ASSESSMENT OF ALLELOPATHIC EFFETS OF COGONGRASS (*IMPERATA CYLINDRICA* L.) ON WHEAT, ONION AND SOME ASSOCIATED WEEDS

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ABSTRACT

Studying the influence of cogongrass (*Imperata cylindrica* L.) extract on germination and seedling growth of some weeds and crops indicated that the dicotyledonous weeds such as goosefoot and sowthistle were the most affected by the aqueous extract of cogongrass (rhizome and foliage) than the monocotyledonous crops, wheat and onion, based on IC_{50} values. Delay in germination was more pronounced in weeds than crops. Dry weight of wheat and onion seedlings were not significantly affected meanwhile the dry weight of goosefoot, fieldbind and sowthistle seedlings were significant, by lower than control. Rhizome extract was generally more effective than foliage extract. Allelochemicals in cogongrass (rhizome and foliage) analyzed by liquid chromatography/mass spectrometry (LC/MS) were identified as phenolic compounds that included 3'-o-methyl-(-)-epicatechin-7-o-sulphate, vanillic, ferulic and coumaric acids in rhizome extract, in addition to (-)-epigallocatechin-3,5-digallate, caffeic and gallic in foliage extract. Chlorogenic acid was the principal phenolic compound in both extracts. These results suggest that cogongrass might have an inhibitory effect. Considering that cogongrass is an allelopathic plant might suggest its use as soil additive material for weed control and could also serve as natural herbicide.

Keywords: Allelopathy, Cogongrass extract, Germination, Seedling growth, Weeds, Crops, Allelochemicals.

INTRODUCTION

Allelopathy is the phenomenon that occurs when one plant in the living or decaying state interfers with growth of another plant via a chemical inhibitors which causes reduction in plant emergence or growth. Chemicals that impose allelopathic activity are called allelochemicals (**Putnam, 1988**). These chemicals are present in all plant or in plant parts; leaves, flowers, seeds, rhizomes and roots and, may be released into the environment (atmosphere or rhizosphere) in sufficient quantities and with enough persistence to affect a neighboring or successional plant. Such release occurs by volatilization or leaching especially from leaves or by exudation from roots and also from decomposition of dead plant parts. The allelohemials have either harmful or beneficial effect but they are generally toxic and cause stress even

death. The allelopathic properties of plants can be exploited successfully as tool for weed reduction and enhancement of crops yield **(Rowshan *et al*., 2014)**. The allelopathic impact of plant extracts on weeds and crops was investigated by many workers **(Al- Mutlaq *et al.* 2002**; **Norsworthy, 2003**; **Ali, 2005**; **Al- Wakeel *et al*. 2007**; **El- Rokiek *et al*. 2010**; **Mubeen *et al.* 2012**; **Won *et al*. 2013 and Javed *et al*. 2014**). The objective of the present work was to study the allelopathic effect of aqueous extract of cogongrass, *Imperata cylindrica* L., rhizome or foliage, on germination and seedling growth of six weed and crop plants to identify the allelochemicals present in cogongrass extract.

MATERIAL AND METHODS

1. Plants used:

Cogongrass, *Imperata cylindrica* L., weed plant was collected from Demo farm, Faculty of Agriculture, Fayoum University. The inhibitory effect of the collected weed on germination and growth characters was tested on two crops (wheat and onion) and four weed namely; sowthistle, fieldbind, wildoat and goosefoot, which identified following **Zaki, 2000**. Of these six tested species, three are dicotyledonous and the other three are monocotyledonous tabulated as follows.

Class	English name	Scientific name	Family
Dicotyledonous plats	Fieldbind	*Convolvulus arvensis* L.	Convolvulaceae
	Sowthistle	*Sonchus oleraceus* L.	Asteraceae
	Goosefoot	*Chenopodium album* L.	Chenopodiaceae
Monocotyledonous plants	Onion	*Allium cepa* L.	Alliaceae
	Wildoat	*Avena fatua* L.	Gramineae
	Wheat	*Triticum aestivum* L.	Gramineae

2. Preparation of cogongrass crude extract:

Foliage and rhizomes of this plant are washed under water and, left to dry under room temperature, away from sunlight. Dried plant material, 700 gm of each part was ground in a grinder and extracted in distilled water, 3 ml per gm plant material as described by **(Kiemnec and McInnis, 2002)**. After 24 hours, the extract was filtered through Whatman No.3 filter paper and eliminated water, to dryness under vacuum at -50°C using Lioalfa 6-50 Freeze Dryer apparatus. The crude extract was kept in the refrigerator till desired used for assay.

