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Amelioration the toxic effect induced by herbicide granstar on barley yield by the cyanbacterium *Nostoc muscorum*

Atef M. Abo-Shady, Mohamed Elanwar H. Osman and Maysa M.F. El-Nagar

Botany Department, Faculty of Science, Tanta University, Tanta 31527, Egypt

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ABSTRACT

The present study aimed to ameliorate the harmful effect induced by granstar herbicide on barley plant through priming the grains in the cyanobacterial suspension of *Nostoc muscorum* before cultivation. The results show that herbicide treatment caused reduction in all yield parameters (number of spikes/plant, spike length, weight of spike, number of grains/spike and weight of 100 grains).

Priming of barley grains in the cyanbacterium *Nostoc muscorum* suspension before cultivation ameliorated the harmful effect induced by herbicide on the plant. Priming treatments induced synthesis of some amino acids which were not present in the control plant and increased the concentration of the amino acids which were already present. This result suggests a unique role of amino acids in the amelioration of the harmful effect induced by the herbicide.

Keywords: *Nostoc muscorum*, Amino acids, Herbicide, Barley, Priming.

Introduction

Method of weeds control are many and variable, each may be used alone or all are usually integrated for any successful weeds control programs (Singh *et al.,* 2006). Herbicide are truly essential to good yield of different crops by suppressing different types of weeds, it is highly affect yield through competing with crops for light, moisture, space and plant nutrients (Crain and Dybzinski, 2013). Herbicides differ in their structure but all exert unfavorable effects after penetration the plant (Spiridonov *et al.*, 2010).

Crops injury may be due to direct or indirect exposure to herbicide molecules. Herbicides are absorbed by crop plants as well as by weeds; certain herbicides are completely metabolized while others are not (Pline and Hatzios, 2003).

Cyanobacteria are a rich source of several fine chemicals of economic value such as vitamins, carotenoids, phycobiliprotein, polysaccharides, amino acids, fatty acids, hormones, etc, with varied properties and possess anti-inflammatory, anticancer, anti-fungal, antioxidant and immune-modu-lator agents (Abd El Baky *et al.,* 2004). In a previous study Osman *et al.* (2015) found an additional role of cyanobacteria, through *Arthrospira platensis*, as an agent for alleviating the adverse effect of fusillade herbicide on faba bean plant.

In the present study a preliminary experiment was carried out to measure the influence of some other cyanobacterial species (*Oscillatoria angustissima, Nostoc muscorum, Anabaena subcylindrica, Anabaena variables* and *Arthrospira platensis*) on some growth parameters of barley seedlings produced from grains presoaked in different cyanobacterial suspensions. The obtained data indicated stimulation of the different growth parameter of the plant in response to treatment with the different cyanobacterial species used. The highest stimulating effect was recorded with *Nostoc muscorum* treatment.

This work was designed to test, if the cyanbacterium *Nostoc muscorum* could act as protective agent to improve tolerance of barley plant against the effect of granstar herbicide through priming grains before cultivation in a suspension of this organism.

Materials and Methods

Algal Isolate

Nostoc muscorum was grown on BG11 medium (Rippka *et al.,* 1979) and harvested at the beginning of stationary growth phase (14 day old) by centrifugation at 3000 rpm (Fisher CenterificTm

Corresponding Author: Maysa M. F. El-Nagar, Botany Department, Faculty of Science, Tanta University, Tanta 31527, Egypt. E-mail: elnagar_maysa@yahoo.com

Centrifuge) for 10 min. A known weight of the *Nostoc muscorum* [1g fresh weight was suspended in 100 ml distilled water (1%)] as described by Lefort-Tran *et al.* (1988), was taken and used for grains treatment.

Plant Material

Pure identified strain of barley (*Hordeum vulgare*: Giza 2000) was used for this study. The grains were obtained from Main Crops Improvement Station (MCIS) in Kafr El-Sheikh (Egypt).

Herbicide Used

Granstar

Common name: DP 75% Tribenuron–methyl (DP: Dustable powder).
Molecular formula: $C_{15}H_{17}N_5O_6S$. Agrochemical type: Sulfonylurea's herbicide.
Mode of action: Amino acids inhibitor.

