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Allelopathic Potential of *Ruta graveolens* L. on Seed Germination and Seedling Growth of two weed species *Panicum turgidum* Forssk. and *Phalaris minor* Retz.

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ABSTRACT

Panicum turgidum and *Phalaris minor* are invasive weeds of crop fields in Egypt. The main objective of the present study was to indicate the potentials for utilization of *Ruta graveolens* shoot aqueous extract and leachate at different concentrations (2.5%, 5.0%, 7.5% and 10.0%) to suppress the germination and growth of *P. turgidum* and *P. minor* in Petri dish experiment. Results indicated that the degree of inhibition on seed germination and growth of both the recipient species was largely dependent on the concentration of *R. graveolens* shoot aqueous extract and leachate. The allelopathic effect was statistically significant at $p \leq 0.05$ for most treatments. The results also showed that the plumule length of *P. turgidum* was more sensitive than that of *P. minor* and responds more strongly to the increase of concentration of *R. graveolens* shoot aqueous extract and leachate. On the contrary, radicle length of *P. minor* was the more sensitive to *R. graveolens* shoot aqueous extract and leachate. Therefore, *R. graveolens* extract and leachate may be offer promises for their usefulness as a tool for weed management.

Key words: allelopathy, *Ruta graveolens*, weed control, *Panicum turgidum*, *Phalaris minor*.

INTRODUCTION

Allelopathy is a phenomenon that includes the direct or indirect harmful or beneficial effects of a chemical from one plant on another (Ashrafi *et al*., 2008). Allelochemicals are usually the secondary metabolites that have a great potential as natural pesticides and as growth promoters (Chon *et al*., 2005). Applied allelopathy is believed to have the potential of being of great help for the management of agro-ecosystems (Hegazy, 1999). Weeds are considered to be a major constraint to agriculture production throughout the world. Allelopathy plays an important role in agricultural ecosystems, and is recognized as appropriate potential technology to control weeds using chemicals released from decomposed plant parts of various species (Naseem *et al*., 2009). The current worldwide demand for environmentally safe for weed management technologies relies on the allelopathic potential of some plant species.

Ruta graveolens L. is an important plant of family Rutaceae. The plant is herbaceous perennial, originally native to the Mediterranean region. It is 0.6 to 0.9 m tall and almost as wide. Leaves are dissected pinnately into oblong segments. The green foliage has a strong, pungent, rather unpleasant scent when bruised. Flowers are yellow colored with four concave notched petals. *R. graveolens* has been used for centuries as herbal remedy in traditional medicine for treatment of various conditions such as eye problems, rheumatism, dermatitis, pain and many inflammatory diseases (Ratheesh & Helen, 2007). Due to its cultural and medicinal value, *R. graveolens* has been introduced in various countries of North, Central and South America, China, India, Middle East and South Africa (Miguel, 2003).

The most commonly known phytochemical compounds from *R. graveolens* are terpenoids, flavonoids, furoquinotines, volatile substances and acridone alkaloids (Kuzovkina *et al*., 2004). Saponin, tannins and glycosides has also been recorded (Hashemi *et al*., 2011). The main active flavonoids of *R.graveolens* are rutin and quercetin (Pathak *et al*., 2003). A high content of aliphatic acids, alcohols and ketones were found in the plant volatile oil (Ivanovaa *et al*., 2003). Rutacridone, rutacridone epoxide and gravacridondiol are acridone alkaloids isolated from the plant root (Meepagala *et al*., 2005), while an alkaloid named graveoline has been isolated from the leaves (Hale *et al*., 2004). Makizadeh *et al*. (2009) studied the allelopathic effects of *R. graveolens* on seed germination and seedling growth of three weeds *Amaranthus retroflexus*, *Descurainia sophia* and *Portulaca oleracea*, they found *R. graveolens* has a sufficient allelopathic effect to suppress seed germination and seedling growth of these weeds so it might be useful as natural herbicides and might also contain numerous growth inhibitors that could be used for the development of biological herbicides.

