Use of *Beta vulgaris* allelopathic properties to control some weeds associated with *Lupinus albus* plant comparing with two recommended herbicides

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Abstract: Two pot experiments were carried out during two successive winter seasons of (2016/2017) and (2017/2018) at the green house of National Research Centre (NRC), Giza, Egypt. The experiments were conducted to compare the allelopathic effect of *Beta vulgaris* (shoot and root aqueous extracts at 3%, 6%, 9%) to chemical herbicides (Topik and Basagran) at recommended doses (140 g fed^{-1} and 1 L fed^{-1}, respectively) in controlling weeds (*Phalaris minor* and *Malva parviflora*) associated with *Lupinus albus* plants. Results showed that all applied aqueous extracts caused reduction in two weeds biomass under investigation. Meanwhile, root aqueous extract was more effective than shoot aqueous extract in controlling weeds. It is worthy to mention that phenolic compounds and flavonoids in *B. vulgaris* root aqueous extract were higher than that in *B. vulgaris* shoot aqueous extract. In addition, all applied aqueous extracts increased growth, yield and yield components of *L. albus* plant. The inhibitory effect of *B. vulgaris* aqueous extract on weeds or its stimulatory effect on *L. albus* plants increased by increasing the extracts concentration. The two applied herbicides gave complete eradication of both weeds depending on selectivity of each herbicide followed by root aqueous extract of *B. vulgaris* at 9%; this in turn reflected on *L. albus* plants by scoring highest results as compared to unweeded treatment.

Keywords: allelopathy, weed control, herbicides, *Lupinus albus* L., *Phalaris minor*, *Malva parviflora*,*Beta vulgaris* L.

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1 Introduction

Lupine (*Lupinus albus* L.) is a poor weed competitor due to its slow vegetative growth and development. However, slow development facilitates light penetration, weed seed germination and subsequent yield loss due to competition (Folgart et al., 2011). Weeds not only compete with crop for nutrients, soil moisture, space and light but also serve as alternative hosts for several insect pests and disease (Yadav et al., 2015). Hand hoeing is still a common method for weed control. Recently, synthetic herbicides become the favorable and effective method due to high cost and scarce of hand labor. Although synthetic herbicides are highly effective in controlling weeds, inexpensive and have a very good selectivity toward crops (Dayan et al., 2009), the excessive and non-judicious use of herbicide may lead to crop injury, human and animal health concerns, soil and water pollution as well as herbicide resistant in weeds (Jabran et al., 2008; Farooq et al., 2011).

In organic farms where weed management is considered a major problem and the application of chemical herbicides is prohibited, safe weed control methods become a useful applied issue. Allelopathy phenomenon can be practically utilized for weed control. Allelopathy is defined as positive or negative effects of one plant on other plant/s through the liberation of

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allelopathic chemical compounds (Allelochemicals) into the surroundings (Rice, 1984). Allelochemicals interfere with physiological processes affecting on plant growth parameters (Kil and Shim, 2006). The use of *Beta vulgaris* as a natural allelopathic herbicide is focused in this investigation.

