ml<sup>-1</sup> (C2) of 10 weeds on germination percentage (GP), seed vigor index (SVI), sprouting index (GI) and coefficient of velocity (CV) of *T. aestivum* and *V. faba*. The data are means± SE, n= 3. The comparison between C1 and C2 of each weed as obtained from One Way ANOVA and LSD for comparison between all weeds are shown.**

Donor plants	Extract	<i>T. aestivum</i>				<i>V. faba</i>			
		GP	SVI	GI	CV	GP	SVI	GI	CV
Control		100±0.00	16.33±0.25	3.0±0.00	33.3±0.17	100±0.00	4.58±0.07	5.0±0.00	20.0±0.12
<i>A. majus</i>	C1	100±0.00	1.79±0.09	3.5±0.14	28.6±0.17	80±2.08	1.76±0.34	5.2±0.12	15.4±0.06
	C2	-	-	-	-	-	-	-	-
		**	**	**	**	**	**	**	**
<i>O. corniculata</i>	C1	100±0.00	5.40±0.39	3.0±0.06	33.3±0.12	80±1.53	2.56±0.45	4.4±0.58	18.2±0.06
	C2	20±1.53	0.06±0.04	1.2±0.06	16.7±0.06	40±1.15	0.01±0.01	2.4±0.57	16.7±0.12
		**	**	**	**	**	**	**	**
<i>P. lagopus</i>	C1	30±1.15	0.17±0.08	0.9±0.03	33.3±0.17	40±0.58	0.32±0.13	2.0±0.11	20.0±0.06
	C2	-	-	-	-	80±1.53	1.04±0.20	5.2±0.23	15.4±0.12
		**	ns	**	**	**	**	**	**
<i>U. urens</i>	C1	100±0.00	9.16±0.84	3.0±0.06	33.3±0.06	100±0.00	3.94±0.06	6.2±0.12	16.1±0.06
	C2	-	-	-	-	100±0.00	1.50±0.10	7.0±0.11	14.3±0.06
		**	**	**	**	ns	**	ns	**
<i>C. dactylon</i>	C1	100±0.00	4.27±0.62	3.7±0.06	27.0±0.06	100±0.00	4.68±0.43	6.0±0.12	16.7±0.12
	C2	20±1.15	0.07±0.04	1.4±0.06	14.2±0.06	40±0.58	0.66±0.27	2.0±0.20	20.0±0.17
		**	**	**	**	**	**	**	**
<i>D. bipinnata</i>	C1	60±1.53	0.90±0.28	1.8±0.06	33.3±0.06	60±0.58	0.90±0.25	3.4±0.23	17.6±0.17
	C2	-	-	-	-	-	-	-	-
		**	**	**	**	**	**	**	**
<i>D. annulatum</i>	C1	100±0.00	5.88±0.68	3.2±0.06	31.3±0.12	80±1.73	3.01±0.59	4.4±0.23	18.2±0.12
	C2	90±1.00	2.64±0.51	5.1±0.06	17.6±0.06	80±0.58	2.29±0.39	4.2±0.05	19.0±0.12
		**	**	**	**	ns	ns	ns	ns
<i>E. colona</i>	C1	30±1.00	0.20±0.10	1.2±0.06	25.0±0.06	20±1.00	0.08±0.05	1.0±0.12	20.0±0.17
	C2	20±1.15	0.07±0.05	1.2±0.12	16.7±0.12	-	-	-	-
		**	ns	ns	**	**	ns	**	**
<i>P. australis</i>	C1	100±0.00	2.56±0.46	3.7±0.12	27.0±0.12	80±0.00	2.50±0.50	4.0±0.57	20.0±0.06
	C2	30±1.73	0.12±0.06	2.0±0.12	15.0±0.29	40±0.58	0.04±0.02	2.4±0.11	16.7±0.12
		**	**	**	**	**	**	*	**
<i>S. virgatum</i>	C1	90±1.00	6.19±0.82	2.7±0.06	33.3±0.06	100±0.00	5.30±0.55	5.4±0.12	18.5±0.06
	C2	30±0.58	0.11±0.06	2.0±0.17	15.0±0.12	80±1.53	2.06±0.38	4.6±0.23	17.4±0.06
		**	**	ns	**	**	**	*	**
LSD		1.22	0.85	0.13	0.20	1.71	0.62	0.37	0.18
		1.56	0.30	0.13	0.20	1.35	0.34	0.22	0.17

