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Cyanobacterial *Arthrospira* (*Spirulina platensis*) as safener against harmful effects of fusilade herbicide on faba bean plant

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Abstract Treatment of faba bean plant by fusilade herbicide (post-emergence herbicide) caused reduction in all measured growth parameters (plant height, root and shoot length, number of leaves/plant, fresh and dry weight of root and shoot and leaf area) and yield parameters (number of pods/plant, number of seeds/pod, number of seeds/plant and weight of 100 seeds). Priming of faba bean seeds in *Arthrospira* (*Spirulina platensis*) suspension before cultivation ameliorated the adverse effects of the herbicide on the plant. Algal suspension treatment caused enhancement in protein and amino acid levels of root and shoot, and ameliorated also the harmful effects of the herbicide on the antioxidant enzymes and reduced the lipid peroxidation and proline content of the plant. The treatment caused marked changes in the profile of amino acids and their concentrations in leaves. The most changes were observed in leucine, isoleucine, valine, serine, phenylalanine, methionine and histidine. The concentrations of these amino acids were reduced in response to herbicide application, while increased in response to algal treatment. According to these results, priming of seeds in the *Arthrospira platensis* suspension induced the biosynthesis of some amino acids which could protect or act as a safener against the adverse effects of the herbicide on the plant.

Keywords Safener · *Arthrospira platensis* · Amino acids · Herbicide · Faba bean

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1 Introduction

Application of herbicides to arable lands is one of the most important practices for controlling weeds growth in modern agriculture (Jevier-Benitez et al. 2006). Herbicides must be toxic to the weeds, but they may have a harmful effect also on the crop species. Herbicides are absorbed by crop plants as well as by weeds; certain herbicides are completely metabolized while others are not (Pline and Hatzios 2003). Different morphological, physiological and biochemical effects have been observed on the treated crop species as results for using herbicides. Because of the crops injury by herbicides, several methods were examined to minimize this injury (Hassan 1998). Herbicides safeners (also known as antidotes) are now extensively used as *chemical compounds* that enhance herbicides selectivity by increasing tolerance in the crop plants, but not in competing weeds (Davies 2001; Hatzios and Burgos 2004).

Faba bean is the main leguminous crop grown in Egypt as its seeds are used for human consumption (Orabi et al. 2013). It is used as green or dried seeds, fresh or canned and it is a common staple food in the Egyptian diet and used as food in various ways.

Fusilade herbicide is used to control weeds in faba bean fields. Fusilade is Aryloxy-phenoxypropionate herbicide that inhibit the enzyme acetyl-CoA carboxylase (ACCase) which catalyzing the first committed step in de novo fatty acid synthesis. Inhibition of fatty acid synthesis presumably blocks the production of phospholipids used in building new membranes required for cell growth.

Beneficial effects of cyanobacterial inoculation (as biofertilizer) were reported for some crops such as rice, wheat, soybean, oat, tomato, radish, cotton, sugarcane, maize, chili, bean, muskmelon and lettuce (Maqubela et al. 2009; Saadatnia and Riahi 2009).

Cyanobacteria can improve plant growth and crop yield due to their release of various biological active substances such as gibberellin, auxin cytokinins (Stirk et al. 2002; Hashtroudi et al. 2011), vitamins, amino acids, polypeptides, antibacterial and antifungal substances, secondary metabolites with various biological actions and antioxidants having safety and effectiveness (Leflaive and Ten-Hage 2007; Priyadarshani and Biswajit 2012). Therefore, in this study we are investigating the additional role of cyanobacteria, through *Arthrospira platensis*, as an agent for alleviating the harmful effects of fusilade herbicide on faba bean plant.

2 Materials and methods

2.1 Algal isolate

Arthrospira platensis was isolated, purified and identified according to Prescott (1962, 1970). *Arthrospira platensis* culture was grown on Modified Zarrouk's medium (Aiba and Ogawa 1977) and harvested at the beginning of stationary growth phase by centrifugation at 3000 rpm (Fisher Centerific™ Centrifuge) for 10 min. Cells pellet was rinsed three times and resuspended in sterilized distilled water to remove traces of growth medium (Rogers and Burns 1994). A known weight of the *A. platensis* [1 g fresh weight was suspended in 100 ml distilled water (1 %)] as described by Lefort-Tran et al. (1988), was taken and used for seeds treatment.

