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Phytotoxicity of *Rosmarinus officinalis* L. and *Salvia officinalis* L. to Control the Noxious Weed, *Panicum turgidum* Forssk.

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PETRI-DISH experiments were applied to investigate the potential allelopathic effects of cold and hot aqueous extracts of *Rosmarinus officinalis* and *Salvia officinalis* on germination percentage, relative germination percentage, and inhibition percentage as well as plumule and radicle lengths of *Panicum turgidum*. The results indicate that the degree of inhibition on seed germination and growth of the recipient species was largely dependent on the concentration of the extracts of both donor species. The effect was statistically significant at $p \leq 0.05$ for most treatments. The present study recommends the use of the two donor species for the biocontrol of weeds like *Panicum turgidum*.

Keywords: Allelopathy – *Rosmarinus officinalis* - *Salvia officinalis* - *Panicum turgidum*.

Known since ancient times, the phenomenon of allelopathy has recently received greater attention from researchers and farmers worldwide (de Albuquerque *et al.*, 2011). Allelopathy is an interference mechanism by which plants release chemicals which affect other plants; while it has often been proposed as a mechanism for influencing plant populations and communities (Gholami *et al.*, 2011). Increasing attention has been given to the role and potential of allelopathy as a management strategy for crop protection against weeds and other pests.

Incorporating allelopathy into natural and agricultural management systems may reduce the use of herbicides, insecticides, and other pesticides, reducing environment/soil pollution and diminish auto-toxicity hazards (Bajalan *et al.*, 2013a). Meanwhile, there is a great demand for compounds with selective toxicity that can be readily degraded by either the plant or by the soil microorganisms which provide new strategies for maintaining and increasing agricultural production in the future (Inderjit and Keating, 1999).

The two donor species are members of the family Lamiaceae. *Rosmarinus officinalis* is a flowering plant that grows wildly in Mediterranean countries, southern Europe and in the littoral region through Minor Asia areas (Derwich

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et al., 2011).The essential oil of *Rosmarinus officinalis* has antioxidant activity (Wang *et al.,* 2008), antibacterial activity (Azfali, 2009), insecticidal toxicity ((Papachristos and Stampoulos, 2004), anti-inflammatory and anti-nociceptive properties (Takaki *et al.,* 2008), antifungal activity (Ozcan and Chalchat, 2008; Pozzatti *et al.,* 2008) and only, in recent years have been commercialized as pest control products (Isman, 2000). Additionally, *Salvia officinalis* L. has been used for centuries as an herbal remedy. It yields a highly effective essential oil with very sweet overtones and can be used in balms, salves, perfumes, cosmetics and topical applications (Bajalan *et al.,* 2013a). Internally, *Salvia officinalis* L. essential oil is believed to be of benefit for a multitude of problems, including stress, exhaustion, headaches, insomnia, depression, colds, digestion, flatulence, upset stomach, liver and gallbladder problems, nervousness and etc. (Omid-Beigi, 2009).

A noxious weed is a plant that has adverse effects on or threatens agricultural production (VanGessel, 1995). *Panicum turgidum* Forssk. (Poaceae) is one of the widely distributed and most drought resistant grasses of the Egyptian desert (Migahid and Elshourbagy, 1961). The success and wide distribution of this plant species indicate its ability to tolerate certain unfavorable conditions (Ismail and Seed, 1983). Accordingly, this species is extremely competitive grass and considered to be one of the most troublesome weed species in cultivated fields. Therefore, the present study aims at assessing the allelopathic effect of two donor species, *Rosmarinus officinalis* and *Salvia officinalis*, on seed germination and seedling growth of *Panicum turgidum*.