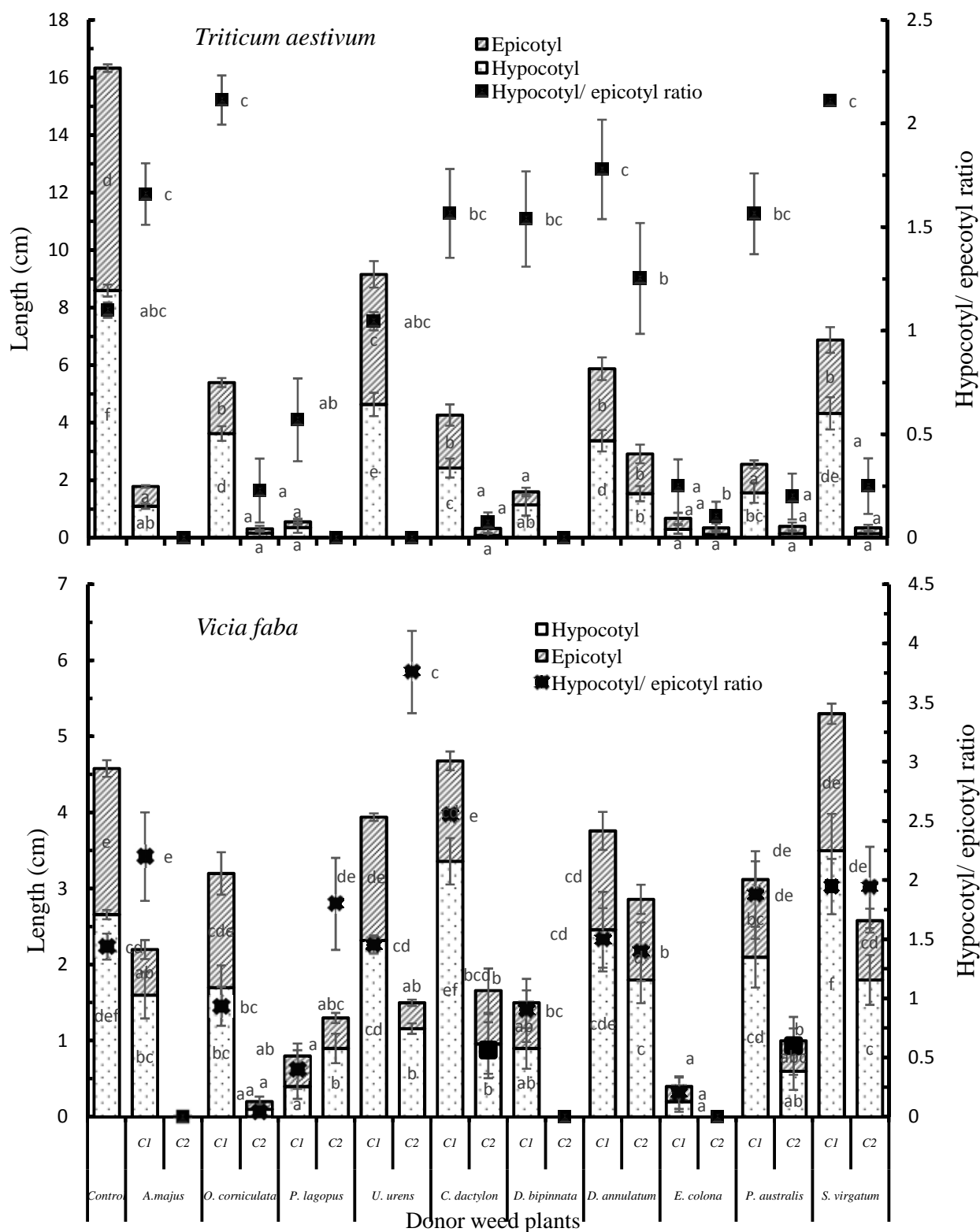
\* & \*\*: Significant at  $P < 0.05$  and  $P < 0.01$ , respectively; ns: nonsignificant.

Estimation of GI and CV reflects the acceleration (decreasing GI values or increasing CV values) or delaying (high values of GI or small values of CV) of seed germination. However, these indices are more valuable in comparison between different donor plants when their GP are approximately similar. Wheat caryopses completely germinated (GP= 100%) when treated with 1 g DW ml<sup>-1</sup> extracts of *A. majus*, *O. corniculata*, *U. urens*, *C. dactylon*, *D. annulatum* and *P. australis*, but the GI was 3.5, 3.0, 3.0, 3.7, 3.2 and 3.7 and the CV was 28.6, 33.3, 33.3, 27, 31.3 and 27, respectively. However, it is obvious that extracts of *A. majus*, *C. dactylon* and *P. australis* delayed the germination of caryopses compared with others (Table

1). The GI in *T. aestivum* was non-significantly differed by both extracts of *E. colona* or *S. virgatum*, while in *V. faba* unchanged significantly by *U. urens* and *D. annulatum*.

#### - Effect on the lengths of hypocotyl and epicotyl:

Results presented in Fig. 1 show the influence of two concentrations of 10 weed extracts collected from cultivated fields on hypocotyl and epicotyl lengths and hypocotyl/ epicotyl ratio of *T. aestivum* and *V. faba*. These parameters were significantly affected by increases in the extract concentration of most weeds. The extracts of *U. urens* and *P. lagopus* significantly decreased lengths of hypocotyl, epicotyl and seedlings of *T. aestivum*, and similarly *E. colona* affected *V. faba*.



**Fig. 1.** Allelopathic effect of two shoots aqueous extracts (C1= 1g ml<sup>-1</sup>; and C2= 2.5 g ml<sup>-1</sup>) of 10 weed plants on hypocotyl and epicotyl lengths (cm) and hypocotyl/ epicotyl ratio of *Triticum aestivum* and *Vicia faba*. Values are means ± SE, n= 3. The means of each parameter with different letters are significantly different at P< 0.05 according to Duncan's test.

Compared to control, the length of the epicotyl and hypocotyl of wheat sprouts were significantly decreased by treatment with both extracts of the ten studied weeds. The longest hypocotyl and epicotyl of *T. aestivum* were shown when treated with 1 g DW ml<sup>-1</sup> extracts of *U. urens* and *S. virgatum* and 2.5 DW g ml<sup>-1</sup> extract of *D. annulatum*. On the other hands, the maximum hypocotyl/epicotyl ratios of *T. aestivum* was estimated when treated with 1g DW ml<sup>-1</sup> extract of *O. corniculata* and *S. virgatum*. Extracts of *E. colona* and *C. dactylon* exerted the maximum reduction in the hypocotyl length of *T. aestivum*, while extracts of *P. lagopus* and *O. corniculata* exerted the maximum reduction in the epicotyl length. On the other hands, the minimum

hypocotyl/epicotyl ratio of *T. aestivum* was estimated when treated with *E. colona* extracts.