Crop cultivation

Barley grains were selected, sterilized in sodium hypochlorite solution (1%) for 15 minutes, washed thoroughly with distilled water, then primed in the following solutions for 2 hours:
1- Distilled water (control). 2- 1% *Nostoc muscorum* suspension

The grains were then sown at 2cm depth, each 15 grains were sown in pots (35-cm diameter filled with clay and sand; 2:1 v/v, pH-7.1, Ec - 0.9 (m mohs cm^{-1}), 2.8 and 2.3 mg/g dry weight of N and P content, respectively). The pots were placed in the green house of the Botany Department (at normal conditions suitable for barley growth). Standard agricultural practices were carried out as recommended. Granstar was applied as post emergence herbicides after 21 days of cultivation (foliar application). The recommended dose of the herbicide (8 g / Fed) was applied per pot and it was calculated according to the surface area as related to the surface area of Feddan (4200 m^2).

Parameters studied

Yield of barley plant was recorded as:
1- Number of spikes/plant 2- Spike length (cm) 3- Weight of spike (g)
4- Number of grains/spike 5- Weight of 100 grains (g).

Yield components of barley plant were determined as:
1- Total carbohydrates (Naguib,1963)
2- Total protein (Bradford, 1976)
3- Total free amino acids (Lee and Takahashi, 1966).
4- Different elements were determined according to Allen *et al.* (1974).
5- The amino acids profile and their content were screened using Amino acid analyzer Model: Dionex ICS-3000)
6- Protein profile was determined for barley grains by methods described by Laemmili (1970).

Statistical Analysis

A complete experimental randomized block design with 3 replicates was adopted. The statistical analysis was 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).

Results

Application of herbicide granstar on barley caused reduction in all yield parameters. This reduction were expressed as decrease in number of spike/plant, spike length, spike wt, number of grains/spike and weight of 100 grains (Table 1).

Priming of grains before cultivation in algal suspension of *Nostoc muscorum* caused significant increase in yield parameters of barley plant in comparison to control (Table 1). A similar trend, was generally obvious in case of dual treatment (granstar + *Nostoc muscorum*) as compared to corresponding single treatment (*Nostoc muscorum*) alone (Table 1).

Table 1: Effect of grains priming in *1% N. muscorum* suspension on some yield parameters of barley plant

Treatment	No of spikes / plant	Spike length (cm)	Spike wt (g)	No of grains / spike	Weight of 100 grains (g)
Control	3.7±0.3[c]	16.2±0.05[g]	1.8±0.001 [g]	20±0 [g]	7.5±0.5 [f]
Granstar herbicide	3±0 [d]	15.3±0.05 [h]	1.5±0.005[h]	17±0 [h]	5.2±0.8 [g]
1% *N. m*	4±0 [c]	17.7±0.08 [d]	2.4±0.005 [d]	25±0[d]	8.8±0.5 [d]
1% *N. m* + Gransta	5.7±0.3[a]	18.9±0.1 [a]	3.2±0.01 [a]	31±0 [a]	10±0.5 [a]
F (value)	13.9***	459.8***	13050.7***	99999.9***	412.8***

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.

Carbohydrates content including direct reducing value (DRV), total reducing value (TRV) and starch of the produced barley grains were decreased in response to of granstar herbicides treatment. The reduction (4.6%, 4.7% and 1.05%) was recorded respectively, as compared to control (Table 2). Protein and total free amino acids content of the produced barley grains also decreased in response to treatment with the post-emergence herbicide granstar by 18.7% and 22.3%, respectively, as compared to control.

All These values showed marked increase in response to grains priming in 1% *N. muscorum* suspension, as compared to control. The combination between algal suspensions and granstar treatment caused further increase in protein and total free amino acids content (Table 2).

Table 2: Effect of grains priming in 1% *N. muscorum* suspension on the measured carbohydrate fractions (DRV, sucrose, starch), protein and free amino acids content of the produced barley grains

Treatment	DRV (mg/g DW)	Sucrose(mg/g DW)	Starch (mg/gDW)	Total protein (mg/g DW)	Total free amino acids (mg/g DW)
Control	40.5±0.3[g]	53.5±0.6[g]	381±1[e]	38.37±0.2[g]	11.5±0.03[g]
Granstar herbicide	38. 7±0.8[h]	50.9±0.3[h]	377±1.2[f]	31.2±0.1[h]	8.9±0.03[h]
1% *N. m*	45.8±0.1[d]	58±0.5[d]	387.9±1.4[c]	56±0.5[d]	16±0.2[d]
1% *N. m* + Granstar	50.2±0.8[a]	64. 2±0.8[a]	392±1[a]	79.5±1.0[a]	26.4±0.1[a]
F (value)	1501.4***	406.8***	79.7***	60925***	59139.8***

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.