3. Germination and bioassay technique

The seeds of the plant species under investigation were sterilized by soaking for 20 minutes in 2.5% sodium hypochlorite solution. Then washed several times with distilled water. Twenty five seeds of each tested species (sowthistle, fieldbind, wildoat, goosefoot, onion and wheat) were placed on Whatman No. 1 filter paper in a 9 cm dia. glass Petri dish. Four

concentrations, 500, 1000, 2000 and 4000 ppm each of crude extract were tested using 5 ml of each concentration/ Petri dish. In the control treatment only distilled water was added. Four replicates were made for each treatment. The seeds tested were incubated at 25°C., except for onion which was incubated at 20°C following **Abdallah *et al.*, 2002**. Germinated seeds were daily counted for 5 days or least germination. Germination percentage (GP %), germination index (GI), and mean germination time (MGT) were recorded after ten days of incubation and were calculated using the formulas:

$$GP\ (\%) = \frac{a}{b} \times 100$$

Where; *a*) is the number of germinated seeds, and *b*) is the total number of seeds.MGT was calculated according to **(Ellis and Roberts, 1981)** as follow:

$$(MGT\ =\ \Sigma\ Dn/\Sigma\ n)$$

Where; *n*) is the number of seeds that had germinated on day, and *D*) is the number of days counted from the beginning of germination. The germination index (GI) was calculated by using the equation of **(Scott *et al.*, 1984):**

$$\mathrm{GI} = \frac{\Sigma \mathrm{TiNi}}{\mathrm{S}}$$

Where; T_i) is the number of days after sowing. N_i) is the number of seeds germinated on day_i, and S) is the total number of seeds planted. The root, shoot lengths and dry weight were also measured after ten days of incubation. Inhibition percentages were calculated according to **(Chung *et al.*, 2001)** as [(control- treatment) / control] ×100 and were analyzed using a probit method **(Finney, 1971)** to estimate the concentration required to inhibit 50% (IC_{50}) of germination (GP %) and seedling growth (root and shoot lengths).

4. Isolation and identification of the allelochemicals

For TLC separation, glass plates (20×20cm) were coated with silica gel (GF_{254}), 0.75mm thickness and left for dryness at room temperature. Aqueous extracts of cogongrass (foliage and rhizome) were applied as a band (fraction) according to **(Nalina and Rahim, 2007)** and developed in solvent system consisting of chloroform: ethanol: acetic acid mixture (92:4:4) by volume. The chromatogram was air dried and sprayed with 25% folin-ciocalteu reagent as chromogenic agent to help in identify phenolic compounds according to **Singleton and Rossi, 1965** and also to calculate rate of flow (R_f values) for each fraction. Each band was separately scraped from the chromatogram, extracted with distilled water, evaporated, and employed in further bioassay tests to elucidate the impact of each on seed germination and seedling growth of goosefoot weed. The bands which exhibited the higher allelopathic activity were selected and subjected to analysis by Liquid chromatography-Mass spectrometry (LC/MS) to identify allelochemicals contents.

5. Statistical analysis

The data obtained were subjected to one- way analysis of variance (SPSS version 20) with means subjected to Duncan multiple range test (**Duncan, 1955**) at 5% probability level.

RESULTS AND DISCUSSION

1. Effect of cogongrass extracts on seed germination

1. 1. Germination inhibition

Data presented in Table (1) indicated that wheat and onion were the least inhibited plants by the aqueous extract of cogongrass rhizome or foliage with IC_{50} being values more than 4000 ppm. In contrast, goosefoot and fieldbind were the least tolerant plants to these extracts showing IC_{50} values of 2246 and 2586 ppm, respectively.

1. 2. Mean germination time (MGT)

Data in Table (2) indicated that the maximum delay in germination was recorded at the higher concentrations of rhizome and foliage extracts. Rhizome extract at 4000 ppm caused significant delay in germination to 1.62, 3.24, 1.59, 4.65, 5.17 and 3.00 days for wheat, wildoat, onion, fieldbind, goosefoot and sowthistle, respectively compared to the control. This delay time was 1.31, 2.51, 1.17, 3.95, 5.03 and 2.66 days respectively by foliage extract at the same concentration. Therefore, it was evident that delay in germination was more pronounced by rhizome extract treatments.

1. 3. Germination index (GI)

Data in Table (3) showed that when concentration of aqueous extract was increased, the value of GI was decreased. These values were significantly reduced for fieldbind to 5.90, 3.04, 2.17 and 1.49 by rhizome extract at 500, 1000, 2000 and 4000 ppm, respectively and 7.23, 5.87, 3.83 and 2.51 by foliage extract at the same concentrations, respectively, compared to GI 9.19 in the control. Because the GI is directly correlated with germination percentage (GP), therefore the higher percentage of germination gave higher values of GI, and consequently, the minimum GI value was observed at the higher concentration (4000 ppm) indicating stronger inhibitory effect on GI and also on GP.