Materials and Methods

Preparation of Donor Species Aqueous Extracts

Rosmarinus officinalis and *Salvia officinalis* (donor species) were collected from cultivated fields at El-Sharquia governorate. Healthy leaves from each species were selected carefully, washed in running tap water for removing the surface contaminants and dust and dried at room temperature for three days in the shade. After drying, leaves were powdered using electric blender. Stock aqueous extract was obtained by soaking 50 g leaf powder in 500 ml of cold and boiled distilled water (10 % w/v) at room temperature (20 ± 2ºC) for 24 hr with occasional shaking. The mixtures were filtered through two layers of cheesecloth and centrifuged for 20 min. at 5000 rpm to remove particulate materials. The purified extracts were kept in the refrigerator at 5ºC and considered to be the full strength concentration (100%). Series of subsequent dilutions (5%, 10%, 20% and 40%) were prepared in addition to the control (distilled water) and tested for their effects on germination and seedling growth of *Panicum turgidum* (recipient species).

Germination Bioassay

Petri-dish experiment was applied to investigate the potential allelopathic effects of cold and hot aqueous extracts of the two donor species on germination percentage (GP), plumule (PL) and radicle (RL) lengths of the recipient species. To accomplish this experiment, 10 seeds of the recipient species were arranged in

9 cm diameter Petri-dishes on 2 discs of Whatman No.1 filter paper under normal laboratory conditions with day temperature range of 25-30°C and night temperature range 20-25°C. 5 ml of cold and hot aqueous extracts of the two donor species were separately added daily to three replicates. GP, PL and RL were recorded daily for successive sixteen days. In addition, relative germination percentage (RGP) and inhibition percentage (IP) were calculated from the GP of the allelopathic treated seeds and GP of the control.

Calculations

Germination percentage (GP)

GP = (number of germinated seeds / total number of seeds)x 100

Relative germination percentage (RGP)

RGP= (allelopathic/control) x100

Inhibition Percentage (IP)

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

Reduction percentage length (RP)

RP= [(control – allelopathic)/control] x100

Statistical Analysis

Data were analyzed by standard analysis of variance (ANOVA) and student's t-test (before and after treatments) with COSTAT 2.00 statistical analysis software (Zar, 1988). Differences were considered to be significant at $p \leq 0.05$.

Results

Figures 1 and 2 represent the GP of *Panicum turgidum* under the effect of cold and hot aqueous extracts of *Rosmarinus officinalis* and *Salvia officinalis* respectively. Generally, there is inverse relationship between GP and concentration of the two extracts. With some exceptions, there is gradual decrease in relative germination percentage (Fig. 3) of the recipient species due to the increase in concentration of the extracts.The same trend was shown from the inhibition percentage (Fig. 4).The hot extract of *S. officinalis* shows the highest allelopathic effect on the germination of the seeds of *P. turgidum.* The inhibition percentages are 22.2, 22.2, 44.4 & 77.8% for the concentrations 5, 10, 20&40% of the hot water extract of *S. officinalis* respectively.

Reduction in both PL and RL of *P. turgidum* is directly proportional to the increase in concentration of both donor aqueous extracts (Fig. 5). Highest reductions were achieved using 40% cold and hot aqueous extracts and reached its maximum (92.21% and 83.43%) for radicle and plumule, respectively, for *R. officinalis.*