The red beet (*Beta vulgaris* L.var. *vulgaris*) is a fresh known vegetable around the world. *B. vulgaris* has been reported to contain many allelopathic compounds. By using high performance liquid chromatography (HPLC) fractionation technique, Kujala et al. (2001) detected four flavonoids (beta grain, betavulgarin, cochliophilin A and dihydroisorhamentin) in 80% aqueous methanol extract of four beetroot cultivars. Moreover, Georgiev et al. (2010) identified four phenolic acids (4-hydroxybenzoic acid, caffeic acid, catechin hydrate and epicatechin) in hairy root cultures of the *B. vulgaris* root. Additionally, beetroot is the primary source of betalains for large-scale food coloring operations. Betalains are water-soluble plant pigments that are widely used as food colorants, and have a wide range of desirable biological activities including antioxidant, antiinflammatory, hepatoprotective, anti-cancer properties (Winkler et al., 2005). However, Condor and Indrea (2010) noticed the presence of allelopathy phenomenon between two species of family chenopodiaceae: Spinach (*Spinacia oleracea* L.) and red beetroot (*Beta vulgaris* L.). Sugar beet (*Beta vulgaris* L.) which belongs to the same red beet beet family (chenopodiaceae) is also known to be allelopathic against weeds (Dadkhah, 2012; Dadkhah, 2013; Dadkhah and Rassam, 2016; Babaeinejad et al., 2017). Allelopathy phenomena in species of family chenopodiaceae have been reported by many authors (Bouchikh-Boucif et al., 2014; Rad et al., 2014; El-Rokiek et al., 2016). Therefore, this study was designed: 1- To evaluate the possibility of using the aqueous extracts of *B. vulgaris*as a bioherbicide to control some weeds (*Phaiaris minor* and *Malva parviflora*); 2- To compare the allelopatheic effect of *B. vulgaris* on growth and yield of *L. albus* plants and some associated weeds with two recommended herbicides.

2 Materials and methods

2.1 Preparation of plant materials

Beta vulgaris plants were collected from the Egyptian fields then washed with tap water. Aerial shoot and underground root parts were separated and dried at room temperature in shadow places for several days. For complete loss of moisture dried plant materials were put in oven at 40°C and weight it successively until reaching to the constant weight. Dried plant tissues were ground separately into a fine powder using an electric mill.

2.2 Preparation of aqueous extract

One hundred grams from each dried plant material (shoot and root) put in 2 L Erlenmeyer flask and 1L of distilled water was added. Two flasks were covered with parafilm and placed on shaker (200 revolution / min.) for 24 hours at room temperature. Both prepared mixtures were filtered through a fine mesh and compressed carefully for complete extraction. The two gained filtrates were filtered again using Whatman No. 1 filter paper to have clear stock solutions. 3%, 6% and 9% (w/v) concentrations were prepared from each stock solution using distilled water.

2.3 Experimental procedure

Two pot experiments were conducted at the green house of National Research Centre (NRC) during the two successive winter season (2016/2017) and (2017/2018). Both experiments were conducted based on complete randomized design with six replicates. Plastic pots (30 cm in diameter) were filled with equal amount of sieved sandy-loam soil. Seeds of *L. albus* (cv. Giza1) were obtained from Agricultural Research Centre, Egypt. After sowing of pots at 2 cm depth from the soil surface ten treatments were conducted in this investigation. Six treatments treated with 3%, 6% and 9% (w/v) aqueous prepared extracts of both shoot and root of *B. vulgaris*. Using hand sprayer extracts were sprayed twice at the rate of 50 mL pot^{-1} 14 and 21 days after sowing (DAS) (plants were at 4 leaf stage) on foliage part of *L. albus* and its associated weeds. Two herbicidal treatments of grass weed Topik 240 EC (Clodinafop-Propargyl) and broad weed Basagran (Bentazon 48 EC) herbicides were sprayed at the recommended rat 140 g fed^{-1} and 1 L fed^{-1}, respectively. Both herbicides are postemergence that Basagran sprayed at 20 DAS and Topik sprayed at 30 DAS. Additionally, two control treatments weed free and unweeded were applied for comparison. All treatments were maintained under green house condition and all cultural practices were applied especially irrigation and fertilization.

2.4 Recorded data

2.4.1 Weeds

In both seasons, three replicates were collected from each treatment at 45 DAS and at the end of the season. Weeds were dried in an oven at 40°C for 48 h to record dry biomass (gpot^{-1}).

2.4.2 *Lupinus albus* plants

A- *Lupinus albus* morphological and physiological parameters

In both seasons, three replicates of *L. albus* were collected from each treatment at 45 DAS to determine the morphological parameters (shoot height (cm), dry biomass/plant and number of leaves/plant). Photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) in fresh *L. albus* leaves were determined as the method described by Moran (1982).