Table (1): Germination inhibition percentages of seeds and IC_{50} Values treated with cogongrass extracts at given concentration.

Test species	Concentration (ppm)				IC_{50} (ppm)	95% Confidence limits		Slope ±S.E.
	500	1000	2000	4000		Lower	Upper	
Rhizome extract								
Wheat	2	4	15	22	> 4000	-	-	-
Wildoat	11	22	36	54	3428	2661	5038	1.46±0.21
Onion	12	19	30	38	>4000	-	-	-
Fieldbind	25	34	42	57	2840	2018	5228	0.92±0.19
Goosefoot	15	36	48	61	2246	1806	2988	1.40±0.20
Sowthistle	13	26	35	61	2939	2334	4090	1.49±0.21
Foliage extract								
Wheat	3	8	17	24	>4000	-	-	-
Wildoat	8	12	27	47	>4000	-	-	-
Onion	6	11	20	31	>4000	-	-	-
Fieldbind	6	27	40	63	2586	2176	3219	1.94±0.23
Goosefoot	26	37	45	53	3045	2022	7320	0.77±0.19
Sowthistle	9	17	22	52	>4000	-	-	-

Table (2): Mean germination time of seeds treated with cogongrass extracts at given concentration.

Treatments		Mean germination time (day)					
		Wheat	**Wildoat**	**Onion**	**Fieldbind**	**Goosefoot**	**Sowthistle**
Rhizome extract (ppm)	500	$2.85\pm0.16^{ab*}$	3.55 ± 0.10^{b}	3.67 ± 0.17^{a}	3.90 ± 0.07^{bc}	3.51 ± 0.50^{ab}	4.45 ± 0.06^{b}
	1000	3.22 ± 0.27^{b}	4.35 ± 0.12^{d}	3.86 ± 0.07^{a}	4.20 ± 0.06^{cd}	4.20 ± 0.12^{b}	4.89 ± 0.10^{c}
	2000	3.92 ± 0.25^{c}	5.25 ± 0.13^{f}	4.85 ± 0.07^{bc}	5.93 ± 0.12^{f}	6.04 ± 0.11^{d}	5.23 ± 0.19^{d}
	4000	4.25 ± 0.05^{c}	6.49 ± 0.07^{h}	5.17 ± 0.04^{c}	7.20 ± 0.19^{h}	8.00 ± 0.23^{e}	6.88 ± 0.33^{g}
Foliage extract (ppm)	500	2.70 ± 0.11^{ab}	3.80 ± 0.08^{bc}	3.62 ± 0.05^{a}	3.85 ± 0.05^{b}	3.36 ± 0.34^{a}	4.20 ± 0.10^{ab}
	1000	3.25 ± 0.20^{b}	3.92 ± 0.10^{c}	3.89 ± 0.17^{a}	4.48 ± 0.10^{d}	5.32 ± 0.07^{c}	4.78 ± 0.10^{c}
	2000	3.88 ± 0.29^{c}	4.68 ± 0.06^{e}	3.92 ± 0.09^{a}	4.95 ± 0.15^{e}	6.46 ± 0.10^{d}	5.67 ± 0.27^{e}
	4000	3.94 ± 0.07^{c}	5.76 ± 0.07^{g}	4.75 ± 0.04^{b}	6.50 ± 0.10^{g}	7.86 ± 0.05^{e}	6.54 ± 0.09^{f}
Control		$\mathbf{2.63\pm0.17^{a}}$	$\mathbf{3.25\pm0.09^{a}}$	$\mathbf{3.58\pm0.21^{a}}$	$\mathbf{2.55\pm0.06^{a}}$	$\mathbf{2.83\pm0.23^{a}}$	$\mathbf{3.88\pm0.19^{a}}$

*Mean in each column followed by the same letter are not significantly different at P = 0.05.

Table (3): Germination index of seeds treated with cogongrass extracts at given concentration.