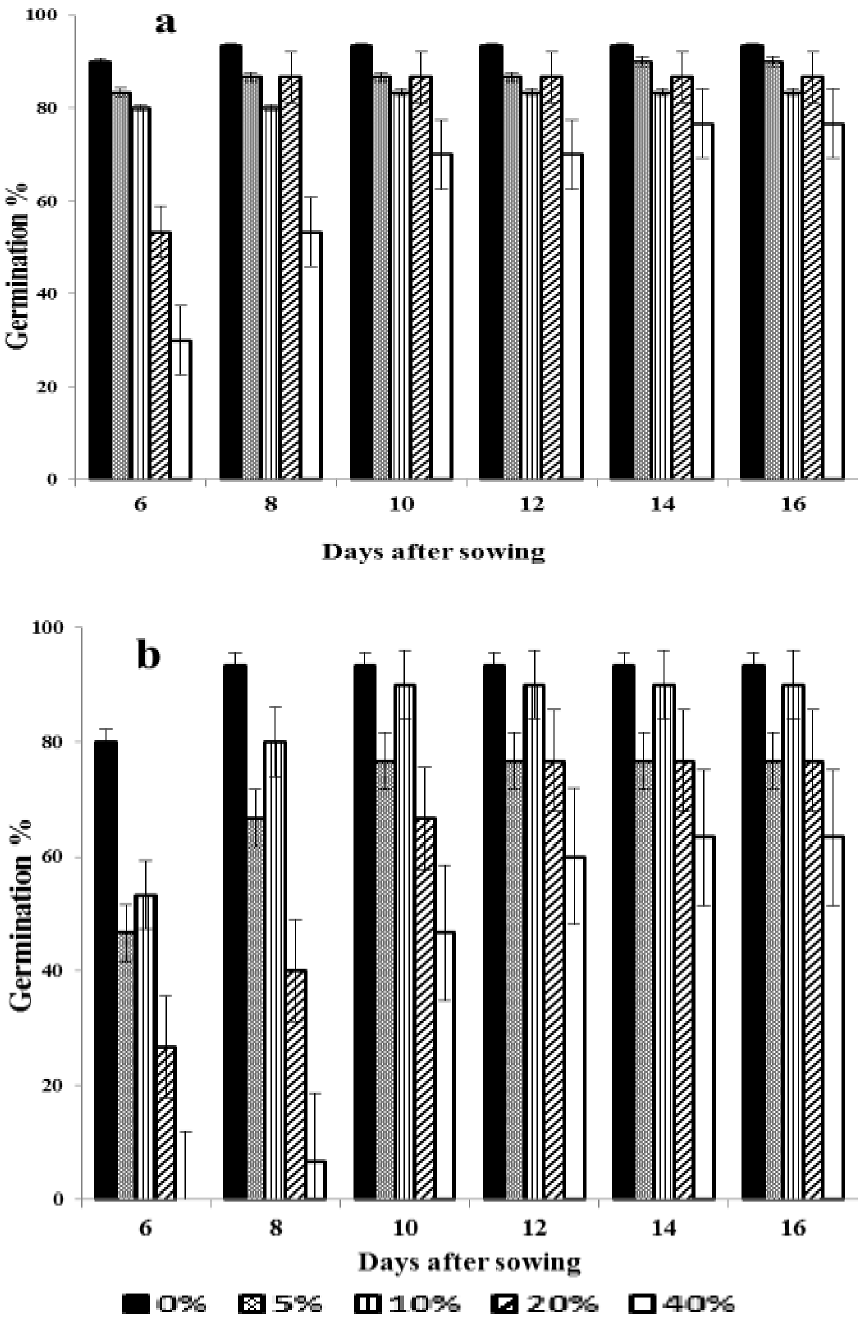

Fig. 1. Germination percentage of *Panicum turgidum* under the effect of aqueous extracts of *Rosmarinus officinalis* (a, cold; b, hot).