The aim of this study was to investigate the possible allelopathic efficiency of *R. graveolens* aqueous extract and leachate obtained from aerial shoots of the plant, and their potential uses as control on weed seed germination and seedling growth of two weed species *P. turgidum* and *P. minor*.

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MATERIALS AND METHODS

Collection and Preparation of Plant Materials

A number of fresh samples from the aerial plant parts (leaves, young stems and flowers) of *R. graveolens* were harvested during the flowering stage of the plant from the botanic garden of the Faculty of Science Alexandria University. Some of the collected samples were air-dried at room temperature for one week, then ground in a Wiley Mill to fine uniform texture and stored in glass jars until use.

Preparation of Aqueous Extract and Leachate

Stock aqueous extract was obtained by soaking 50 g air-dried plant material in 500 ml of cold distilled water (10 % w/v) at room temperature (20 ± 2ºC) for 24 hours with occasional shaking. Stock leachate was obtained by soaking 50 g fresh samples of aerial shoots in 500 ml of cold distilled water (10 % w/v) at room temperature (20 ± 2ºC) for 24 hours with occasional shaking. The mixtures of both the extract and the leachate were filtered through two layers of cheese cloth and centrifuged for 20 minutes at 5000 rpm to remove particulate materials. The supernatants were adjusted to 6.8 with 1M HCl and were kept in the refrigerator at 5ºC until used. Different concentrations (2.5, 5.0, 7.5, and 10.0%) were prepared from the stock solution of both the extract and the leachate, in addition to the control (distilled water).

Preparation of Seeds for Germination Bioassay

The seeds were surface sterilized with 2% sodium hypochlorite for 2 min before sowing, then rinsed four times with distilled water. The sterilized seeds were soaked in aerated distilled water for 24 h.

Germination Bioassay

In order to study the allelopathic effect of aqueous extract and leachate of *R. graveolens* on germination and seedling growth of *P. turgidum* and *P. minor*, an experiment was conducted in a plan with 5 treatments and 3 replications of both the extract and leachate for each of the recipient weed species. Ten seeds of each the weed species (*P. turgidum* and *P. minor*) were arranged in 9 cm diameter Petri-dishes lined with two discs of Whatman No.1 filter paper under laboratory conditions with day temperature ranging from 20-22 ºC and night temperature from 14-16 ºC. Ten ml of the prepared aqueous extract from various concentrations were added to each of the recipient species. The first count of the germinated seeds was done five days later. The germination percentage, plumule and radicle lengths were recorded every two days until the end of the experiment after 14 days.

Calculations

(1) *Germination percentage (GP)*

GP = number of germinated seeds / total number of seeds X 100

(2) *Inhibition percentage (IP)*

Inhibition percentage (IP) was calculated according to the general equation:

IP= [1- (allelopathic/control) 100]

(3)*The Reduction percentage in plumule and Radicle Length*

Reduction percentage (RP) was calculated according to the general equation:

RP= [(control-allelopathic)/control] x 100

Statistical Analysis

Data concerning the effect of different concentrations of *R. graveolens* shoot aqueous extract (RGSAE) and leachate (RGSAL) on germination percentage (GP), hypocotyl (HL) and radicle (RL) lengths, were subjected to standard analysis of variance (ANOVA) using COSTAT 2.00 statistical analysis software manufactured by Cohort software company (Zar, 1984).

RESULTS

Percentage germination of the two tested weed species *Panicum turgidum* and *Phalaris minor* decreased as the *Ruta graveolens* shoot aqueous extract concentration increased from 2.5% to 10% (Table 1). A significant decrease in germination percentage (P = 0.036) is noticed in *P. turgidom*. The aqueous leachate of *R. graveolens* showed considerable effect on the germination percentage of *P. minor* reducing it to 50% when using a concentration of 10%of the aqueous leachate. On the contrary, it seems that *R. graveolens* aqueous leachate in concentrations 2.5%, 5.0% & 7.5% promoted the germination of *P. turgidum* seeds. The inhibition percentage (Fig. 1) showed the same trend; the aqueous extract of *R. graveolens* showed the highest allelopathic effect on the germination of the seeds of both the recipient weed species. The inhibition percentages of the seeds of *P.turgidum* were 31%, 12% & 16% for the concentrations 5.0%, 7.5% & 10%of the aqueous extract of *R. graveolens* respectively. For *P. minor*, the inhibition percentages were 9%, 9%, 39% & 48%for the concentrations 2.5%, 5.0%, 7.5% & 10% of *R. graveolens* aqueous extract respectively.