B- Yield and yield attributes

At harvest, three replicates of *L. albus* plants were taken from each treatment to determine plant height, number of pods/plant and seeds weight/plant.

2.4.3 Chemical analysis of *B. vulgaris* aqueous extracts

In both aqueous extracts (shoot and root) of *B. vulgaris*, total phenolic compounds and total flavonoids were determined according to Srisawat et al. (2010).

2.5 Statistical analysis

The experiments were carried out in completely randomized design. All obtained data were subjected to proper statistical of variance according to Snedecor and Cochran (1980). The mean values were analyzed using Duncan's multiple range test to compare the recorded means at 0.05 probability level (Duncan 1955).

3 Results and discussion

Recorded statistical analyzed data showed that there were significant differences between the applied weed control treatments.

3.1 Weeds

Table 1 showed that the aqueous extracts of both shoot and root of *Beta vulgaris* at concentrations 3%, 6% and 9% in addition to the two herbicide treatments significantly reduced fresh and dry weight of both weeds. Topik and Basagran herbicides at recommended doses at 45 DAS and at the end of season completely eradicated weeds depending on selectivity of each herbicide. Aqueous root extract of *B. vulgaris* at 9% and 6% concentration followed these perfect treatments and caused reduction in *Phalaris minor* reached to 80.4% and 64.6%. The corresponding reduction in *Malva parviflora* dry biomass were equal about 83.7% and 77.2% at harvest as compared with unweeded pots. From the recorded results it was noticed that root aqueous extract was more effective than shoot aqueous extract in controlling weeds. Generally, inhibiting the reduction in *M. parviflora* biomass was higher than that in *P. minor*.

Table 1 Effect of weed control treatments on fresh and dry biomass of *P. minor* and *M. parviflora* (g) at 45 DAS and at the end of season. (Average of two seasons)

Treatments			45 days after sowing At				At the end of the season	
			P. minor (g) / pot		*M. parviflora* (g) /pot		*P. minor* (g)/pot	*M. parviflora* (g)/pot
			Fresh biomass	Dry biomass	Fresh biomass	Dry biomass	Fresh biomass	Dry biomass
Control	Unweeded		22.23 f	5.30 g	35.20 e	4.40 d	80.00 g	80.33 g
	Weed free		0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
Herbicides	Topik		0.00 a	0.00 a	1.46 a	0.20 a	0.00 a	27.07 ed
	Basagran		11.17 dc	2.27 d	0.00 a	0.00 a	12.50 b	0.00 a
B. vulgaris aqueous extracts	3%	Shoot	16.87 e	3.47 f	18.93 d	2.93 c	53.33 f	45.00 f
	6%		13.57 d	2.85 e	16.27 dc	2.20 b	40.20 ed	34.1 e
	9%		12.13 dc	2.38 d	13.73 c	1.77 b	36.00 d	21.33 dc
	3%	Root	16.73 e	3.33 f	19.53 d	3.90 d	44.00 e	43.27 f
	6%		10.10 c	1.60 c	16.07 dc	2.80 c	28.33 c	18.33 cb
	9%		4.53 b	1.15 b	8.53 b	0.48 a	15.67 b	13.10 b

These results are in agreement with Hegab et al. (2008) who ensured that the highly sensitive response to the inhibition effect of the applied allelopathic extract accompanied with the increment in allelochemicals concentration. Kujala et al. (2001) detected four flavonoids (beta grain, betavulgarin, cochliophilin A and dihydroisorhamentin) in 80% aqueous methanol extract of four beetroot cultivars. Moreover, Georgiev et al. (2010) identified four phenolic acids (4-hydroxybenzoic acid, caffeic acid, catechin hydrate and epicatechin) in hairy root cultures of the *B. vulgaris* root. These phenolic acids may be the active agent in allelopathic interactions by affecting on growth of crops and associated weeds (Chung et al., 2002). Plant growth parameters were affected by allelochemicals in various mechanisms such as reduction in mitotic activity, photosynthesis, nutrient uptake, respiration, permeability of cell membrane as well as inhibition of enzyme action and protein formation (Rice, 1984; Wu et al., 2000; Xuan, 2004). Reduction in photosynthetic area or assimilation rate may be other reasons of dry matter reduction (Dadkhah, 2012).