2.2 Plant materials

Pure identified strain of faba bean (*Vicia faba*: Giza 843) was used for this study. The seeds were obtained from Main Crops Improvement Station (MCIS) in Kafr El-Sheikh (Egypt).

2.3 Herbicide used

2.3.1 Fusilade

Common name: EC 12.5 % fusilade DX/fluazifop-*p*-butyl (EC: Emulsifiable concentrate).

Chemical name: (*R*)-2-[4-[[5-trifluoromethyl]-2-pyridinyl] oxy] phenoxy] propanoic acid.

Agrochemical type: Aryloxy-phenoxypropionate herbicide.

Mode of action: Inhibition of lipid formation.

2.4 Cultivation and treatments

Faba bean seeds were selected, sterilized in sodium hypochlorite solution (1 %) for 15 min, washed thoroughly with distilled water, then primed in distilled water (as control) or 1 % *A. platensis* suspension for 12 h. Cultivation took place under normal environmental conditions of light and temperature in pots (35-cm diameter containing equal amounts of clay and sand loamy soil; 2:1 v/v, pH 7.1, Ec 0.9 (m mohs/cm). Each treatment was replicated three times. Ten seeds were sown in each pot. The pots were placed in the green house of the Botany Department. The dose of the chemical fertilizers (super phosphate 100–150 kg/fadden) and (ammonium sulfate 15 kg/fadden) were added in doses recommended by the Ministry of Agriculture. Fusilade was applied (foliar application) as post emergence herbicide after 21 days of cultivation. The recommended dose of the herbicide (500 cm^3/fadden) was applied per pot and it was calculated according to the surface area as related to the surface area of fadden (4200 m^2). The pots were arranged in rows and the treatments were control (not treated), fusilade herbicide treatment alone, 1 % *A. platensis* treatment alone and 1 % *A. platensis* + fusilade herbicide treatment.

2.5 Growth and yield measurements

Plant samples were taken at different stages: seedling stage (15 day old)—vegetative stage (45 day old). Plant height, root and shoot length, number of leaves/plant, fresh and dry weight of root and shoot and leaf area were recorded at the vegetative stage. Leaf area was recorded using Ushikata x-plan 360d Planimeter (Featonby-Smith and Van Staden 1983). Yield of faba bean plant was recorded as number of pods/plant, number of seeds/pod, number of seeds/plant and weight of 100 seeds.

2.6 Biochemical analyses

Photosynthetic pigments were estimated in the fresh leaves according to Metzner et al. (1965). The photosynthetic activity was followed by measuring chlorophyll fluorescence of leaves as described by Gonçalves and Santos Junior (2005) using modulated light MINI-PAM portable fluorescence (ADC Fim 1500). The carbohydrates content was measured using the method reported by Naguib (1963); total protein was estimated according to Bradford (1976). Total free amino acids were determined according to Lee and Takahashi (1966). The activity of the antioxidant enzymes (peroxidase, catalase) were measured according to Kato and Shimizu (1987) and ascorbic acid oxidase according to Oberbacher and Vines (1963).

The proline content was determined according to Bates (1973). Lipid peroxidation was measured as concentration of malondialdehyde (Uchiyama and Mihara 1978). The amino acids profile and their content were screened using Amino acid analyzer Model: Dionex ICS-3000) in seedling and vegetative stages.

2.7 Statistical analysis

A complete experimental randomized block design with three replicates was adopted. The statistical analysis carried out using SAS program version 6.12. Data obtained were analyzed statistically to determine the degree of significance between treatments using one way analysis of variance (ANOVA). Additionally, the least significant differences (LSD) test was used to determine treatment differences comparing with control at $P \leq 0.05$ level of significance (Snedecor 1970).