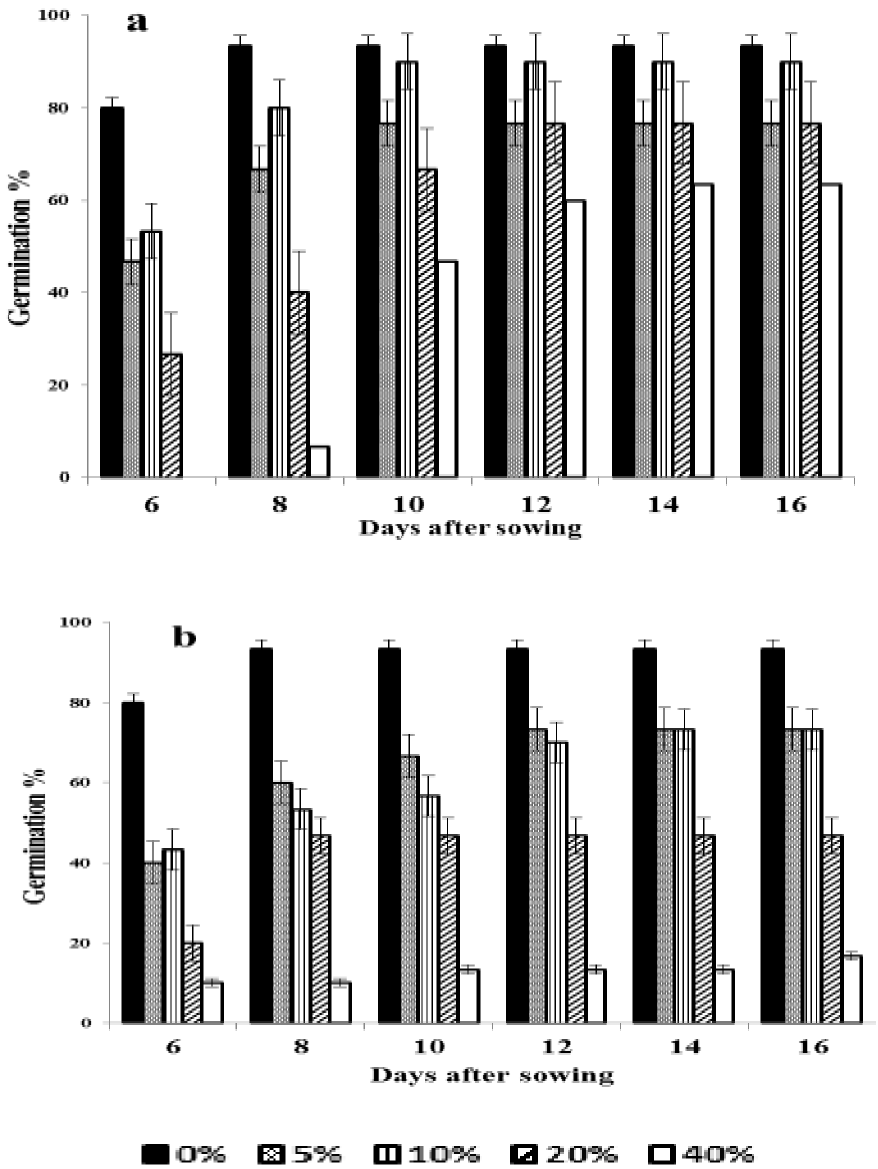

Fig. 2. Germination percentage of *Panicum turgidum* under the effect of aqueous extracts of *Salvia officinalis* (a, cold; b, hot).

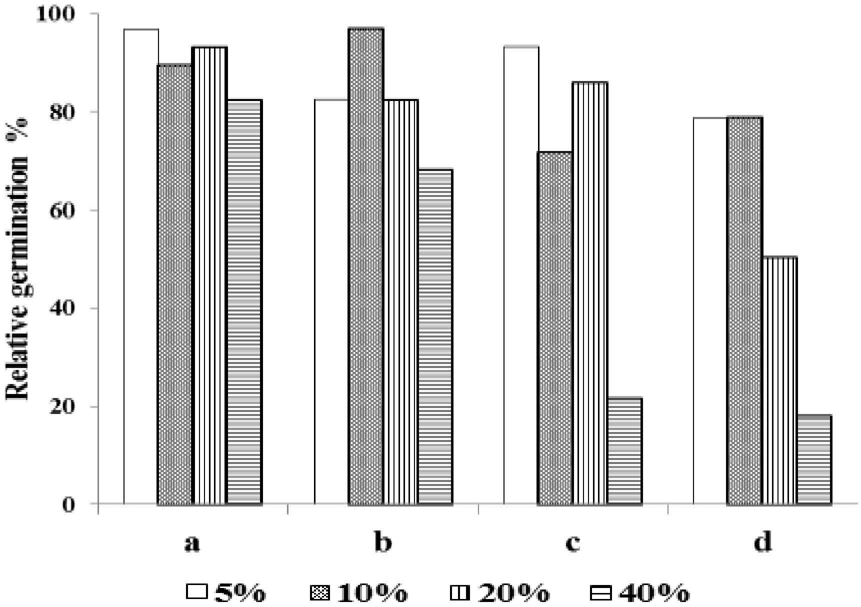

Fig. 3. Relative germination percentage of *Panicum turgidum* under the effect of aqueous extracts of *Rosmarinus officinalis* (a, cold; b, hot) and *Salvia officinalis* (c, cold; d, hot).