Non-significant effect was recorded for the final cumulative germination rate of both tested weed species after fourteen days treatment in response to the concentration (Fig. 2).

The highest concentration (10%) treatment of *R. graveolens* extract and leachate attained with the greatest reduction in the radicle and plumule lengths of *P. turgidum* after fourteen days. Plumule and radicle lengths of *P. turgidum* at extract treatment 10% were reduced by 51% and 56% respectively, while they were reduced by 39% and 53% respectively in the leachate (Fig. 3). In *P. minor*, 7.5% *R. graveolens* aqueous extract and leachate attained with the greatest reduction in the radicle and plumule lengths after fourteen days. Plumule and radicle lengths of *P. minor* at extract treatment 7.5% were reduced by 23% and 76% respectively, while at leachate they were reduced by 38% and 57% respectively (Fig. 4).

Analysis of variance (ANOVA) of the allelopathic effect of different concentrations of *R. graveolens* aqueous extract and leachate on radicle and plumule lengths of *P. turgidum* and *P. minor* was presented in Tables (2 and 3) respectively. The results are statistically significant at $P \leq 0.05$ except aqueous leachate on the plumule length of *P.minor* which show a slightly lower effect (P= 0.056).

Generally, a considerable decrease in the fresh weight of seedlings is notable after fourteen days for both the recipient species. The highest concentration (10%) of *R. graveolens* aqueous extract and leachate attained with the greatest reduction in seedlings fresh weight of both the recipient species; *P. turgidum* and *P. minor* ,as shown in (Fig. 5).

Table (1): Allelopathic effect of different concentrations of *R. graveolens* (RG) aqueous extract and leachate on germination percentage of the two selected weed species.

Concentrations	Germination (%)			
	Phalaris minor		*Panicum turgidum*	
	RG Extract	RG leachate	RG Extract	RG leachate
0%	33.3 ± 15.3	40.0 ± 20.0	83.3 ± 5.8	76.7 ± 11.5
2.5%	30.0 ± 10.0	33.3 ± 5.8	93.3 ± 5.8	93.3 ± 11.5
5.0%	30.0 ± 10.0	43.3 ± 25.2	56.7 ± 15.3	90.0 ± 0.0
7.5%	20.0 ± 10.0	30.0 ± 10.0	73.3 ± 11.5	93.3 ± 5.8
10.0%	16.7 ± 5.8	20.0 ± 10.0	70.0 ± 17.3	76.7 ± 15.3
F	1.382	0.987	3.932*	2.094
P	0.308	0.458	0.036*	0.157
LSD 5%	19.38	28.98	22.05	18.81