3 Results

3.1 Effect on growth and yield

Table 1 indicates that the application of herbicide fusilade to faba bean caused significant reductions in the measured growth parameters (9 % in root length, 6 % in shoot length, 14 % in number of leaves/plant and 18 % in total area of leaves). The recorded reduction was accompanied by marked decrease in fresh and dry weight of both root and shoot. The fresh weight of root and shoot of plant was reduced by 57 and 12 %, whereas the dry weight was decreased by 50 and 41 %, respectively. The changes in the growth potential of faba bean plant were similarly reflected on their yield (Table 2). This reduction was expressed as decrease in number of pods/plant (5 %) and weight of 100 seeds (13 %). Priming of seeds in algal suspension of *A. platensis* before germination caused significant increase in the different measured growth parameters of the plant in comparison to control (Table 1). Results show that priming of seeds in the algal suspension *A. platensis* before cultivation has alleviated the adverse effects induced by the herbicide on the above mentioned growth parameters of the plant, with an appreciable extent in case of the dual treatment (algal suspension and herbicide fusilade) compared with the corresponding single treatment (algal suspension alone). The percentage of increase in the weight of 100 seeds produced from the algal primed seeds reached 30 % (Table 2).

Table 1 Effect of seeds priming in algal suspension (1 % *A. platensis*) on some growth parameters of the produced faba bean plant (45 day old) treated with fusilade herbicide

Treatment	Root length (cm)	Shoot length (cm)	No. of leaves/plant	Leaf area (cm^2)	Fresh weight (g)		Dry weight (g)	
					Root	Shoot	Root	Shoot
Control	20.2 ± 0.5^{g}	31.7 ± 0.5^{g}	18.3 ± 0.3^{e}	16.8 ± 0^{g}	2.8 ± 0.03^{g}	18 ± 0.3^{g}	0.2 ± 0.005^{f}	1.7 ± 0.0^{g}
Fusilade herbicide	18.3 ± 0.5^{h}	29.9 ± 0.8^{h}	15.8 ± 0.3^{f}	13.7 ± 0.05^{h}	1.2 ± 0.01^{h}	15.9 ± 0.1^{h}	0.1 ± 0.005^{g}	1 ± 0.03^{h}
1 % *A. platensis*	27.2 ± 0.3^{d}	40.5 ± 0.3^{d}	22.0 ± 0.3^{c}	18.4 ± 0.08^{e}	4.9 ± 0.05^{d}	24.4 ± 0.5^{d}	0.4 ± 0.003^{d}	2.9 ± 0.08^{d}
1 % *A. platensis* + Fusilade	30.6 ± 0.5a	48.3 ± 0.1^{a}	24.3 ± 0^{a}	24.7 ± 0^{a}	6.2 ± 0.2^{a}	31.3 ± 0.5^{a}	0.7 ± 0.05^{a}	4.4 ± 0.3^{a}
F (value)	63823.1***	393.1***	143.9***	7930.6***	44695.4***	20984.1***	754.8***	41551.7***

Each value is the mean value of three values ± standard error

ns no significant difference at $P > 0.05$; * Significant at $P < 0.05$; ** Significant at $P < 0.01$; *** Highly significant at $P < 0.001$ *F* (value). Means with the same letters are not significantly different

Table 2 Effect of seeds priming in algal suspension (1 % *A. platensis*) on some yield parameters of faba bean plant produced from faba bean seeds treated with fusilade herbicide

Treatment	No. of pods/plant	No. of seeds/pod	No. of seeds/plant	Weight of 100 seeds (g)
Control	6 ± 0.3^{c}	3 ± 0.05^{b}	18 ± 0.1^{b}	53.7 ± 0^{g}
Fusilade herbicide	5.7 ± 0^{d}	3 ± 0.05^{b}	18 ± 0.5^{b}	47 ± 0^{h}
1 % *A. platensis*	6.3 ± 0^{c}	3.3 ± 0.05^{a}	21.3 ± 0.3^{a}	60.4 ± 0^{d}
1 % *A. platensis* + Fusilade	7 ± 0.3^{a}	3.7 ± 0.1^{a}	25.7 ± 0.005^{a}	70 ± 0^{a}
F (value)	4.3**	1.6*	3.7**	3532.3***

Each value is the mean value of three values ± standard error

ns no significant difference at $P > 0.05$; * Significant at $P < 0.05$; ** Significant at $P < 0.01$; *** Highly significant at $P < 0.001$ *F* (value). Means with the same letters are not significantly different