The longest hypocotyl and epicotyl of *V. faba* sprouts was estimated when treated with extracts of *C. dactylon* and *S. virgatum* (1 g DW ml<sup>-1</sup>) or *D. annulatum* (2.5 g DW ml<sup>-1</sup>). In addition, the maximum ratios of hypocotyl/epicotyl were estimated when treated with 1 g DW ml<sup>-1</sup> extract of *C. dactylon* and 2.5 g DW ml<sup>-1</sup> extract of *U. urens*, while the minimum ratios resulted by extracts of *O. corniculata* and *E. colona*.

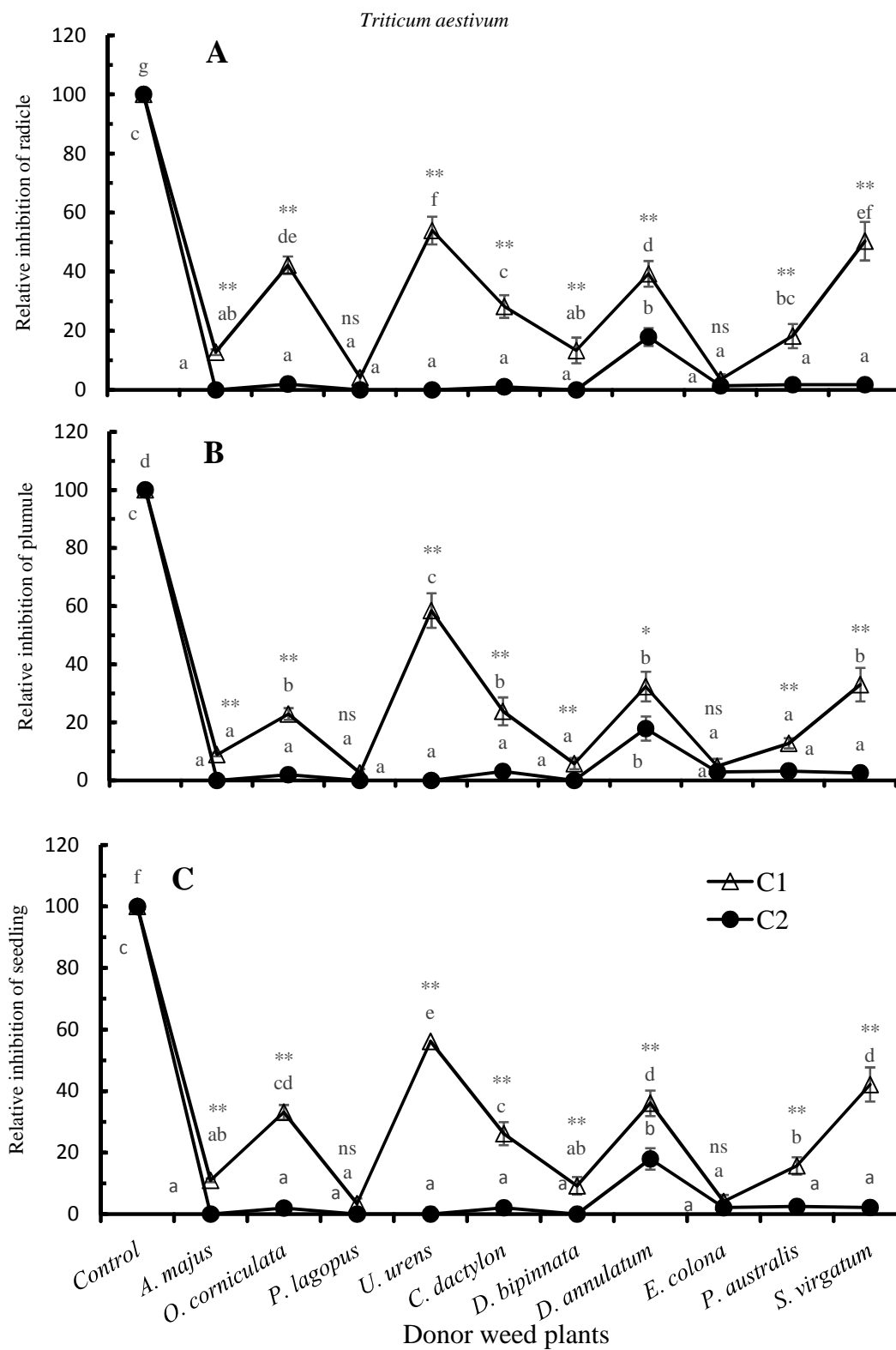
*B. Effect on elongation rates*

The effect of weed shoots extracts on the rates of elongation of hypocotyls and epicotyls (mm day<sup>-1</sup>) is shown in Table 2.

**Table 2: Allelopathic effect of 1g DW ml<sup>-1</sup> (C1) and 2.5g DW ml<sup>-1</sup> (C2) extracts of 10 weeds on elongation rates of radicle, plumule and seedling of *T. aestivum* and *V. faba*. The data are means± SE, n= 3. The comparison between C1 and C2 of each weed as obtained from One Way ANOVA and LSD for comparison between all weeds are shown.**