Concerning to the efficiency of chemical herbicides such as Topik for controlling grassy weeds,similar findings have been reported by El-Wakeel(2015), Ali et al.(2016), and Khan et al. (2018). Topik interacts with [acetyl co-enzyme A carboxylase (Accase)] and inhibits it, which is essential for the production of lipids (fatty acids) needed for plant growth (EPA, 2000). Whereas, Basagran is a member of the benzothiadiazole group of herbicides, which acts to inhibit photosynthesis at photosynthesis II and can selectively control broad leaved weeds (Khajehpour, 2004). These results are in conformation with those of Baghestani et al. (2008) as well as Aboali and Saeedipour (2015) who reported that Basagran offers sizeable increase in crop production corresponding to it's weed control spectrum.

3.2 *Lupinus albus* plants

A- *Lupinus albus* morphological and physiological parameters

As shown in Table 2 all the applied weed control treatments caused a significant progressin shoot height and dry biomass of *L. albus*at 45 DAS. Statistical analysis of data revealed that the used herbicides at recommended doses as well as weed free (control) treatment gave the highest *L. albus* shoot height and *B. vulgaris* aqueous extract of both shoot and root followed these treatments with no significant differences between them. However, in the same trend the previous mentioned ideal treatments increased *L. albus* dry biomass with observing significant differences between them. Number of *L. albus* leaves not significantly affected by different weed control treatments under investigation.

Table 2 Effect of weed control treatments on morphological parameters of *L. albus* plants at 45 DAS (Average of two seasons)

Treatments			Shoot height (cm)	Dry biomass (g)	No. of leaves
Control	Unweeded		18.57 b	1.63 e	7.40 a
	Weed free		26.70 a	7.75 a	7.77 a
Herbicides	Topik		25.03 a	6.74 a	7.77 a
	Basagran		25.77 a	6.66 a	7.63 a
B. vulgaris aqueous extracts	3%	Shoot	23.70 a	2.31 cd	7.40 a
	6%		23.23 a	2.92 cd	7.40 a
	9%		24.77 a	3.14 c	7.43 a
	3%	Root	24.90 a	1.94 de	7.47 a
	6%		24.67 a	2.90 cd	7.45 a
	9%		25.47 a	4.71 b	7.45 a

Chlorophyll a, chlorophyll b, carotenoids and consequently total photosynthetic pigments in fresh leaves of *L. albus* significantly increased by the application of weed control treatments over that in unweeded pots (Table 3). The highest values in photosynthetic pigments were recorded in weed free plants supplemented by Basagran and Topik herbicides. *B. vulgaris* aqueous extract of root part at 3% concentration followed these recommended applied treatments. However, by increasing the concentration of the allelopathic extract the photosynthetic pigments decreased but still higher than unweeded control.

Table 3 Effect of weed control treatments on Photosynthetic pigment contents (mg/g fresh leaves) of *L. albus* plants at 45 DAS (Average of two seasons)

Treatments			Photosynthetic pigment (mg/g fresh leaves)			
			Chl. A	Chl. B	Cartenoids	Total photosynthetic pigments
Control	Unweeded		0.711 d	0.195 c	0.095 d	0.975 d
	Weed free		1.795 a	0.485 a	0.305 a	2.435 a
Herbicides	Topik		1.510 ab	0.390 ab	0.230 ab	2.035 ab
	Basagran		1.605 ab	0.405 ab	0.280 ab	2.150 ab
B. vulgaris aqueous extracts	3%	Shoot	1.115 c	0.335 ab	0.195 c	1.745 bc
	6%		1.145 c	0.275 bc	0.205 bc	1.520 cd
	9%		1.300 bc	0.290 bc	0.190 c	1.500 cd
	3%	Root	1.475 ab	0.370 ab	0.230 ab	1.960 ab
	6%		1.340 bc	0.350 ab	0.240 ab	1.795 bc
	9%		1.230 bc	0.340 ab	0.205 bc	1.775 bc

Generally, it is worthy to mention that *B. vulgaris* aqueous extract of root part increased photosynthetic pigments than that of shoot. Additionally, the application of herbicides induced the increment in photosynthetic pigments than that of applied aqueous extracts. These results are in agreement with Dadkhah and Rassam (2016).