Treatments		Germination index					
		Wheat	**Wildoat**	**Onion**	**Fieldbind**	**Goosefoot**	**Sowthistle**
Rhizome extract (ppm)	500	$9.47\pm0.88^{a*}$	4.90 ± 0.54^{b}	4.93 ± 0.75^{b}	5.90 ± 0.48^{c}	6.83 ± 0.37^{b}	5.37 ± 0.37^{bc}
	1000	7.76 ± 0.95^{bc}	3.45 ± 0.27^{c}	3.99 ± 0.32^{bc}	3.04 ± 0.09^{de}	4.76 ± 0.30^{c}	4.67 ± 0.14^{cd}
	2000	6.38 ± 0.30^{cde}	2.47 ± 0.24^{d}	3.09 ± 0.07^{cd}	2.17 ± 0.05^{ef}	1.98 ± 0.16^{f}	4.46 ± 0.17^{d}
	4000	5.18 ± 0.39^{e}	1.54 ± 0.23^{e}	2.28 ± 0.15^{d}	1.49 ± 0.22^{f}	1.63 ± 0.07^{f}	3.60 ± 0.08^{e}
Foliage extract (ppm)	500	9.26 ± 0.37^{ab}	4.33 ± 0.36^{b}	5.52 ± 0.52^{ab}	7.23 ± 0.10^{b}	6.20 ± 0.57^{b}	6.05 ± 0.46^{b}
	1000	7.35 ± 0.06^{cd}	4.07 ± 0.10^{bc}	4.88 ± 0.78^{b}	5.87 ± 0.34^{c}	4.37 ± 0.52^{cd}	5.35 ± 0.23^{bc}
	2000	5.92 ± 0.68^{de}	3.46 ± 0.22^{c}	4.33 ± 0.39^{bc}	3.83 ± 0.29^{d}	3.43 ± 0.08^{de}	4.47 ± 0.16^{d}
	4000	5.02 ± 0.09^{e}	2.08 ± 0.04^{de}	3.15 ± 0.14^{cd}	2.51 ± 0.21^{ef}	3.21 ± 0.31^{e}	3.37 ± 0.22^{e}
Control		$\mathbf{10.88\pm0.34^{a}}$	$\mathbf{6.19\pm0.09^{a}}$	$\mathbf{6.67\pm0.59^{a}}$	$\mathbf{9.19\pm0.97^{a}}$	$\mathbf{9.89\pm0.22^{a}}$	$\mathbf{7.15\pm0.30^{a}}$

*Mean in each column followed by the same letter are not significantly different at P = 0.05

2. Effect of cogongrass extracts on seedling growth

2. 1. Root length

Data in Table (4) revealed that root lengths of goosefoot and sowthistle plants were the most reduced by rhizome and foliage aqueous extracts with IC_{50} values 560 and 747 ppm, respectively. Wheat and onion roots were the least affected with IC_{50} values 2228 and 3243 ppm, respectively. Thus, goosefoot and sowthistle roots were 3.98 and 4.34 folds more sensitive than wheat and onion roots in this respect.

2.2. Shoot length

Data in Table (5) showed that shoot length of goosefoot plants was the most reduced by rhizome and foliage extracts with 1016 and 1493 ppm IC_{50} values, while wheat and onion were the least affected with IC_{50} values more than 4000 and 3778 ppm respectively by rhizome extract and more than 4000 ppm by foliage extract. Generally, rhizome extract was more effective on seedling growth than foliage extract.

2. 3.Dry weight

Data given in Table (6) showed that the dry weights of onion and wheat seedlings were not significantly affected by rhizome and foliage aqueous extracts of cogongrass except for 4000 ppm for wheat. However, significant effects occurred with goosefoot, sowthistle, fieldbind and wildoat seedlings, except for wildoat and sowthistle at low concentration of foliage extract 500 ppm compared with the control.

These results obtained revealed that seed germination and seedling growth of dicotyledonous weeds namely goosefoot and sowthistle were the most affected by the aqueous extract of cogongrass (rhizome or foliage) whereas monocotyledons crops namely wheat and onion were the least affeted. Similar results were repated by (**Al-Mutlaq et *al*., 2002**) whom found that the dicotyledonous plants (alfalfa and wildradish) were more sensitive to the alkaloidal extract isolated from *Rhazya stricta* than the monocotyledonous plants (Italian ryegrass and wheat). Roots were more sensitive than shoot length in all tested plants. Moreover, **(Norsowrthy, 2003)** reported that the broadleaf weeds; sicklepod and pricklysida, showed higher sensitivity to the aqueous extract of wildradish than wheat and corn. Also, **Sabh and Ali (2010)** showed that shoot length of London rocket and root length of sowthistle were the most sensitive to the aqueous extract of nightshade based on IC_{50} values, while wheat was the least sensitive plant.

Table (4): Effect of cogongrass rhizome and foliage aqueous extracts on root length of the tested plants.