Donor plants	Extract	<i>T. aestivum</i>			<i>V. faba</i>		
		Radicle	Plumule	Seedling	Radicle	Plumule	Seedling
Control		1.89 ± 0.09	1.70 ± 0.07	3.59 ± 0.16	1.05 ± 0.09	0.72 ± 0.05	1.77 ± 0.12
<i>A. majus</i>	C1	0.39 ± 0.02	0.23 ± 0.02	0.63 ± 0.04	1.40 ± 0.04	0.51 ± 0.02	1.90 ± 0.07
	C2	-	-	-	-	-	-
		**	**	**	**	**	**
<i>O. corniculata</i>	C1	0.82 ± 0.06	0.39 ± 0.03	1.21 ± 0.08	1.36 ± 0.00	1.22 ± 0.04	2.59 ± 0.04
	C2	0.38 ± 0.03	0.34 ± 0.02	0.71 ± 0.04	0.18 ± 0.02	0.18 ± 0.02	0.35 ± 0.04
		**	ns	**	**	**	**
<i>P. lagopus</i>	C1	0.26 ± 0.01	0.14 ± 0.01	0.40 ± 0.02	0.35 ± 0.04	0.35 ± 0.04	0.69 ± 0.07
	C2	-	-	-	0.77 ± 0.05	0.39 ± 0.01	1.12 ± 0.04
		**	**	**	**	ns	**
<i>U. urens</i>	C1	0.88 ± 0.10	0.84 ± 0.09	1.73 ± 0.19	1.73 ± 0.05	1.18 ± 0.02	2.90 ± 0.04
	C2	-	-	-	0.92 ± 0.12	0.26 ± 0.04	1.18 ± 0.16
		**	**	**	**	**	**
<i>C. dactylon</i>	C1	0.75 ± 0.10	0.67 ± 0.16	1.42 ± 0.25	2.02 ± 0.11	0.79 ± 0.05	2.80 ± 0.16
	C2	0.38 ± 0.04	1.00 ± 0.10	1.38 ± 0.14	0.73 ± 0.03	0.53 ± 0.03	1.27 ± 0.06
		*	ns	ns	**	*	**
<i>D. bipinnata</i>	C1	0.40 ± 0.04	0.16 ± 0.02	0.54 ± 0.07	0.86 ± 0.12	0.62 ± 0.05	1.48 ± 0.16
	C2	-	-	-	-	-	-
		**	**	**	**	**	**
<i>D. annulatum</i>	C1	0.67 ± 0.08	0.48 ± 0.10	1.16 ± 0.18	1.31 ± 0.04	0.71 ± 0.04	2.03 ± 0.08
	C2	0.98 ± 0.15	0.86 ± 0.24	1.85 ± 0.38	0.89 ± 0.04	0.49 ± 0.03	1.38 ± 0.07
		ns	ns	ns	*	*	*
<i>E. colona</i>	C1	0.32 ± 0.03	0.40 ± 0.05	0.71 ± 0.08	0.35 ± 0.04	0.35 ± 0.04	0.69 ± 0.07
	C2	0.42 ± 0.02	0.80 ± 0.04	1.19 ± 0.06	-	-	-
		*	*	*	**	**	**
<i>P. australis</i>	C1	0.54 ± 0.05	0.41 ± 0.03	0.95 ± 0.07	0.78 ± 0.07	0.37 ± 0.04	1.15 ± 0.10
	C2	0.34 ± 0.11	0.54 ± 0.15	0.88 ± 0.26	0.52 ± 0.06	0.35 ± 0.04	0.87 ± 0.09
		ns	ns	ns	ns	ns	ns
<i>S. virgatum</i>	C1	1.08 ± 0.08	0.52 ± 0.06	1.60 ± 0.04	1.69 ± 0.21	1.02 ± 0.03	2.71 ± 0.22
	C2	0.36 ± 0.02	0.47 ± 0.03	0.84 ± 0.04	0.91 ± 0.04	0.40 ± 0.02	1.30 ± 0.05
		**	ns	**	*	**	**
LSD <sub>0.05</sub>	C1	0.11	0.12	0.22	0.16	0.07	0.20
	C2	0.10	0.16	0.26	0.09	0.05	0.13

\* & \*\*: Significant at P< 0.05 and P< 0.01, respectively; ns: non-significant.



**Fig. 2.** Allelotoxic effect of two extract strengths (C1= 1g ml<sup>-1</sup>; and C2= 2.5g ml<sup>-1</sup>) of 10 donor weeds on radicle (A), plumule (B) and seedling (C) of *Triticum aestivum* as relative to control. The data are means  $\pm$  SE, n= 3. The means of each line with different letters are significantly different at  $P < 0.05$  according to Duncan's test. For each panel, C1 and C2 values that show statistically significant differences are marked with asterisks (\*:  $P < 0.05$  or \*\*:  $P < 0.01$ ); otherwise, the difference between means is not significant (ns).

Compared to control, both extracts of all weeds reduced the elongation rate of wheat radicle by 43%-86%, plumule by 40%-92% and seedling by 48%-89% (Table 2). Generally, the elongation rates in wheat negatively affected more than that in faba beans. The extracts of some weeds exerted more reduction on the rate of elongation of radicle than plumule (e.g. *C. dactylon* and *E. colona*), while others (e.g. *O. corniculata*, *P. lagopus* and *D. annulatum*) exerted more reduction on plumule. On the other hand, the extract of *U. urens* and *P. australis* have approximately the same magnitude of reduction of elongation rates of radicle, plumule and seedling of wheat. The situation was different in case of faba bean where the 1 g DW ml<sup>-1</sup> extract of five (*O. corniculata*, *U. urens*, *C. dactylon*, *D. annulatum* and *S. virgatum*) weeds increased the rate of elongation of seedlings (radicle and plumule), while 2.5 g DW ml<sup>-1</sup> extracts significantly decreased the rate of elongation (Table 2).

The low extract strength of *A. majus* increased the elongation rate of hypocotyl, while decreased the elongation rate of epicotyl. The low and high extract strengths of *P. lagopus* and *P. australis* significantly decreased the elongation rates of radicle, plumule and seedling of faba bean. Generally, the rate of epicotyl elongation was allelopathically reduced more than the hypocotyl.