Table 3 Effect of seeds priming in algal suspension (1 % *A. platensis*) on total pigments, photosynthetic activity and carbohydrates content of the produced faba bean plant (45 day old) treated with fusilade herbicide

Treatment	Total pigments (mg/g DW)	Photosynthetic activity (F_v/F_m)	Carbohydrate (mg/g DW)			
			DRV		TRV	
			Root	Shoot	Root	Shoot
Control	4.2 ± 0.1^g	2450 ± 1^b	44 ± 0.5^g	58.2 ± 0.5^g	53.3 ± 0.5^f	60 ± 0.5^c
Fusilade herbicide	3.4 ± 0.1^h	2468 ± 1.5^a	42.9 ± 0.5^h	57 ± 0.5^h	50 ± 0.8^g	59.3 ± 0.2^c
1 % *A. platensis*	9.1 ± 0.3^d	2342.7 ± 1.5^e	46.9 ± 0.6^d	60.4 ± 0.3^d	55 ± 0.3^d	63.5 ± 0.3^b
1 % *A. platensis* + Fusilade	14.9 ± 0.5^a	2203 ± 1^h	48.9 ± 0.3^a	66.5 ± 0.2^a	57.3 ± 0.3^a	69.6 ± 0.2^a
F (value)	99999.99***	44695***	1970***	630***	215.2***	12.1***

Each value is the mean value of three values ± standard error

Reduction in F_v/F_m value means higher photosynthetic activity

DW dry weight, F_v variable fluorescence, F_m maximum fluorescence

ns no significant difference at $P > 0.05$; * Significant at $P < 0.05$; ** Significant at $P < 0.01$; *** Highly significant at $P < 0.001$ *F* (value). Means with the same letters are not significantly different

Table 4 Effect of seeds priming in algal suspension (1 % *A. platensis*) on total carbohydrates, protein and free amino acids content of the faba bean seeds (fruiting stage) produced from faba bean plant treated with fusilade herbicide

Treatment	DRV (mg/g DW)	Sucrose (mg/g DW)	Starch (mg/g DW)	Total protein (mg/g DW)	Total free amino acids (mg/g DW)
Control	39 ± 0.8^g	45.2 ± 0.5^g	100 ± 1^g	83.1 ± 1^h	32 ± 0.2^h
Fusilade herbicide	36.6 ± 1^h	43. ± 60.5^h	97.3 ± 0.8^h	92 ± 1.1^g	36.8 ± 0.2^g
1 % *A. platensis*	42.4 ± 0.5^d	47.6 ± 0.8^d	107.2 ± 1.1^d	115 ± 0.8^d	46.2 ± 0.3^d
1 % *A. platensis* + Fusilade	47.4 ± 0.3^a	52.4 ± 1.2^a	112 ± 0.6^a	121.8 ± 1.1^a	51.2 ± 0.2^a
F (value)	97.3***	1860.3***	1239.4***	16291.9***	20027.8***

DW dry weight

Each value is the mean value of three values ± standard error

ns no significant difference at $P > 0.05$; * Significant at $P < 0.05$; ** Significant at $P < 0.01$; *** Highly significant at $P < 0.001$ *F* (value). Means with the same letters are not significantly different

3.2 Effect on metabolites

Results showed that application of fusilade caused reduction in total pigments and photosynthetic activity of leaves (Table 3) and also caused reduction in carbohydrates content of root, shoot and produced seeds (Tables 3, 4). On the other hand, total protein and free amino acids were increased for both root, shoot and produced seeds (Tables 4, 5). On the other hand, priming of seeds in the algal suspension *A. platensis* ameliorated the adverse effects on the different metabolites (Tables 3, 4, 5). This was true for both the vegetative and the fruiting stages, with further increase in case of the dual treatment (Tables 3, 4, 5).

Results in Table 6 show a significant increase in proline content in both root and shoot in response to herbicide treatment. Results show also an increase in lipid peroxidation (calculated as MDA accumulation) as a result of herbicide application (Table 6). Application of the herbicide caused also an increase in peroxidase activity and ascorbic acid oxidase, while the activity of catalase was decreased (Table 6). Again the priming of seeds in the algal suspension *A. platensis* caused a reduction in proline content, lipid peroxidation and changes in antioxidant enzyme activities.