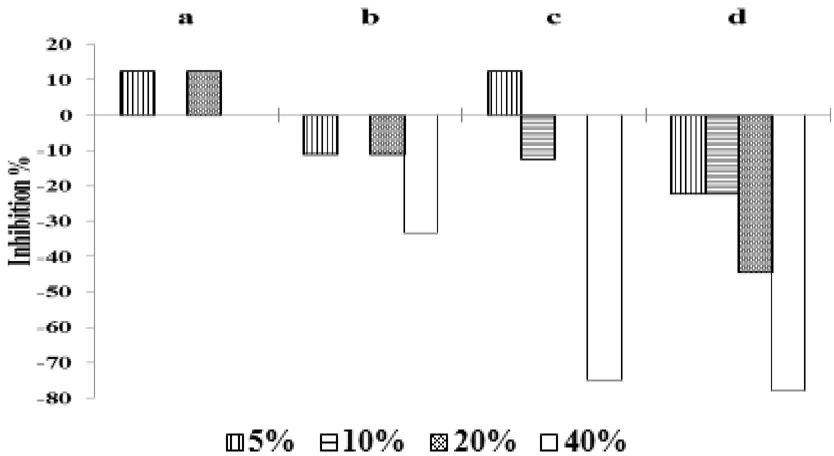

Fig. 4. Inhibition percentage of *Panicum turgidum* under the effect of aqueous extracts of *Rosmarinus officinalis* (a, cold; b, hot) and *Salvia officinalis* (c, cold; d, hot).