Fig. 2 represents the relative allelopathic inhibition of radicle, plumule and seedling of *T. aestivum* by extracts of donor weeds. It is obvious that all weeds extracts have phytotoxic effects on wheat seedlings. The high extract level severely inhibited the growth of radicle and plumule, and hence reflected on the whole seedling. The relative inhibition of plumule was generally higher than that of radicle. The highest inhibition was exerted by extracts of *P. lagopus* and *E. colona* with non-significant differences between the extract concentrations. There was no significant

differences in the relative inhibition of radicle, plumule or seedlings of wheat caused by 2.5 g DW ml<sup>-1</sup> extracts of all studied weeds, except *D. annulatum*. The situation was greatly difference by using 1 g DW ml<sup>-1</sup> extracts, where there were significant variations between donor weeds.

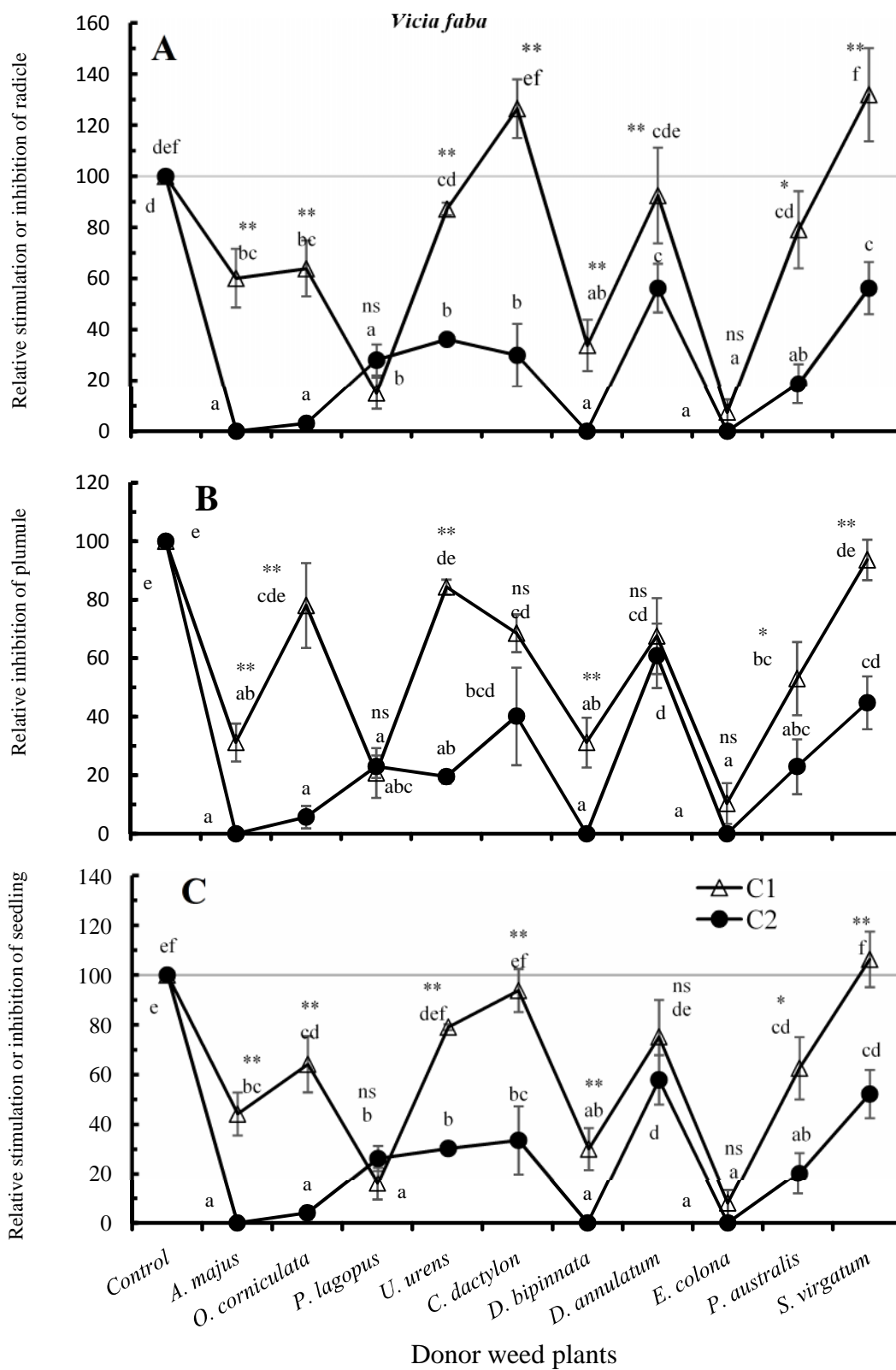
The results of relative inhibition of radicle, plumule and seedlings of faba bean (Fig. 3) were greatly different from that of wheat. The inhibition in case of faba bean was less magnitude than in wheat (Fig. 2 with Fig. 3). There were no significant differences in relative inhibitions between the low and high extract concentrations of each of three weeds: *P. lagopus*, *D. annulatum* and *E. colona*. Otherwise, a significant differences are shown either between both extract levels or between different donor weeds. More important that the low extract concentration of the grass *C. dactylon* and *S. virgatum* stimulated the growth of faba bean radicle after emergence by about 26% and 32% over that of control, respectively. The extract of *S. virgatum* stimulated also the growth of seedling of faba bean by about 6%, as the plumule unchanged significantly from control (Fig. 3).