Test species	Concentration(ppm)				IC_{50} (ppm)	95% Confidence limits		Slope ±S.E.	Fold**
	500	1000	2000	4000		Lower	Upper		
Rhizome extract									
Wheat	21*	35	50	60	2228	1729	3166	1.18±0.20	1.00
Wildoat	35	51	61	85	986	767	1211	1.46±0.20	2.26
Onion	26	46	59	71	1383	1089	1748	1.30±0.20	1.61
Fieldbind	38	53	77	91	800	638	956	1.84±0.21	2.79
Goosefoot	46	71	80	96	560	412	693	1.84±0.24	3.98
Sowthistle	43	68	84	95	604	465	732	1.95±0.24	3.69
Foliage extract									
Wheat	22	30	45	62	2409	1871	3455	1.21±0.20	1.35
Wildoat	29	38	54	66	1697	1300	2316	1.10±0.19	1.91
Onion	24	32	40	55	3243	2247	6558	0.91±0.20	1.00
Fieldbind	33	44	65	80	1104	872	1354	1.45±0.20	2.94
Goosefoot	35	46	57	73	1208	885	1585	1.08±0.19	2.68
Sowthistle	40	57	74	86	747	550	932	1.48±0.21	4.34

*Percent inhibition in root length

**No. of folds compared with wheat in rhizome extract and onion in foliage extract

Table (5): Effect of cogongrass rhizome and foliage aqueous extracts on shoot length of the tested plants.

Test species	Concentration(ppm)				IC_{50} (ppm)	95% Confidence Limits		Slope ±S.E.
	500	1000	2000	4000		Lower	Upper	
extract Rhizome								
Wheat	2*	21	22	47	> 4000	-	-	-
Wildoat	20	35	63	78	1468	1244	1738	1.86±0.20
Onion	13	21	30	55	3778	2851	5922	1.38±0.21
Fieldbind	27	47	60	80	1228	998	1487	1.56±0.20
Goosefoot	33	49	64	85	1016	807	1233	1.55±0.20
Sowthistle	15	38	67	88	1327	1161	1512	2.45±0.23
Foliage extract								
Wheat	9	19	30	39	> 4000	-	-	-
Wildoat	17	30	44	65	2315	1872	3058	1.46±0.20
Onion	3	14	20	32	> 4000	-	-	-
Fieldbind	25	30	45	79	1744	-	-	2.06±0.22
Goosefoot	23	38	59	74	1493	1230	1823	1.56±0.20
Sowthistle	14	22	50	76	1965	1690	2334	2.08±0.22

*Percent inhibition in shoot length

Table (6): Effect of cogongrass rhizome and foliage aqueous extracts on the dry weight of the tested plants.

Treatments		Dry weight (mg/seedling)					
		Wheat	Wildoat	Onion	Fieldbind	Goosefoot	Sowthistle
Rhizome extract (ppm)	500	10.25 ± 0.43^{a}*	14.54 ± 0.92^{bc}	3.00 ± 0.54^{a}	5.60 ± 0.22^{bc}	2.00 ± 0.25^{b}	2.00 ± 0.20^{b}
	1000	9.00 ± 0.35^{ab}	12.16 ± 2.30^{bcd}	2.58 ± 0.66^{a}	4.64 ± 0.39^{cd}	1.70 ± 0.12^{bc}	1.25 ± 0.14^{c}
	2000	8.75 ± 0.66^{ab}	9.51 ± 0.46^{d}	2.50 ± 0.74^{a}	3.52 ± 0.37^{d}	1.25 ± 0.10^{cd}	0.70 ± 0.09^{d}
	4000	6.25 ± 0.32^{c}	4.76 ± 0.31^{e}	1.86 ± 0.25^{a}	1.84 ± 0.30^{e}	0.25 ± 0.03^{e}	0.30 ± 0.07^{d}
Foliage extract (ppm)	500	10.50 ± 0.54^{a}	15.86 ± 2.37^{ab}	3.20 ± 0.45^{a}	6.00 ± 0.54^{b}	2.20 ± 0.36^{b}	2.25 ± 0.32^{ab}
	1000	9.50 ± 1.70^{ab}	11.23 ± 0.60^{cd}	2.90 ± 0.45^{a}	5.12 ± 0.42^{bc}	1.79 ± 0.18^{bc}	2.00 ± 0.20^{b}
	2000	9.00 ± 0.35^{ab}	10.04 ± 0.78^{d}	2.50 ± 0.20^{a}	3.60 ± 0.22^{d}	1.30 ± 0.12^{cd}	1.40 ± 0.09^{c}
	4000	7.50 ± 0.33^{bc}	5.81 ± 0.48^{e}	2.00 ± 0.35^{a}	2.40 ± 0.23^{e}	0.90 ± 0.17^{d}	0.60 ± 0.14^{d}
Control		11.00 ± 0.98^{a}	18.50 ± 0.72^{a}	3.5 ± 0.35^{a}	8.00 ± 0.20^{a}	3.00 ± 0.20^{a}	2.70 ± 0.12^{a}

*Mean in each column followed by the same letter are not significantly different at P = 0.05.