Data presented in Table 3 show that the content of N, K, Ca, Mg and Fe was decreased in response to granstar application by 31.5%, 7.8%, 55.8%, 25.1% and 40.8%, respectively, while P and Na content increased by 21.3%, 42.1% respectively, in comparison to control.

On the other hand , the content of different elements N, K, Ca, Mg and Fe were increased , whereas P and Na content were decreased in response to grains priming in 1% *N. muscorum* suspension, as ompared to control. However, the combination between priming and granstar treatment caused further increase in their content (Table 3).

Table 3: Effect of grains priming in 1% *N. muscorum* suspension on different elements content of the produced barley grains

Treatment	N (mg/g)	P (mg/g)	K (ppm)	Na (ppm)	Ca (ppm)	Mg (ppm)	Fe (ppm)
Control (H_2O)	4.96±0.5 ^{g}	1.41±0.5 ^{b}	10.30±0.5 ^{g}	7.60±0.5 ^{b}	5.20±0.5 ^{g}	2.03±0.5^{g}	1.74±0.05 ^{e}
Gra (8g/F)	3.40±0.5 ^{h}	1.71±0.6 ^{a}	9.50±0.5^{h}	10.80±0.5 ^{a}	2.30±0.5 ^{h}	1.52±0.5 ^{h}	1.03±0.01 ^{f}
1% *N. m*	6.37±0.3 ^{d}	0.98±0.03^{e}	12.80±0.5^{d}	5.20±0.5 ^{e}	5.87±0.5 ^{d}	2.62±0.2^{d}	1.98±0.05 ^{c}
1% *N. m* + Gra	8.30±0.5^{a}	0.90±0.08 ^{a}	15.83±0.8 ^{a}	3.03±0.3 ^{h}	7.46±0.1^{a}	3.20±0.2^{a}	2.50±0.05^{a}
F (value)	1568.56***	6001.89***	1378.81***	1509.09***	4067.73***	361.96***	130.56***

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 4 shows the changes in the amino acids content of the harvested grains of barley produced from plants of algal treated grains or plants treated with granstar. Granstar application increased the amount of arginine, threonine and leucine, while amino acids: proline, cystine, valine, isoleucine and phenyl alanine were decreased in response to granstar application.

Table 4: Amino acids content (mM/g D.wt) of harvested barley grains, as affected by herbicide (granstar), 1% *N. muscorum* treated grains and 1% *N. muscorum* treated grains + herbicide in comparison with control

Type of amino acids	mM /g D.wt			
	Control	Granstar (herbicide)	1% *N. muscorum*	1% *N. muscorum* + granstar (herbicide)
Arginine	0.22	0.49	0.55	0.22
Threonine	1.51	1.84	Nd	Nd
Proline	3.5	3.48	0.55	1.23
Glutamic acid	0.04	0.04	Nd	0.04
Cystine	0.65	0.60	2.03	1.4
Tyrosine	4.14	4.14	2.0	3.84
Alanine	Nd	Nd	1.5	0.06
Aspartic acid	Nd	Nd	3.01	0.52
Lysine	Nd	Nd	0.13	0.26
Histidine	Nd	0.01	0.06	0.29
Glycine	Nd	Nd	Nd	0.01
Valine	5.01	4.71	8.10	12.26
Leucine	0.1	0.2	0.31	0.8
Isoleucine	0.08	0.06	0.26	0.9
Phenyl alanine	0.8	0.74	0.98	2.08
Serine	Nd	0.03	0.08	0.50
Total	16.31	16.34	19.56	24.41

Nd= Non detectable

On the other hand, no change in amino acids content of tyrosine and glutamic acid was observed. Amino acids serine and histidine were not present in control but appeared in the grains treated with granstar herbicide. Priming the grains in 1% *N. muscorum* suspension caused increase in total amino acids content by 20% as compared to control value. Priming treatment increased the content of some amino acids (arginine, cystine, valine, isoleucine, leucine, and phenyl alanine) in the harvested grains, as compared to control and grains treated with granstar alone, while proline and tyrosine content were decreased as compared with control and grains treated with granstar alone.