Table 3 show the values of eta square ( $\eta^2$ ) which calculated from factorial analysis of variance to achieve the magnitude of effect of donor species, extract strength, receiving species or the interaction between them on different parameters. It is obvious that the donor plants have the highest magnitude of effect on the changes in SVI, and hypocotyl, epicotyl and sprout length followed by the interaction donor\* target plants and then the extract strength. The target plants, target plant \* extract and donor \* target \* extract have weak role in changes ( $\eta^2 < 0.04$ ). The donor plants followed by extract strength and target plants as single factors were have the highest magnitude of phytotoxicity effect on the hypocotyl, epicotyl and sprout.

**Table 3: The partial eta square ( $\eta^2$ ) for the magnitude of effect of donor weeds, target species, extract concentration and the interaction between them on different parameters.**

Factors	Parameters							
	SVI	Length			Hypoc. / Epic. ratio	Phytotoxicity		
		Hypoc.	Epic.	Sprout		Hypoc.	Epic.	Sprout
Donor plants	0.52	0.47	0.50	0.50	0.14	0.37	0.46	0.45
Extract strength	0.09	0.12	0.07	0.09	0.10	0.14	0.09	0.11
Target plants	0.03	0.01	0.04	0.03	0.03	0.11	0.10	0.10
Donor plants * Extract	0.07	0.06	0.05	0.06	0.14	0.07	0.06	0.06
Donor*Target plants	0.19	0.18	0.22	0.20	0.10	0.07	0.04	0.05
Target plant * Extract	0.01	0.01	0.01	0.01	0.03	0.01	0.01	0.01
Donor * Target * Extract	0.02	0.02	0.02	0.02	0.07	0.02	0.01	0.01





**Fig. 3.** Allelotoxic effect of donor weeds extracts on radicle (A), plumule (B) and seedling (C) of *Vicia faba*. For more details see Fig. 2.

C. Phytochemical compounds

The concentration of some phytochemical compounds of the studied ten donor weeds are summarized in Table

4. Generally, phenolics, flavonoids, alkaloids and terpenoids were detected with significant differences, in all studied weeds.

**Table 4: Concentration of the phytochemical compounds total phenolics, flavonoids, alkaloids and terpenoids (mg g<sup>-1</sup> DW) in the ten studied weeds. Data are means± SE, n= 3. LSD for comparison between different species is shown.**

Donor plants	Phytochemical compounds (mg g <sup>-1</sup> DW)			
	Total phenolics	Flavonoids	Alkaloids	Terpenoids
<i>Ammi majus</i>	10.11±0.27	0.51±0.01	1.85±0.04	0.23±0.02
<i>Oxalis corniculata</i>	1.68±0.03	0.11±0.01	2.62±0.04	0.21±0.01
<i>Plantago lagopus</i>	6.64±0.11	0.48±0.02	3.75±0.15	0.16±0.01
<i>Urtica urens</i>	2.54±0.08	0.09±0.01	1.29±0.08	0.88±0.00
<i>Cynodon dactylon</i>	2.22±0.10	0.04±0.01	1.37±0.10	0.17±0.01
<i>Desmostachya bipinnata</i>	5.63±0.37	0.38±0.01	1.19±0.09	0.16±0.00
<i>Dichanthium annulatum</i>	3.10±0.07	0.26±0.02	1.19±0.01	0.11±0.00
<i>Echinochloa colona</i>	3.44±0.05	0.47±0.01	1.04±0.01	0.19±0.01
<i>Phragmites australis</i>	4.28±0.14	0.34±0.02	0.64±0.04	0.22±0.01
<i>Sorghum virgatum</i>	4.55±0.08	0.41±0.02	1.38±0.02	0.23±0.01
LSD <sub>0.05</sub>	0.26	0.03	0.12	0.02

**Table 5: r-values of correlation analyses between concentrations of phytochemicals of donor plants and different parameters in target plants. C1, extract strength 1g DW ml<sup>-1</sup>; C2, extract strength 2.5 g DW ml<sup>-1</sup>.**

Parameter	Extract	Phytochemicals of donor plants							
		Total phenolics	Flavonoids	Alkaloids	Terpenoids	Total phenolics	Flavonoids	Alkaloids	Terpenoids
		<i>Triticum aestivum</i>				<i>Vicia faba</i>			
GP	C1	-0.27	-0.60	-0.40	0.17	-0.33	-0.65*	-0.36	0.23
	C2	-0.58	-0.43	-0.55	-0.47	-0.49	-0.49	-0.03	0.25
SVI	C1	-0.67*	-0.76*	-0.51	0.08	-0.50	-0.66	-0.42	0.15
	C2	-0.51	-0.50	-0.52	-0.38	-0.51	-0.48	-0.40	-0.15
GI	C1	-0.18	-0.51	-0.43	0.11	-0.23	-0.62*	-0.31	0.33
	C2	-0.49	-0.30	-0.46	-0.43	-0.43	-0.45	0.05	0.39
CV	C1	-0.14	-0.24	0.27	0.15	-0.33	0.13	-0.07	-0.50
	C2	-0.73*	-0.50	-0.56	-0.51	-0.61*	-0.64*	-0.03	-0.04
Hypocotyl length	C1	-0.68*	-0.74**	-0.49	0.04	-0.39	-0.57	-0.42	0.08
	C2	-0.53	-0.51	-0.53	-0.39	-0.51	-0.48	-0.38	-0.12
Epicotyl length	C1	-0.66*	-0.73*	-0.54	0.12	-0.64*	-0.75**	-0.37	0.17
	C2	-0.53	-0.51	-0.53	-0.39	-0.57	-0.55	-0.47	-0.34
Hypocotyl/epicotyl ratio	C1	0.01	-0.20	-0.06	-0.10	0.06	-0.30	-0.34	0.06
	C2	-0.50	-0.38	-0.44	-0.42	-0.24	-0.29	0.01	0.66*
Seedling length	C1	-0.67*	-0.74**	-0.51	0.08	-0.50*	-0.66*	-0.42	0.11
	C2	-0.52	-0.50	-0.52	-0.39	-0.54	-0.51	-0.42	-0.20
Hypoc elongation rate	C1	-0.67*	-0.71*	-0.52	-0.09	-0.20	-0.54	-0.16	0.35
	C2	-0.65*	-0.52	-0.57	-0.49	-0.48	-0.50	-0.16	0.14
Epic elongation rate	C1	-0.70*	-0.74**	-0.62*	-0.01	-0.47	-0.62*	0.01	0.47
	C2	-0.69*	-0.54	-0.62*	-0.50	-0.58	-0.60	-0.24	-0.24
Seedling elongation rate	C1	-0.70*	-0.74**	-0.58	-0.05	-0.32	-0.61*	-0.11	0.42
	C2	-0.68*	-0.54	-0.61*	-0.51	-0.53	-0.56	-0.21	-0.01