A- Yield and yield attributes

From the recorded results in Table 4, it's clear that all the applied weed control treatments significantly increased yield and its attributes of *L. albus* plants (number of pods/plant and seeds weight/plant). Weed free treatment and aqueous extract at 9% viewed the greatest plant height. Both of herbicides as well as shoot aqueous extract at 9% followed these superior treatments. The same trend in turn was recorded in number of pods/plant. Root aqueous extract at 9% concentration and Topik herbicide recorded the same increment in seed weight/plant which equal about 63.5% followed by Basagran herbicide and root aqueous extract at 6% concentration with increment percentage reached to 59.6% and 51.9% over unweeded control.

Table 4 Effect of weed control treatments on yield and it's attributes of *L. albus* plants (Combined analysis of two seasons)

Treatments			Plant height (cm)	No. of pods/plant	Seed weight/ plant (g)
Control	Unweeded		26.00 d	2.67 b	2.60 e
	Weed free		58.00 a	5.00 a	4.90 ab
Herbicides	Topik		49.50 bc	4.00 a	4.25 b
	Basagran		48.00 bc	4.00 a	4.15 bc
B. vulgaris aqueous extracts	3%	Shoot	44.00 c	4.00 a	3.55 cd
	6%		47.00 bc	4.50 a	3.50 cd
	9%		48.00 bc	4.50 a	3.25 d
	3%	Root	43.50 c	4.00 a	3.75 bc
	6%		46.50 bc	4.50 a	3.95 bc
	9%		53.50 ab	5.00 a	4.25 b

The negative inhibition response of competitor associated weeds whether for chemical herbicides or allelopathic *B. vulgaris* aqueous extract owed to increment in recorded growth parameters of *L. albus*, which in turn increased yield and its attributes recorded results. Many scientists ensured that controlling weeds accompanied by high growth and yield recorded results (El-Wakeel, 2015; Ali et al., 2016; Babaeinejad et al., 2017) as weeds compete with plant on water, nutrients, light and space (Yadav et al., 2015).

3.3 Quantitative determination of total phenolic compounds and flavonoids in the applied *B. vulgaris* aqueous extracts

Theresults (Table 5) revealed that the most abundant phenolic compounds and flavonoid recorded in root aqueous extract with a percentage reached to 2.17% and 1.74%, successively in the lowest applied 3% concentration. Moreover, it was noticed that phenolic compounds and flavonoids concentration are higher in root aqueous extract than in shoot aqueous extract.

Table 5 Quantitative amount of total phenolic compounds and flavonoids in shoot and root *B. vulgaris* aqueous extract

Extracts		% Total phenolic compounds	% Total Flavonoids
Shoot aqueous extract	3%	0.701	0.997
Rootaqueous extract		2.172	1.748

4 Conclusion

The allelopathic properties of *B. vulgaris* aqueous extracts caused a great inhibition of *M. parviflora* and *P. minor* weeds. This inhibition depended upon the source of extract (root part of *B. vulgaris* was more effective than shoot part), the extract concentration (the higher aqueous extract concentration, the higher allelopathic inhibition effect) and the weed species tested (*M. parviflora* broad weed was more sensitive than *P. minor* grass weed). Moreover, red beet aqueous extracts stimulated growth and yield of *L. albus* plants. Additional research is required to test the efficacy of *B. vulgaris* root aqueous extract in controling weeds under field conditions striving to have eco-friendly herbicide.

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