3-Isolation and identification of allelochemicals

3.1. Different bands isolated by TLC and their inhibitory effects on goosefoot.

Data in Table (7) showed that the crude aqueous extract of rhizome and foliage cogongrass exhibited five fractions with R_f values of 0.40, 0.64, 0.77, 0.87 and 0.96 by rhizome extract and 0.42, 0.65, 0.73, 0.80 and 0.96 by foliage extract. The band No.2 (R_f = 0.64) in rhizome extract and (R_f = 0.65) in foliage extract were the most effective and caused considerable inhibitory effect on seed germination and seedling growth of goosefoot. Both fractions significantly inhibited germination of goosefoot seeds to 53.8% by rhizome extract and 47.3% (foliage extract). Also, the same fractions caused significant reduction in shoot and root lengths that reached to 60.0-66.1% by rhizome extract, 46.7 - 62.7% by foliage extract, respectively. These two fractions also gave a positive reaction with folin-ciocalteu reagent indicating the presence of phenolic compounds.

3.2. Phenolic compounds and its derivatives identified by LC/MS.

The results in Tables (8 and 9) and Figs. (1and 2) reveal the presene of six phenolic acids and derivatives in rhizome and foliage extracts of cogongrass as identified by LC/MS. For the rhizome extract, the dominant compound detected in the 2nd band (R_f =0.64), was chlorogenic acid, representing 27.81% of the total compounds, followed by vanillic acid, 3'-o- methyl- (-)-epicatechin-7-o-sulphate and ferulic acid (12.87, 9.67 and 7.84% respectively). Only traces of coumaric acid (1.01%) were detected as shown in Table (8) and Fig (1). Meanwhile, in the foliage aqueous extract, principal compounds in the 2 nd band (R_f=0.65) were chlorogenic acid (53.49%) and caffeic acid (31.48%), forming 84.97% of the total compounds deteted. In contrast, two phenolic compounds: (-)-epigallocatechin-3,5-digallate and gallic acid were present in lower percentages , 9.22 and 1.89% respectively as given in Table (9) and Fig. (2).The chemical structures of phenolic compounds identified are listed in Fig. (3).

These results are in accordance to the findings of **Ali (2005)** that aqueous extract of *Cyperus rotundus* exhibited allelopathic activity on germination and growth of *Echinochloa crus-galli* and *Phalaris minor* due to the presence of phenolic compounds identified as kaempferol, p.hydroxybenzoic, chlorogenic and ferulic acids. In thisrespet, **Saleem *et al.* (2013)** mentioned that 4- hydroxybenzoic, vanillic, p-coumaric, caffeic, gallic and protocatechuic acids were phenolic compounds identified in mango leaves extract, which could be used as a herbicide to suppress canarygrass and to enhance wheat growth. Also, **Won *et al.* (2013)** identified five phenolic compounds (P-coumaric acid, P-hydroxybenzoic acid, ferulic acid, trans-cinnamic acid and kaempferol) in sorghum leaf extract. This extract from grinding plus boiling for 24h caused 100% suppression of broadleaf weed species and 52% suppression in grass weed species, whereas the extract from cutting plus soaking (24h) resulted in similar degrees of inhibition in both broadleaf and grass weed species. Generally, the presence of phenolic compounds in plant extracts was also reported by **Eussen and Niemann (1981)** using *Imperata cylindrica* extract ; by **Al-Wakeel *et al*. (2007)** using *Acacia nilotica* extract, by **El Rokiek *et al.* (2010)** using *Cyperus rotundus* extract and **Yousaf *et al.* (2013)** using *Psidium guajava* extract. They showed that these extracts contain phenolic acids which have allelopathic activity on seed germination and growth of weed species.

Table (7): Effect of various bands isolated from cogongrass aqueous extracts by TLC on seed germination (SG%), shoot length (SL) and root length (RL) of goosefoot.

Treatments	Rhizome extract				Foliage extract			
	R_f	SG (%)	SL(cm)	RL(cm)	R_f	SG (%)	SL(cm)	RL(cm)
Control	-	91^{a*}	3.0^{a}	5.9^{a}	-	91.0^{a}	3.0^{a}	5.9^{a}
Band 1	0.40	50.2^{d} (45.0)**	2.9^{ab} (3.3)	2.3^{c} (61.0)	0.42	50.0^{c} (45.1)	1.8^{b} (40.0)	3.5^{bc} (40.7)
Band 2	0.64	42.0^{e} (53.8)	1.2^{c} (60.0)	2.0^{c} (66.1)	0.65	48.0^{c} (47.3)	1.6^{b} (46.7)	2.2^{c} (62.7)
Band 3	0.77	70.6^{c} (22.7)	2.5^{b} (16.7)	3.2^{bc} (45.8)	0.73	69.1^{b} (24.1)	2.4^{a} (20.0)	2.5^{c} (57.6)
Band 4	0.87	80.0^{b} (12.1)	2.7^{ab} (10.0)	4.5^{ab} (23.7)	0.80	80.4^{ab} (11.6)	2.8^{a} (6.7)	3.1^{c} (47.5)
Band 5	0.96	89.0^{a} (2.2)	3.0^{a} (0.0)	5.8^{a} (1.7)	0.96	88.7^{a} (2.5)	2.8^{a} (6.7)	4.8^{ab} (18.6)

*Mean in each column followed by the same letter are not significantly different at P=0.05.
** Each figure between bracket represents the percentage of inhibition

Table (8): Phenolic compounds and its derivatives in rhizome aqueous extract of cogongrass (R_f = 0.64) identified by LC/MS.