\*: significant r-value at P< 0.05.

\*\* : significant r-value at P< 0.01.

The contents of total phenolics in *A. majus*, *P. lagopus* and *D. bipinnata* were significantly higher than that in other weeds. The highest content of flavonoids was estimated in *A. majus*, *P. lagopus* and *E. colona*, while the highest content of alkaloids was found in *P. lagopus* and *O. corniculata*. *Urtica urens*, *A. majus* and *S. virgatum* were characterized by increasing amount of terpenoids. Most of secondary metabolites were stored in the 10 weeds in form of phenolic compounds which represented by a minimum 37.2% of secondary metabolites in *O. corniculata* to a maximum 83.3% in *P. australis*. In contrast, alkaloids were represented by the minimum percent in *P. australis* (12.4%) and the maximum percent in *O. corniculata* (58.1%).

Total phenolic contents and flavonoids of the donor weeds were greatly varied from species to another. Total phenolics were ranging between 1.68 and 10.11 mg GAEq g<sup>-1</sup> dry weight, while flavonoids were ranging between 0.04 and 0.51 mg g<sup>-1</sup>DW.

The content of total terpenoids in different donor weeds was ranging between 0.11 and 0.88 mg Linalool Eq g<sup>-1</sup> DW, and was significantly different from species to another. The highest content of terpenoids was found in *U. urens* which was 4- to 8-fold of that in other weeds. In this species, terpenoids represented about 18.68% of the secondary metabolites estimated here (phenolics, alkaloids and terpenoids), while represented less than 5% in the other 9 weeds.

Table 5 show r-values of correlation analyses between estimated phytochemicals in the donor weeds and different parameters of seed germination or lengths and elongation rates of hypocotyl, epicotyl and seedlings of *T. aestivum* and *V. faba*. Approximately, all parameters were negatively correlated with total phenolics, flavonoids and alkaloids, and about 50% from these negative r-values were significant. Despite most r-values of correlation between different parameters and total terpenoids were positive especially at low concentration, only one positive r-value was significant when correlated with hypocotyl/ epicotyl ratio of *V. faba*.

## DISCUSSION

Weed plants are characterized by their ability to grow rapidly through the generation of large quantities of biomass in a short time and their ability to tolerate environmental stress and modification (Qasem, 2017, Qasem and Foy, 2001). In Egypt, weeds in agricultural lands increased to represent approximately 22.5% of the total flora (El-Hadidi, 1993). Weeds allelopathy plays an important role in agroecosystems leading to a wide array of interactions between crop-crop, crop-weed and tree-crops (Singh *et al.*, 2001). The results of this study showed that different strengths of shoot extracts of 10

field weeds have allelopathic effects on germination and seedling growth of target plants and the inhibition increased with increasing extract concentration. *Eruca sativa* seems to be a very sensitive species and all attempts to germinate its seeds in presence of any weed extract have been failed, despite the control seeds were perfectly germinated. In a separate experiment, seeds of this species treated with more diluted extracts of weeds, but also did not germinate. However, we suggest that *E. sativa* (dicotyledonous plant) may be an ideal plant for allelopathic studies. The results indicate, in general, that *T. aestivum* negatively affected by weeds extracts more than *V. faba*. Accordingly, there are no clear evidences to support what Soltys *et al.* (2013) concluded that monocotyledonous plants are more resistant to allelochemicals than dicotyledonous ones.

As no caryopses of wheat or seeds of faba bean noticed emergent before the 3<sup>rd</sup> or 5<sup>th</sup> day from planting, however by applying the equation of (Scott *et al.*, 1984) the maximum limit of CV in this study will be 33.33 and 20, respectively. These values will resulting if the total or any number of seeds emerged only at the 3<sup>rd</sup> (for *T. aestivum*) or 5<sup>th</sup> (for *V. faba*) day, and no further seeds have emerged after that. These values of CV obtained in untreated (control) planted seeds and in many other treatments. In *V. faba*, no any weed extract exerted 100% seed germination and CV 20, while two weeds exerted 100% germination and CV 33.33 on *T. aestivum* caryopses. However, the main effect of these two weeds, *O. corniculata* and *C. dactylon*, was suppressing of the rate of elongation and hence the whole seedling length at low extract strengths and reducing germination by increasing the extract strengths. Homa and Mitra (2014) found that the extracts from dry shoots of *C. dactylon* had slight inhibitory effect on the germination of seeds of *T. aestivum*.