Amino acids (alanine, aspartic acid and lysine) were present in grains produced from 1% *N. muscorum* treated grains but not present in grains produced from treated plant with granstar alone, while amino acids threonine and glutamic acid were not present in grains treated with 1% *N. muscorum* as compared to control. The amount of serine and histidine increased in grains treated with 1% *N. muscorum* suspension as compared to the grains treated with granstar herbicide alone. Combination between 1% *N. muscorum* treated grains and granstar herbicide application increased the amino acids (valine, isoleucine, leucine and phenyl alanine as compared with control, granstar alone and 1% *N. muscorum* alone. Content of arginine, proline, cystine and tyrosine were decreased in response to combined treatment (combination between 1% *N. muscorum* treated grains and granstar herbicide) as compared with control. The total amino acids content show marked increase (50%) in response to combined treatment.

Concerning the protein profile of the grains, scanning of the gel indicated the occurrence of 10 protein bands in the barley grains control ranging from 507 to 10 KDa. Application of different treatment on barley caused appearance or disappearance of some protein bands in the harvested barley grains (Fig. 1). New protein band with 90 KDa was recorded in response to granstar herbicide treatment. This protein band may act as protector against the toxic action of the herbicide. Our results revealed that application of cyanbacterium suspension *Nostoc muscorum* alone induced the appearance of protein bands having molecular weight of 168-137-112 and 105 KDa. The combination with granstar herbicide caused appearance of some protein bands in the harvested barley grains having molecular weight of 209-141-125-77 and 40 KDa (Fig.1).

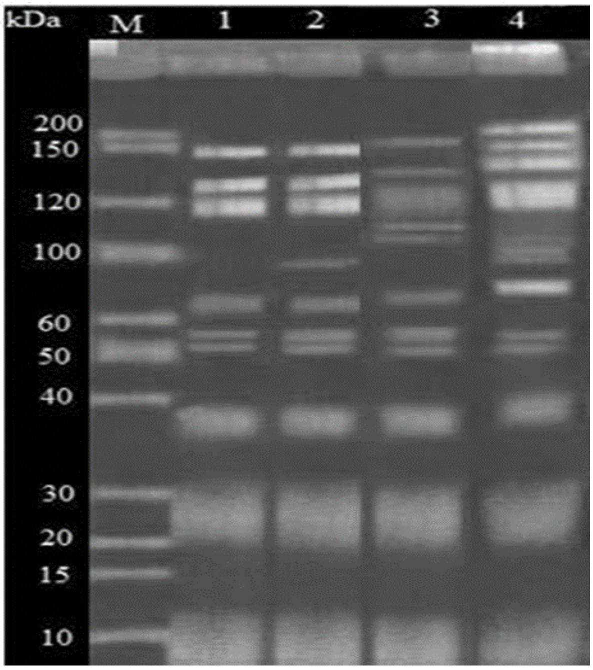

Fig. 1: The SDS-PAGE gel illustration shows the results of protein banding of produced barley grains from different treatments:
(M) Marker (1) Control (2) Granstar herbicide (3) 1% *N. muscorum* (4) 1% *N. muscorum* + granstar herbicide

Discussion

Several methods were examined to minimize crops injury caused by herbicides (Hassan, 1998). Herbicides induce different morphological, physiological and biochemical effects on the injury plants. The present results show that granstar herbicide caused reduction in all the measured yeild parameters (Table 1), these results are in accordance with those of Yazdanpak *et al.,* 2014 and Larbi *et al.,* 2013 who reported that the application of the herbicide 2, 4-D resulted in a significant reduction in yield of maize. Priming of barley grains in *Nostoc muscorum* suspension caused increased in all growth and yield parameters (Table 1), These results are consistent with those obtained by other workers with various plants treated with different algal suspensions (Haroun and Hussein, 2003 and Osman *et al.,* 2015).

Reduction in carbohydrates value, protein and free amino acids of the produced grains were also recorded in response to granstar application (Table 2). These results are in agreement with the data obtained by other workers (Scarponi *et al.,* 2005 and Rad *et al.,* 2011).

The reduction in carbohydrates indicates inhibition of the CO_2 fixation in Calvin cycle and/or inhibition of the photosynthetic electron transport; these findings are in conformity with data obtained by other workers (Alvie *et al.,* 2003 and Kumar, 2012).