NO	Compounds	RT *	M.W.**	M. F.***	% of total
1	**Vanillic acid**	1.20	168.04	$C_8H_8O_4$	12.87
2	**Ferulic acid**	1.35	194.18	$C_{10}H_{10}O_4$	7.84
3	**Chlorogenic acid**	36.76	354.10	$C_{16}H_{18}O_8$	27.81
4	**Coumaric acid**	40.81	164.16	C_9H_8O	1.01
5	**3'-O-methyl-(-)-epicatechin-7-O-sulphate**	41.98	367.35	$C_{16}H_{15}O_8S$	9.67

* Retention time (min) **Molecular weight *** Molecular formula

Table (9): Phenolic compounds and its derivatives in foliage aqueous extract of cogongrass (R_f = 0.65) identified by LC/MS.

NO.	Compounds	RT *	M.W.**	M. F.***	% of total
1	**Chlorogenic acid**	36.46	354.10	$C_{16}H_{18}O_8$	53.49
2	**(-)-epigallocatechin-3,5-digallate**	37.74	610.47	$C_{29}H_{22}O_{15}$	9.22
3	**Caffeic acid**	42.43	180.16	$C_9H_8O_4$	31.48
4	**Gallic acid**	43.46	170.12	$C_7H_6O_5$	1.89

* Retention time (min) **Molecular weight *** Molecular formula

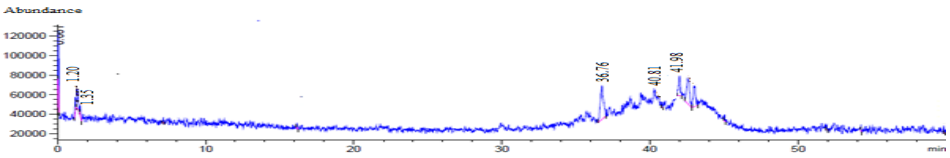


Fig. (1): Chromatogram of compounds identified in most active fraction (Rf = 0.64) of rhizome aqueous extract by LC/MS.

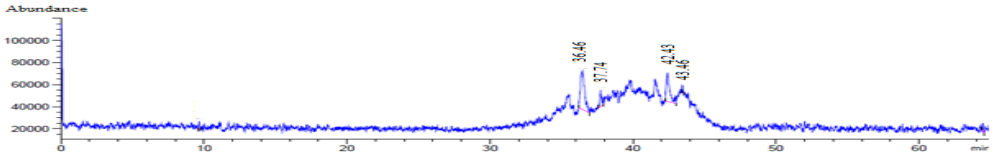
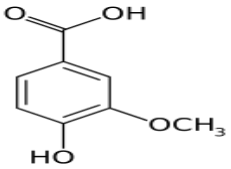
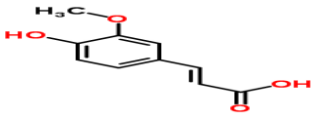


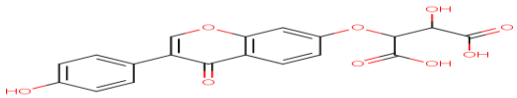
Fig. (2): Chromatogram of compounds identified in most active fraction (Rf = 0.65) of foliage aqueous extract by LC/MS.



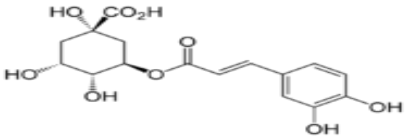
Vanillic acid



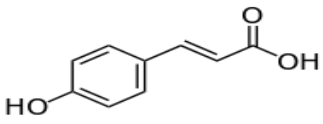
Ferulic acid



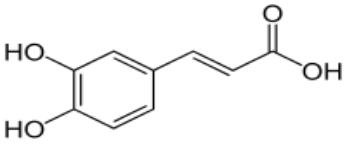
(-)-Epigallocatechin -3,5-di gallate



Chlorogenic acid



Coumaric acid



Caffeic acid

Fig. (3): The chemical structure of some the phenolic compounds identified through LC/MS analysis.