Concerning the amino acids profile of seedlings (without herbicide treatment; Table 7), the results show that seed priming caused significant changes in amino acids profile and their concentrations. These changes were reflected in the disappearance of the amino acids Argnine and glutamic acid and the appearance of some amino acids; proline, isoleucine, leucine, methionine, aspartic acid, cyctein, serine and phenylalanine which were not present in control. On the other hand, the concentration of threonine and valine was increased, whereas those of lysine and histidine was decreased. At the vegetation stage (45 day old), fusilade treatment caused a reduction in the concentration of threonine, tyrosine, histidine, valine, phenylalanine and serine, whereas the concentration of proline, glutamic acid, cyctein, alanine, aspartic acid and isoleucine) were increased. Algal

Table 5 Effect of seeds priming in algal suspension (1 % *A. platensis*) on protein and amino acids content of the produced faba bean plant (45 day old) treated with fusilade herbicide

Treatment	Total protein (mg/g DW)		Total free amino acids (mg/g DW)	
	Root	Shoot	Root	Shoot
Control	22.7 ± 0.3^{h}	35.3 ± 0.1^{h}	6.8 ± 0.2^{h}	10.6 ± 0.4^{h}
Fusilade herbicide	28.8 ± 0.3^{g}	43.5 ± 0.3^{g}	9.5 ± 0.1^{g}	13 ± 0.1^{g}
1 % *A. platensis*	41.7 ± 0.6^{d}	58.2 ± 0.5^{d}	12.5 ± 0.2^{d}	17.5 ± 0.3^{d}
1 % *A. platensis* + Fusilade	50 ± 0.5^{a}	65 ± 0.1^{a}	16 ± 0.3^{a}	20.8 ± 0.2^{a}
F (value)	34813.4***	22668***	32679***	30743***

DW dry weight

Each value is the mean value of three values ± standard error

ns no significant difference at $P > 0.05$; * Significant at $P < 0.05$; ** Significant at $P < 0.01$; *** Highly significant at $P < 0.001$ *F* (value). Means with the same letters are not significantly different

Table 6 Effect of seeds priming in algal suspension (1 % *A. platensis*) on proline, antioxidant enzymes activities and lipid peroxidation (measured as MDA) level of the faba bean of the produced faba bean plant (45 day old) treated with fusilade herbicide

Treatment	Proline content (mg/g DW)		Leaves			
	Root	Shoot	Peroxidase enzyme (Um/g FW min)	Catalase enzyme (Um/g FW min)	Ascorbic acid oxidase enzyme (Um/g FW min)	MDA (mg/g DW)
Control	0.6 ± 0.05^{b}	1.9 + 0.01^{b}	1.2 ± 0.05^{b}	1.1 ± 0.005^{b}	0.3 ± 0.01^{b}	1.5 ± 0.05^{b}
Fusilade herbicide	0.7 ± 0.04^{a}	2.4 ± 0.03^{a}	2.8 ± 0.06^{a}	0.03 ± 0.001^{a}	0.6 ± 0.02^{a}	2.3 ± 0.06^{a}
1 % *A. platensis*	0.5 ± 0.03^{c}	1.7 ± 0.03^{d}	1.1 ± 0.01^{e}	0.08 ± 0.003^{b}	0.2 ± 0.03^{e}	0.7 ± 0.03^{d}
1 % A. *platensis* + Fusilade	0.4 ± 0.03^{f}	1.6 ± 0.02^{f}	0.9 ± 0.05^{g}	0.05 ± 0.002^{b}	0.15 ± 0.02^{h}	0.03 ± 0.02^{g}
F (value)	349.4***	1158.8***	4148.7***	1.2*	99999.9***	387.5***

DW dry weight, *FW* fresh weight, *MAD* malondyaldehyde concentration

Each value is the mean value of three values ± standard error

ns no significant difference at $P > 0.05$; * Significant at $P < 0.05$; ** Significant at $P < 0.01$; *** Highly significant at $P < 0.001$ *F* (value). Means with the same letters are not significantly different

treatment caused a reduction in threonine, proline, glutamic acid, alanine, lysine, histidine, methionine and glycine, while the concentration of aspartic acid, valine, leucine, isolucine, phenyl alanine and serine were increased, resulting in a marked increase in the total amino acids content. Dual treatment (priming in algal suspension + treatment with herbicide) caused an increase in the concentration of arginine, threonine, glutamic acid, tyrosine, alanine, aspartic acid, glycine, valine, leucine, isoleucine, phenyl alanine, methionine and serine leading to a marked increase in the total amino acids content. These changes in the type of amino acids and their levels were observed in plants produced from primed seeds or the dual treated plants.