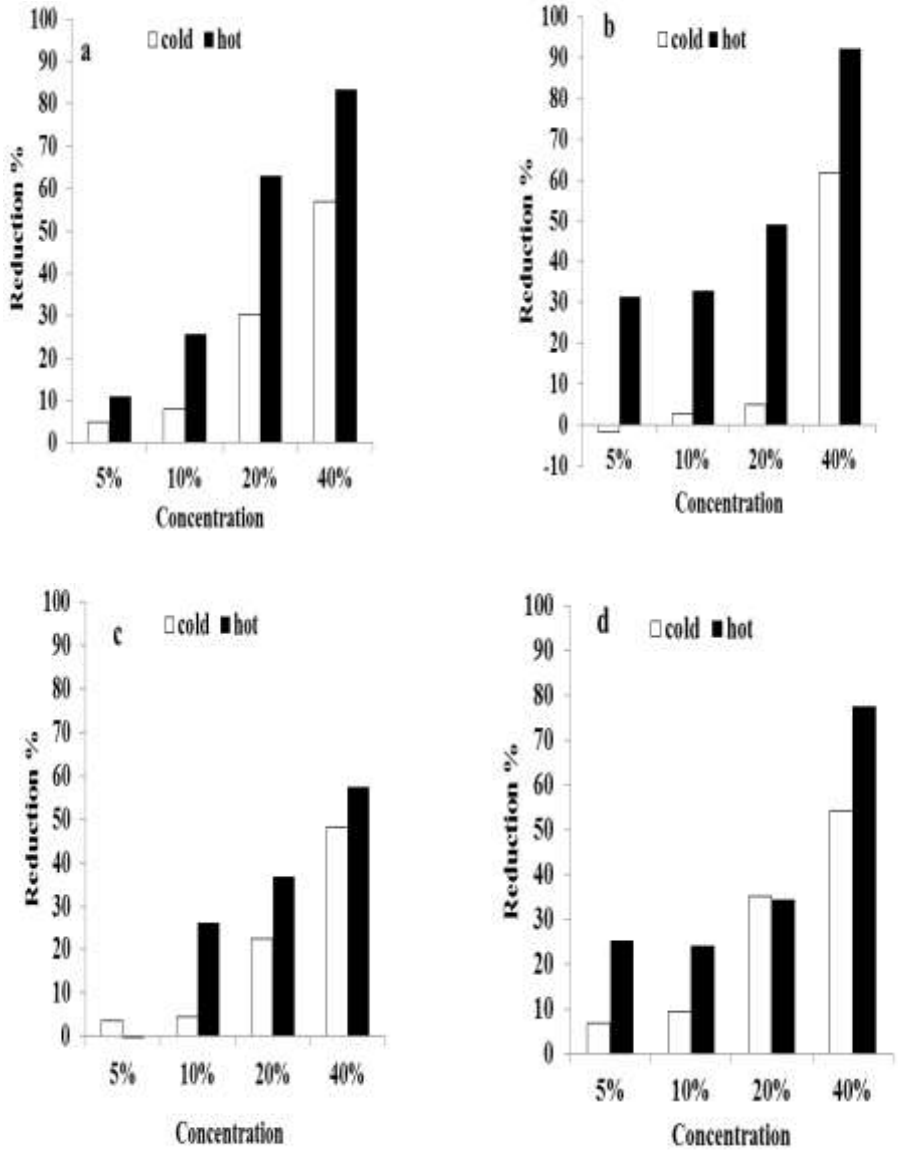

Fig. 5. Reduction percentage of *Panicum turgidum* under the effect of aqueous extracts of *Rosmarinus officinalis* (a, plumule; b, radicle) and *Salvia officinalis* (c, plumule; d, radicle).

Tables 1, 2 and 3 represent comparison between analysis of variance (ANOVA) of the studied treatments according to germination percentage, plumule length and radicle length, respectively. The effect is statistically significant at p ≤ 0.05 according to radicle and plumule length for both *Rosmarinus officinalis* and *Salvia officinalis*. Meanwhile, it is significant according to germination percentage for *Salvia officinalis* only. Also, Tables 4, 5 and 6 show repeated paired t-test before and after treatments according to germination percentage, plumule length and radicle length, respectively. The t-test revealed that all concentrations of the two species are significant for germination percentages. Concerning plumule length, most cold and hot concentrations of *Rosmarinus officinalis* are significant, while most those of *Salvia officinalis* are non-significant. Reverse trend was noted for radicle length where, most cold and hot concentrations of *Salvia officinalis* are significant, while most those of *Rosmarinus officinalis* are non-significant.

TABLE 1. Comparison between the studied treatments according to germination%.

Treatment (%)	Germination %			
	Rosmarinus officinalis		*Salvia officinalis*	
	Cold	Hot	Cold	Hot
0	79.7 ± 5.8	93.3 ± 11.5	83.3 ± 11.5	93.3 ± 11.5
5	90.0 ± 0.0	76.7 ± 15.3	86.7 ± 5.8	73.3 ± 5.8
10	83.3 ± 5.8	90.0 ± 10.0	66.7 ± 5.8	73.3 ± 30.6
20	86.7 ± 5.8	76.7 ±5.8	80.0 ± 0.0	46.7 ± 25.2
40	76.7 ± 15.3	63.3 ± 20.8	20.0± 17.3	16.7 ± 5.8
F	1.600	2.321	22.733*	7.472*
p	0.249	0.128	<0.001*	0.005*
LSD at 0.05	1.487	2.488	1.821	3.423

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

TABLE 2. Comparison between the studied treatments according to plumule length.