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التأثير الأليلوباثي لحشيشة الحلفا علي القمح والبصل وبعض الحشائش المصاحبة لهما

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اجري هذا البحث لدراسة تأثير المستخلص المائي من ريزومات وأوراق حشيشة الحلفا علي إنبات ونمو بذور ست انواع من المحاصيل والحشائش وهي القمح – البصل – الزمير– الزربيح – الجعضيض – العليق وكذلك فصل وتعريف المركبات ذات التأثير الأليلوباثي لمستخلص الحلفا من الريزومات والأوراق باستخدام Thin layer chromatography (TLC) و Liquid chromatography/Mass spectrometry (LC/MS). وقد أظهرت النتائج: وجود اختلافات في الحساسية ما بين الانواع النباتية المختبرة في الانبات والنمو للمستخلص المائي من الحلفا علي اساس قيم IC_{50}. كانت الحشائش ذات الفلقتين مثل الزربيح والجعضيض والعليق اكثر حساسية من المحاصيل ذات الفلقة الواحدة مثل القمح والبصل مما يعطي إمكانية الاستفادة من التاثير النوعي (المتخصص) للحلفا في مكافحة الحشائش عريضة الأوراق النامية في المحاصيل ذات الفلقة الواحدة. بذور القمح والبصل كانت الأكثر مقاومة للمستخلص المائي من الحلفا بقيم IC_{50} اكبر من ٤٠٠٠ جزء في المليون بينما كانت بذور الزربيح والعليق أقلهم مقاومة بقيم IC_{50}= ٢٢٤٦ و ٢٥٨٦ جزء في المليون لمستخلص الريزومات والأوراق علي الترتيب. بقياس طول الجذير والريشة كان الزربيح والجعضيض الاكثر حساسية للمستخلص في حين كان القمح والبصل أقلهم حساسية علي اساس قيمة IC_{50}. تقل سرعة الانبات مع زيادة مستوي تركيز المستخلص وعموماً كان التأخير في إنبات بذور الحشائش اكثر وضوحاً من بذور المحاصيل كذلك انخفضت قيمة معامل إنبات بذور الحشائش خاصة الزربيح والعليق بدرجة كبيرة عند التركيزات العالية من المستخلص وقد أدي ذلك الي انخفاض النسبة المئوية للانبات في حين كانت بذور القمح هي الأقل انخفاضاً. لم يتأثر معنوياً الوزن الجاف لبادرات القمح والبصل بواسطة مستخلص الريزومات والأوراق من الحلفا وعلي العكس من ذلك تأثرت الأوزان الجافة معنوياً لبادرات حشائش الزربيح والجعضيض والعليق مقارنة بالكنترول. يحتوي المستخلص المائي من الحلفا (الريزومات والأوراق) خمس مكونات مفصولة بواسطة TLC كانت ذات قيم R_f ٠,٤٠، ٠,٦٤، ٠,٧٧، ٠,٨٧ و ٠,٩٦ من مستخلص الريزومات و ٠,٤٢، ٠,٦٥، ٠,٧٣، ٠,٨٠ و ٠,٩٦ من مستخلص الأوراق وبدراسة تأثير كل مكون علي حدة ضد بذور وبادرات الزربيح. وتبين النتائج ان المكونات ذات قيم R_f ٠,٦٤ و ٠,٦٥ كانت الأكثر تأثيراً علي بذور الزربيح حيث أدت الي تثبيط معنوي في إنبات البذور بلغ ٥٣,٨ و ٤٧,٣% علي الترتيب كما أدت نفس المكونات الي نقص واضح في طول الريشة والجذير لبادرات الزربيح بلغت ٦٠,٠ و ٦٦,١% من المكون (R_f=٠,٦٤) المفصول من مستخلص الريزومات و ٤٦,٧ و ٦٢,٧% من المكون (R_f =٠,٦٥) المفصول من مستخلص الأوراق علي التوالي. ولذلك تم وضع هذه المكونات ذات التأثير الأليلوباثي للتحليل من خلال جهاز LC/MS بغرض تعريف مركباتها الكيميائية. وقد أوضحت نتائج هذا التحليل ان المستخلص من ريزومات و أوراق الحلفا يحتوي علي المركبات الفينولية التالية :

coumaric acid, vanillic acid, ferulic acid, (-)-epigallocatechin-3,5-digallate, 3'-o-methyl-(-)-epicatechin-7-o-sulphate, caffeic acid, gallic acid and chlorogenic acid

وقد تكون هذة المركبات الفينولية هي المسئولة عن النشاط الأليلوباثي لمستخلص الحلفا علي إنبات بذور الأنواع النباتية المختبرة ونمو بادراتها خاصة الحشائش منها.

الكلمات الدالة: الاليلوباثي– مستخلص الحلفا– الإنبات– نموالبادرات– الحشائش– المحاصيل– المثبطات الكيميائية.