4 Discussion

Application of herbicide fusilade super to faba bean caused a reduction of some growth parameters (plant height, root length, shoot length, number of leaves/plant and total area of leaves), these results are in accordance with those obtained by other workers using different plants treated with other herbicides such as: beans, sesame and cabbage (Miller et al. 2003; Nacheva et al. 2012). These reductions in the different growth parameters of faba bean caused also a reduction in their yield. Such results coincided with those of Ahsan et al. (2008) in rice plant treated with glyphosate herbicide and Bondada (2011) in grapevine plant treated with 2,4-D herbicide.

Priming of seeds in algal suspension of *A. platensis* caused significant rise in the values of the different measured growth parameters of the plant in comparison to control. These results are consistent with those obtained by other workers (Likhitkar and Tarar 1995 in cotton; Haroun and Hussein 2003 in *Lupinus termis* seeds; Faheed and Abd-El Fattah 2008 in lettuce plant).

Results showed that application of fusilade caused a reduction in total pigments, carbohydrate content and photosynthetic activity. On the other hand, total protein and free amino acids were increased for both roots, shoots and

Table 7 Amino acids content (mM/g DW) of faba bean leaves (15 day old) produced from seeds suspended 1 % *A. platensis* and faba bean plant leaves (45 day old) produced from seeds treated with (fusilade super alone, 1 % *A. platensis* alone and 1 % *A. platensis* + fusilade herbicides) in comparison with control

Type of amino acid (mM/g DW)	Seedling (15 day old)		Vegetative (45 day old)			
	Control	1 % *A. platensis*	Control	Fusilade (herbicide)	1 % *A. platensis*	1 % *A. platensis* + Fusilade
Arginine	0.2	Nd	0.5	0.52	0.5	0.7
Threonine	8.8	54.5	0.5	0.28	0.4	0.6
Proline	Nd	14.2	0.4	0.48	0.3	0.4
Glutamic acid	4.2	Nd	0.7	1.02	0.6	0.8
Cystine	Nd	11.5	0.003	0.01	Nd	Nd
Tyrosine	Nd	Nd	1.0	0.32	0.9	1.4
Alanine	Nd	Nd	0.6	0.64	0.5	0.8
Aspartic acid	Nd	100.4	1.3	4.1	3.6	4.1
Lysine	1.8	0.8	0.96	1.0	0.6	0.1
Histidine	13.1	0.7	0.62	0.4	0.5	0.6
Glycine	Nd	Nd	0.47	0.5	0.4	0.7
Valine	38.7	60	0.9	0.8	1.0	1.3
Leucine	Nd	0.08	0.61	0.6	0.9	1.5
Isoleucine	Nd	0.005	0.83	1.0	2.1	2.2
Phenyl alanine	Nd	40	0.60	0.4	0.8	1.2
Methionine	Nd	12.4	0.09	0.02	0.05	0.1
Serine	Nd	70	0.52	0.4	1.1	1.6
Total	66.7	364.6	10.7	11.9	14.2	18.2

DW dry weight, *Nd* non detectable

produced seeds. These results are in accordance with those obtained by other workers (Fayez et al. 2011 in peanut treated with fusilade and basagran herbicide; Kumar 2012 in *Triticum Aestivum* treated with 2,4-D and isoproturon herbicide and Badr et al. 2013 in *vicia faba* treated with metosulam herbicide).

The above mentioned reductions in the measured growth parameters can be attributed to the interference of fusilade with one or more of the metabolic process in faba bean plant. This can be realized from the reduction of the photosynthetic activity and the significant reduction in the level of carbohydrate.