F: F test (ANOVA)
*: Statistically significant at p ≤ 0.05

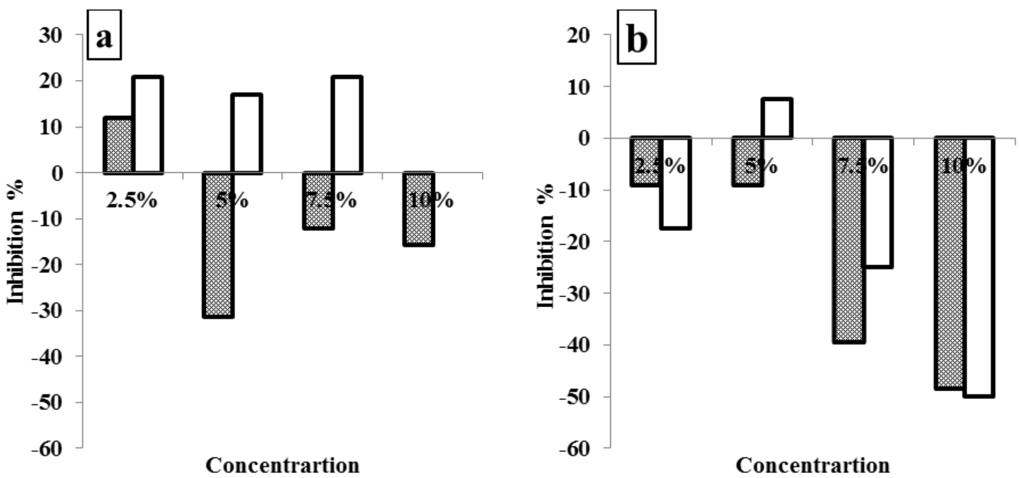

Figure (1): Inhibition percentages of *P. turgidum* (a) and *P. minor* (b) seeds under the different concentrations of *R. graveolens* aqueous extract and aqueous leachate.

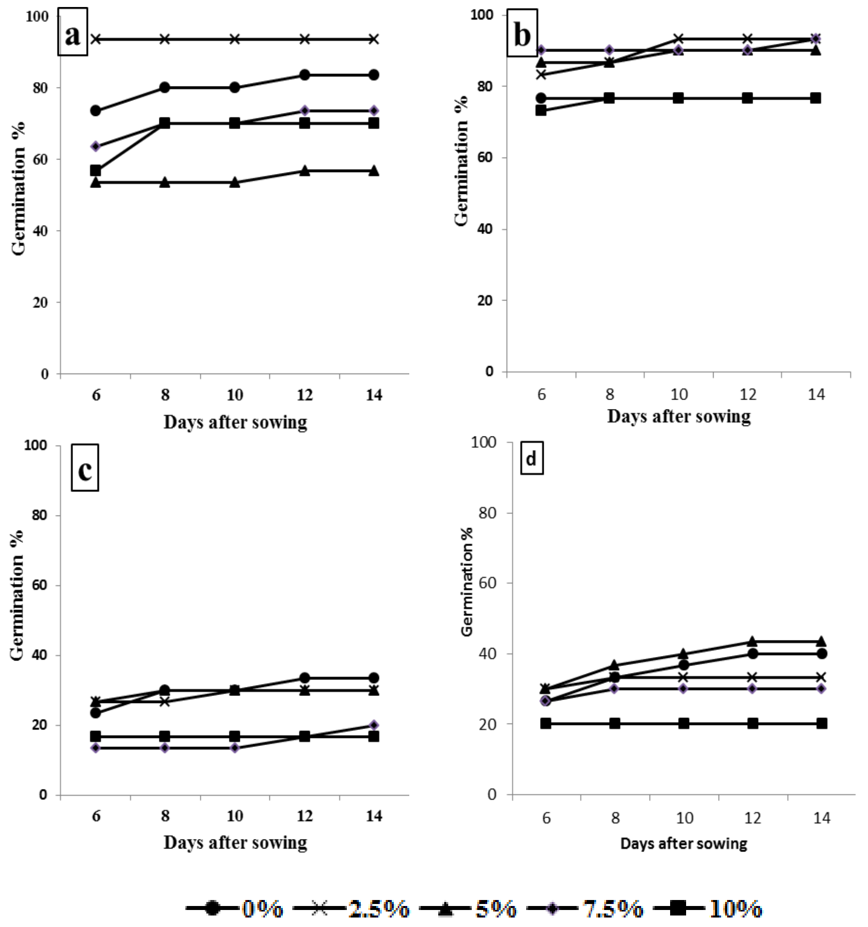

Figure (2): Germination percentages of *P. turgidum* (a & b) and *P. minor* (c & d) seeds under the different concentrations of *R. graveolens* aqueous extract (a & c) and aqueous leachate (b & d).