Treatment	Plumule length (cm)			
	Rosmarinus officinalis		*Salvia officinalis*	
	Cold	Hot	Cold	Hot
0%	1.81 ± 0.53	1.81 ± 0.53	2.31 ± 0.23	2.31 ± 0.23
5%	1.72 ± 0.27	1.61 ± 0.08	2.22 ± 0.27	2.36 ± 0.44
10%	1.66 ± 0.14	1.35 ± 0.39	2.20 ± 0.45	1.71 ± 0.52
20%	1.26 ± 0.25	0.67 ± 0.05	1.79 ± 0.47	1.46 ± 0.14
40%	0.78 ± 0.24	0.30 ± 0.02	1.20 ± 0.28	0.98 ± 0.43
F	5.586*	13.898*	3.836*	7.167*
p	0.013*	<0.001*	0.044*	0.005*
LSD 5%	0.573	0.543	0.662	0.685

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

TABLE 3. Comparison between the studied treatments according to radicle length.

Treatment	Radicle length (cm)			
	Rosmarinus officinalis		*Salvia officinalis*	
	Cold	**Hot**	**Cold**	**Hot**
0%	4.45 ± 0.36	4.45 ± 0.36	5.39 ± 0.43	5.39 ± 0.43
5%	4.23 ± 0.56	3.06 ± 0.53	3.50± 0.50	4.03 ± 0.64
10%	4.33 ± 0.29	2.99 ± 0.60	4.88 ± 0.15	4.09 ± 1.00
20%	4.52 ± 0.44	2.26 ± 0.85	5.03 ± 0.89	3.54 ± 0.60
40%	1.70± 0.40	0.35 ± 0.13	2.46 ± 0.16	1.20 ± 0.56
F	24.962*	22.358*	13.163*	15.574*
p	<0.001*	<0.001*	0.001*	<0.001*
LSD 5%	0.761	0.999	0.978	1.225

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

TABLE 4. Repeated t-test before and after treatments according to germination%.

Treatment (%)	P values			
	Rosmarinus officinalis		*Salvia officinalis*	
	Cold	**Hot**	**Cold**	**Hot**
5% vs Control	3.062*	3.897*	2.449*	3.266*
10% vs Control	3.801*	2.449*	3.690*	2.887*
20% vs Control	3.320*	3.690*	3.897*	4.025*
40% vs Control	4.382*	4.382*	4.382*	4.382*

*: Statistically significant at $p \leq 0.05$

TABLE 5. Repeated t-test before and after treatments according to plumule length.

Treatment (%)	P values			
	Rosmarinus officinalis		*Salvia officinalis*	
	Cold	**Hot**	**Cold**	**Hot**
5% vs Control	0.816	2.858*	1.677	2.021
10% vs Control	2.887*	3.753*	0.612	1.225
20% vs Control	3.578*	3.578*	0.866	2.012
40% vs Control	4.017*	4.382*	2.465*	2.739*

*: Statistically significant at $p \leq 0.05$

TABLE 6. Repeated t-test before and after treatments according to radicle length.

Treatment (%)	P values			
	Rosmarinus officinalis		*Salvia officinalis*	
	Cold	**Hot**	**Cold**	**Hot**
5% vs Control	2.008	0.816	2.683*	3.266*
10% vs Control	2.021	0.722	3.175*	3.464*
20% vs Control	2.449	2.571*	0.000	3.578*
40% vs Control	2.012	3.469*	3.104*	4.382*

*: Statistically significant at $p \leq 0.05$

Discussion

The results indicate that the degree of inhibition on seed germination and growth of the recipient species is largely dependent on the concentration of the extracts of both donor species. These results are in agreement with those of Bajalan *et al.* (2013 a & b) who found that *Salvia officinalis* has strong allopathic effect on germination of barley (*Hordeum vulgare*) and Purslane (*Portulaca oleracea*) seeds (2013a) and wheat (*Triticum aestivum* L.) and velvet flower *(Amaranthus retroflexus*) seeds (2013b) in such a way that the statistical comparison indicates the reduction of germination percentage of treated seeds in comparison with control in the level of 5 percent. The results also are in consistence with those of de Almeida *et al.* (2010) who found that the germination and radicle growth of seeds of *Raphanus sativus, Lactuca sativa* and *Lepidium sativum* were affected by twelve essential oils from Mediterranean aromatic plants, of which *Salvia officinalis* is one of them. Results of *Rosmarinus officinalis* are also in complete harmony with many previous works. For example, Arouiee *et al.* (2010) reported that *Rosmarinus officinalis* leaf extract has allelopathic effect on seed germination and some growth characteristics of *Solanum nigrum* and *Amaranthus retroflexus*.