Reduction in pigment content could be attributed to the photo-oxidation of chlorophylls by the herbicide and/or inhibition of biochemical reactions involved in their biosynthesis.

However, priming of seeds in the algal suspension *A. platensis* has ameliorated the adverse effects of herbicide on the different metabolites. Similar results were reported by Kannaiyan (2000) in rice; Ayyappan et al. (2002) in sunflower. The promotion of carbohydrate biosynthesis which is related to the increase in the photosynthetic activity induced by algal treatment may be responsible for the rise in amino acid and protein contents. It could be suggested that increase in carbohydrate content being accompanied with an increase in respiration rate causing a rise in α- keto-acids (Kreb's cycle) which are the main precursor for amino acid biosynthesis.

Results show an increase in the proline content in both root and shoot of the plant in response to herbicide treatment, which is in agreement with those obtained by Fayez (2000) in soybean treated with diuron herbicide; Fayez et al. (2011) in peanut treated with fusilade and basagran herbicide and Badr et al. (2013) in *vicia faba* treated with metosulam herbicide. Increased levels of proline in plants are correlated with enhanced stress tolerance as suggested by Molinari et al. (2007) and Banu et al. (2009).

Increase in lipid peroxidation (calculated as MDA accumulation) as a result of herbicide are in accordance with data obtained by many workers using different plants treated with other herbicides (Nemat-Alla and Hassan 2006; Sergiev et al. 2006 in maize).

Reduction or increase in the activities of antioxidant enzymes were recorded by other workers with many crop plants in response to herbicides application (Nemat-Alla and Hassan 2006 in maize; Kim and Jung 2013, in rice). These changes could be tentatively interpreted on the basis of the balance between the production and scavenging of reactive oxygen species (ROS) and the relative sensitivities of each antioxidant enzyme to other cell signals (Mittler 2002). These results support that priming of faba bean seeds before cultivation can promote the synthesis of some substances acting as safener against the adverse effects of the herbicide and/or exert a protective effect by enhancing herbicide detoxification.

Amino acid profile and their contents were changed in plants produced from primed seeds (Table 7). Among these changes were the appearance of the leucine, isoleucine, valine, serine, phenylalanine, methionine and histidine which were not present in the control. These amino acids are already used as chemical safeners against the adverse effects of some herbicides (Ray 1984; Shaner and Reider 1986; Mugnier 1988; Coruzzi and Last 2000; Davien and Preston 2000; Forgacs et al. 2000). Accordingly it could be suggested that synthesis of these amino acids in response to algal treatment is the main cause correlated with the amelioration of the adverse effects of the herbicide. Similar effects have also been observed in cucumber plants inoculated with Azospirillum brasilense, suggesting that amino acids biosynthesis might also represent a plant response to bacterial determinants (Pii et al. 2015).

The increase in some amino acid contents; arginine, threonine, glutamic acid, tyrosine, alanine, aspartic acid, glycine, valine, leucine, isoleucine, phenyl alanine, methionine and serine accompanied with the reduction in others; threonine, proline, glutamic acid, alanine, lysine, histidine, methionine and glycine in response to algal treatment suggesting an enhancement in the transaminase activities leading to the synthesis of new amino acids; proline, isoleucine, leucine, methionine, aspartic acid, cyctein, serine and phenylalanine whereas, the increase in the content of the already present ones could support our suggestion that they act as safener against the harmful action of the herbicide (fusilade) on the target plant (faba bean).

5 Conclusion

According to the obtained results, priming of faba bean seeds in algal suspension (1 % *A. platensis*) before cultivation has induced the biosyntheses of some amino acids or may be other compounds required as safener against the harmful action of herbicides. However, the produced amino acids can play a unique role in the regulation of stress signaling pathways, leading to build up tolerance mechanisms in plants under the adverse effects of the herbicides. It is known that algae contain many compounds: growth regulators, vitamins, amino acids, polypeptides and fatty acids which can stimulate the different metabolic processes. So priming of faba bean seeds in algal suspension *A. platensis* (instead of chemical safeners) could be a fruitful method to minimize crop injury by herbicide, through the induction of biosyntheses of the substances required for the resistance of the harmful effects of the herbicide.

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