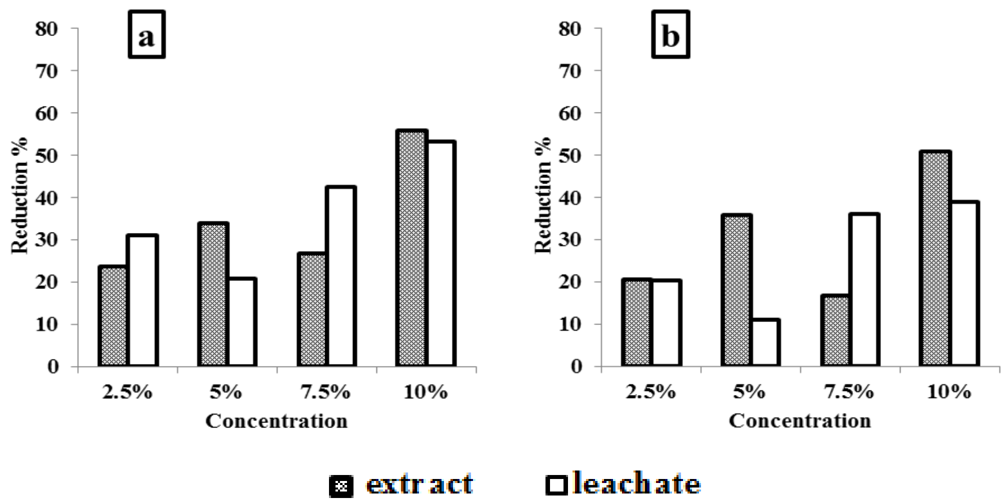

Figure (3): Reduction percentages of *P. turgidum* radicle and plumule (a & b) under the different concentrations of *R. graveolens* aqueous extract and aqueous leachate.

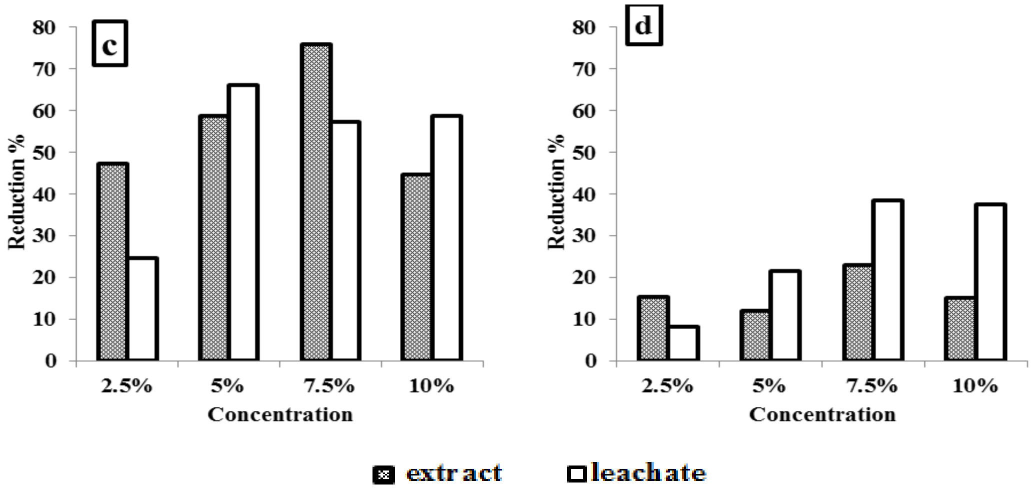

Figure (4): Reduction percentages of *P. minor* radicle and plumule (c & d) under the different concentrations of *R graveolens* aqueous extract and aqueous leachate.