The changes in hypocotyl/ epicotyl ratios reflect the allelopathic influence on each organ. Results of this study indicates that the increasing hypocotyl/ epicotyl ratios of both receiving species by extracts of some weeds were due to the increasing magnitude of effect on epicotyl rather than increasing the length of hypocotyl. According to González and Reigosa (2001), the toxicity of substances and the degree of interaction between plant-plant depend on the stage of growth of the donor and receiving species alike. Both extracts of all weeds reduced the elongation rate of wheat seedling by more than 50% from that of control, with differential effect on hypocotyl and epicotyl. The reduction in faba bean seedling was less than 40% by most high extract concentration of weeds, otherwise many extracts stimulated elongation rate after seeds emergence.

However, as the donor weeds have different allelopathic influence on target plants, the target plants and their different organs and stages differentially respond to the same donor plant. Cai and Mu (2012) found that higher concentrations of the *Datura stramonium* extracts inhibited primary root elongation and lateral root development, decreased root hair length and density, inhibited cell division in root tips of soybean. From the various effects of flavonoids reported by Weston and Mathesius (2013), it plays important roles in transport of auxin and root and shoot development. They concluded that flavonoid glycosides have active roles in regulation of indole-3-acetic acid oxidase which could lead to changes in auxin accumulation.

Shoots of some weeds contain allelopathic substances that when reached to a definite bioactive concentrations prevented the seeds of receiver plants from germination. The bioactive compounds are mainly secondary metabolites which accumulated in all plant cells, but their concentration varies according to the plant organ (Ram *et al.*, 2015). The high extract concentrations of shoots of *A. majus* and *D. bipinnata* completely prevented the seeds of wheat and faba bean from germination. The same extract level of *U. urens* hampered germination of wheat, while that of *E. colona* hampered germination of faba bean. However, the effect depends on the concentration and allelopathic substances of donor plant and on the target species, but the donor plant being have the highest magnitude of effect. Chen *et al.* (2017) studied the effect of root extract of *Caragana intermedia* on the germination and seedling growth of two dicot and two monocot crops. They found that all root extracts inhibited the germination of both dicotyledonous plants, while in monocotyledonous species the germination inhibited by high concentrations of extract but stimulated by low concentrations. Lovett and Houlst (1998) concluded that some compounds that are toxic or inhibitory at high concentrations are stimulatory at low concentrations. Also, Nikneshan *et al.* (2011) reported that with increasing extract concentration the inhibitory effect on germination indices increased, while the low concentration have incitement effects on seed germination. The prevention of seed germination in the present study by extracts of some weeds was basically due to the inhibitory effect of allelochemicals such as water soluble phenolics, alkaloid or terpenoids that found with considerable amounts in shoots of these weeds. Alkaloids among a group of at least 50 tested compounds possess phytotoxicity, acting to inhibit germination and/or seedling growth in neighboring plants (Haig, 2008). The hypothesis that weeds contain more total phenolics, alkaloids or terpenoids will have more allelopathic effect than others is documented by the results of this study. Therefore, most estimated parameters of germination and rates of elongation in

target plants, as expected, have been negatively correlated with the contents of total phenolics, flavonoids and alkaloids in donor weeds. Most r-values of correlation analysis between terpenoids and different parameters were weak positive. It seems that terpenoids have a positive allelopathic effect on both target plants especially at low concentration. On the other hand, the total phenolics, alkaloids or terpenoids were not indicators for the magnitude of allelopathic effect on different organs or processes in all plants. The specific secondary compounds appear to be more important in allelopathy, and plants contain such compounds even in low concentration exert more effect than others containing high content of secondary metabolites but not including these compounds. After that, the allelopathic influence of a donor plant will increase as the content of specific compounds increase in its tissues. In the present study, increasing the extract concentration of some donor weeds caused a dramatic decrease in lengths of seedling (epicotyl and hypocotyl), SGI and SVI of both *T. aestivum* and *V. faba*. Inhibitory effects of weeds tested in this study were different on receiving plant species. The variation might be attributed to the differences in type, total amount as well as properties of allelochemicals produced by different weeds. Ben-Hammouda *et al.* (1995) found that the inhibition of wheat (*T. aestivum*) hypocotyl growth was positively correlated with concentrations of total phenolics contained in sorghum (*Sorghum bicolor*) plant parts. In another study (Bhowmik and Doll, 1982), both sorghum and corn residues stimulated the growth of soybean, indicating a lack of allelopathic activity associated with some dried and decomposing sorghum shoot tissues. These and our studies strongly support the plant-plant specification in the allelopathic relationship.

In conclusion, the results show that: 1) All studied weeds prevented seeds germination of *E. sativa* and delayed germination of *T. aestivum* and *V. faba*. 2) High extract concentrations of some weeds completely prevented germination of either both target plants (e.g. *A. majus* and *D. bipinnata*) or only wheat (e.g. *O. corniculata* and *P. lagopus*) or faba bean (e.g. *E. colona*). 3) Some weeds suppressed cell division and elongation in the apical meristem (may be by growth inhibitors) even by low extract concentrations (e.g. *P. lagopus*, *D. bipinnata* and *E. colona*). 4) Low level of some weeds extracts stimulated elongation after emergence of faba bean seeds (e.g. *A. majus*, *O. corniculata*, *U. urens*, *C. dactylon*, *D. annulatum* and *P. australis*). Regarding the negative effect, the studied weeds can be categorized into: 1) Competitive and inhibitory allelopathic weeds (those inhibit cell division, elongation and seedling growth), 2) Germination preventing allelopathic weeds (those completely prevent germination of a defined receiving plant).

A donor plant may be competitive for receiving plant and germination preventing for another, and the effect depends on the corresponding competitiveness and sensitivity of the receiving plant. The results represented in this study also brings out the general need for comparative allelopathy either between receiving or between donor plants, or between the effects of purely separated allelopathic compounds with exactly known structures on donor plants.

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