The chemical compositions of *Rosmarinus officinalis* essential oils have been reported mainly as α-pinene, 1.8-cineole and camphor (Azfali *et al.*, 2009). *Salvia officinalis* essential oil is characterized by high amounts of α-thujone, camphor, β-pinene and α-pinene, (Karaaslan and Özguven, 2001). In particular, a high presence of oxygenated monoterpenes is related to a potent phytotoxic activity (de Almeida *et al.*, 2010). Moreover, Vokou *et al.* (2003) studied the allelopathic activities of 47 monoterpenoids belonging to different chemical groups, estimating their effects on seed germination and subsequent growth of *Lactuca sativa* seedlings and found that the most active compounds against both processes belonged to the groups of ketones and alcohols, followed by the group of aldehydes and phenols.Our data agree with this findings where significant effects were reported for germination percentage, plumule and radicle lengths but at different levels of activity.

In addition, it is well known that monoterpenes in the essential oils have phytotoxic effects that may cause anatomical and physiological changes in plant seedlings leading to accumulation of lipid globules in the cytoplasm, reduction in some organelles such as mitochondria, possibly due to inhibition of DNA synthesis or disruption of membranes surrounding mitochondria and nuclei (Zunino & Zygadlo, 2004 and Nishida *et al.*, 2005). However, the specific structural factors, that operate and determine the activity of monoterpenoid and essential oils, remain still obscure (de Almeida *et al.*, 2010).

In conclusion, we have demonstrated that *Rosmarinus officinalis* and *Salvia officinalis* have significantly phytotoxic effect on seed germination and growth of *Panicum turgidum*. Thus, the present study recommends the use of the two donor species for the biocontrol of harmful weeds like *Panicum turgidum*. At the same time the study alerts for the inhibitory effect of these species on the growth of economic plants. Additional work is required to test the efficacy of residues or extracts from these plants on weed control under field conditions and to isolate and identify allelochemicals involved. This information may allow the development of biosynthesized herbicides and other biologically based weed control methods.

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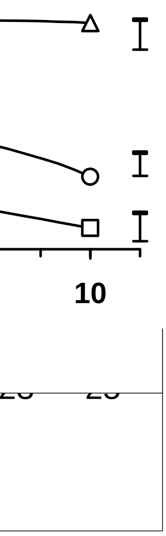

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السمية النباتية لإكليل الجبل والمرامية للسيطرة على عشبة القصيبة الضارة

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التباعد التضادى الكيميائي هو آلية تداخلية تطلق من خلالها النباتات بعض المواد الكيميائية التي تؤثر على نمو وانتشار النباتات الأخرى، وكثيرا ما يقترح كآلية للتأثير على المجتمعات النباتية. وللإستفادة من هذه الظاهرة أجريت تجارب معملية لمعرفة قدرة المستخلصات المائية الباردة والساخنة لنباتي إكليل الجبل والمرامية على التباعد التضادى الكيميائي لنسبة إنبات بذور عشبة القصيبة الضارة بالإضافة إلى تأثير هذه المستخلصات على بعض صفات نمو النبتة مثل طول الريشة والجذير. أوضحت النتائج أن إنبات ونمو بذور العشبة الضارة تأثرت بدرجة كبيرة بمستخلصات النباتات المانحة، وأن درجة التأثر إعتمدت على تركيز المستخلصات. وتوصي الدراسة الحالية استخدام نباتي إكليل الجبل والمرامية للمكافحة الحيوية للأعشاب الضارة مثل القصيبة. وفي نفس الوقت تنبه الدراسة على التأثير المثبط لنباتي إكليل الجبل والمرامية على نمو النباتات الاقتصادية.