Table (2): Allelopathic effect of different concentrations of *R. graveolens* (RG) aqueous extract and leachate on radicle and plumule length (cm) of *P. turgidum.*

Concentrations	*Panicum turgidum*			
	Radicle		Plumule	
	RG Extract	RG leachate	RG Extract	RG leachate
0%	3.70 ± 0.26	3.70 ± 0.26	3.16 ± 0.34	3.16 ± 0.34
2.5%	2.82 ± 0.71	2.55 ± 0.37	2.51 ± 0.21	2.52 ± 0.39
5.0%	2.44 ± 0.71	2.93 ± 1.20	2.03 ± 0.21	2.81 ± 0.20
7.5%	2.71 ± 0.26	2.13 ± 0.16	2.63 ± 0.15	2.02 ± 0.26
10.0%	1.63 ± 0.34	1.73 ± 0.49	1.55 ± 0.52	1.93 ± 0.51
F	6.627*	4.490*	11.180*	6.344*
P	0.007*	0.025*	0.001*	0.008*
LSD 5%	0.911	1.126	0.575	0.652

F: F test (ANOVA)
*: Statistically significant at $p \leq 0.05$

Table (3): Allelopathic effect of different concentrations of *R. graveolens* (RG) aqueous extract and leachate on radicle and plumule length (cm) of *P. minor*.

Concentrations	*Phalaris minor*			
	Radicle		Plumule	
	RG Extract	RG leachate	RG Extract	RG leachate
0%	7.22 ± 0.20	7.22 ± 0.20	16.37 ± 0.65	16.37 ± 0.65
2.5%	3.80 ± 0.75	5.44 ± 0.77	13.84 ± 0.23	15.04 ± 0.12
5.0%	2.98 ± 0.28	2.45 ± 1.69	14.42 ± 0.61	12.83 ± 3.77
7.5%	1.73 ± 0.47	3.08 ± 0.50	12.60 ± 0.46	10.08 ± 0.97
10.0%	4.0 ± 0.50	2.98 ± 1.37	13.90 ± 1.49	10.21 ± 4.51
F	53.396*	10.954*	8.567*	3.318
P	<0.001*	0.001*	0.003*	0.056
LSD 5%	0.878	1.932	1.477	4.882

F: F test (ANOVA)
*: Statistically significant at $p \leq 0.05$

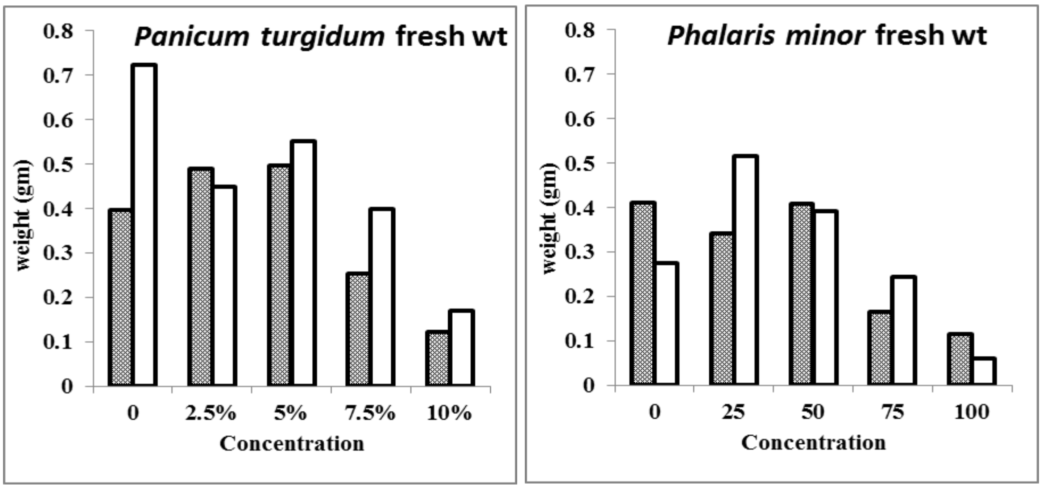

Figure (5): Effect of different concentrations of *R. graveolens* aqueous extract and aqueous leachate on the fresh weight of *P. turgidum* and *P. minor* seedlings.

DISCUSSION

Panicum turgidum and *Phalaris minor* are native weeds of Mediterranean region, and has spread at many parts of the world. They are serious grass weeds, which consider the most troublesome grassy weeds (Travlos *et al*., 2012; Abd El-Ghani *et al*., 2013). In many cases, crop fields are heavily infested by *P. turgidum* and *P. minor* which emerges with the germinating crops, competes for water and nutrient requirement, and significantly reduces the crops yield (Afentouli and Eleftherohorinos 1996; Abd El-Ghani *et al*., 2013). Efforts are being made to find out new environmental-friendly means for weed management. In this respect, the present study is an attempt to evaluate the allelopathic effect of *R. graveolens* shoot aqueous extract and leachate as a control of germination and seedling growth of *P. turgidum* and *P. minor*.

Germination percentage of *P. turgidum* and *P. minor* seeds were differentially affected by the *R. graveolens* shoot aqueous extract and leachate and the reduction was concentration dependent. El-Darier *et al*. (2014) found a difference between the species germination percentages in response to allelopathic materials coming from aqueous extract of *Haplophyllum tuberculatum* (HTAE) which agrees with the findings of Al-Zahrani & Al-Robai (2007), and Faravani *et al*. (2008) who found a difference between the species germination percentages in response to allelopathic materials coming from *Calotropis gigantea* leaves and *Melastoma malabathricum*. The highest concentration (10%) *R. graveolens* shoot aqueous extract and leachates exert the highest allelopathic effect on the germination percentages of the two tested species seeds. Germination percentage of *P. turgidum* and *P. minor* seeds were more sensitive to *R. graveolens* shoot aqueous extract than to *R. graveolens* shoot aqueous leachate. Reduction in germination percentages of the two current studied species may be due to secondary metabolic compounds present in *R. graveolens* shoot aqueous extract and leachate such as glycosides, flavonoids (rutin), alkaloids (quinolones), furocoumarins (psoralens), essential oil with methyl ketones and alcohols (Pathak *et al*., 2003). These compounds reduce the seed germination percentage (Williams and Hoagland, 1982; Al-Charchafchi *et al*., 1987), due to their interference with indol acetic acid metabolism, or synthesis of protein and ion uptake by the plant (Hussain and Khan, 1988). Inhibition of seed germination was attributed to the disruption of mitochondrial respiration (Podesta and Plaxton, 1994) and disruption of the activity of metabolic enzymes involved in glycolysis and in oxidative pentose phosphate pathway (OPPP) (Muscolo *et al*., 2001). Some allelopathic compounds interact with the mitochondrial membrane and directly impair the mitochondrial respiration (Abrahim *et al*., 2003).

The current study inferred that the plumule length of *P. turgidum* was found more sensitive than *P. minor* and responds more strongly to the increase of R. *graveolens* shoot aqueous extract and leachate concentrations. On contrary in radicle length, where *P. minor* was found more sensitive than *P. turgidum* under the effect of *R. graveolens* shoot aqueous extract and leachate. Impaired metabolic activities caused by allelochemicals decreased root and shoot lengths and allelochemicals decreased elongation, expansion and division of cells which are growth prerequisite (Einhellig, 1996). Also, allelochemicals inhibit absorption of ions (Qasem and Hill, 1989) and therefore, resulted in arrested growth (Dos Santosh *et al*., 2004). Radicle length of *P. minor* was more sensitive to allelochemicals from *R. graveolens* shoot aqueous extract and leachate than plumule length, that agree with many studies such as Chung and Miller (1995), Ashrafi *et al*. (2008) and El-Darier and Zein El-Dien (2011) who found that the radicle length of the recipient species were more sensitive than the plumule length. On the contrary plumule length of *P. turgidum* was more sensitive to allelochemicals from *R. graveolens* shoot aqueous extract and leachate than radicle length on concordance with many studies (Kadioglu and Yanar, 2004, Khan *et al*., 2013; Algandaby *et al*., 2014; El-Darier *et al*., 2014).

CONCLUSION

Our results revealed that *R. graveolens* shoot aqueous extract and leachate have allelopathic effects sufficient to reduce germination and plumule length of *P. turgidum* respectively, while germination and radicle length of *P. minor* suppressed under the allelopathic potential of *R. graveolens* shoot aqueous extract and leachate correspondingly. The authors hope that this research may aid practically in using allelopathy as an alternative natural solution for weeds management in crop fields.

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