There are evidences suggesting that herbicides besides controlling weeds induce phytotoxic effect on the plant resulted in a reduction in carbohydrate, protein and amino acids content of the plant. Some workers applied exogenous growth agents (such as nutrient elements or amino acids) with the herbicides in attempts to avoid the phytotoxic action of these herbicides without affecting the herbicide selectivity on weeds (Abouziena *et al.,* 2011 and Moran *et al.,* 2011).

Our results are in close conformity with Nowicka (1991), who observed a reduction in yield of different wheat varieties treated with chlorosulfuron and metoxuron herbicide. Herbicides also have toxic effect on the photosynthetic pigments and photo-system II electron transport and therefore, the process of photosynthesis which affect the carbohydrate metabolism and yield of crops (Osman *et al.,* 2015).

Priming of grains in *Nostoc muscorum* suspension caused increase in carbohydrates, protein and amino acids of the grains as compared to control (Table 2). These indicate that priming treatment alleviated the toxic action induced by the herbicide on the different metabolic process of the plant.

Granstar application to barley plants reduced the content of N and increased P of the harvested grains (Table 3), more or less similar data were observed by Malik and Tesfai, (1985) who found that Metribuzin and alachlor at recommended and five times more of recommended rates caused a significant reduction in nodulation, nitrogenase activity and total N content of soybean. In a similar study, the recommended and double the rates of metribuzin inhibited growth, nodulation and N_2 fixation in cowpea without adversely affecting the N uptake by the plant (Silva *et al.,* 1998). On the contrary, Billore *et al.* (2001) observed that fluchloralin when applied at normal rates with soybean, showed a considerable increase in nodulation on root system of groundnuts while the same rate of benefin, dinitroamine and nitralin decreased nodule mass, nitrogenase activity, yield and total N content, under field and greenhouse conditions (Durgesha, 1994). Higher content of P was reported by many authors due to the application of herbicides in wheat grains (Rachon and Szumieo, 2009 and Kraska, 2011), furthermore, herbicide treatment decreased the content of K, Na, Ca, Mg and Fe in the grains and seeds, respectively, as compared to control (Table 3). These results may indicate that application of the tested herbicides cause reduction in the process of ion uptake, as reported by Rachon and Szumieo (2009) and Kraska (2011). They reported that application of atrazine amounting, 10 to 100 ppm resulted in decrease in, potassium and sodium content of root and shoot of wheat (*Triticum Amestivum SSp. Spelta).*

Priming treatment caused changes in some elements content (Table 3). Algal extract as a new bio-fertilizer containing N, P, K, Ca, Mg and S as well as Zn, Fe, Mn, Cu, Mo and Co, some growth regulators, polyamines and vitamins are applied to improve nutritional status, vegetative growth, yield and fruit quality (Abd-Alla and El-Khoshiban, 2012 and Abd El-Moniem and Abd-Allah, 2008).

The changes in amino acids (profile and content) in the response to different treatments were investigated in this study. Priming of grains in *Nostoc muscorum* suspension (alone or in dual treatment) increased the amino acids content (Table 4). This may be attributed to increase in respiration leading to production of α-Keto acids (Krebs's cycle) which are required for amino acids biosynthesis.

The rise in the content of total amino acids in response to priming in the cyanobacterial suspension could suggest a direct role of such amino acids in the protection of plant against the adverse effect of the granstar herbicide and/or these amino acids may share in the synthesis of specific type of protein which act as protector against the toxic action of the herbicide. Furthermore, the increased in amino acid cystein which are considered as one of the precursor compounds for glutathione biosynthesis, which play an unique role in the defense mechanism against the toxic action of some inhibitory effects induced by some compounds in plant (Nemat-Alla and Hassan, 1998 and Riechers *et al.,* 2005).

The changes in Protein profile in response to priming treatment are in agreement with the data obtained by Haroun and Hussein, (2003), who found that protein banding patterns of *Lupinus termis* shoot soaked in culture filtrate of two blue-green algae namly *Cylindrospermum muscicola* and *Anabaena oryzae* showed disappearance of 34 and 42 KDa protein and appearance of 129, 3, 8 KDa by application of *Cylindrospermum muscicola*, meanwhile certain 18 KDa protein was only induced following treatment with *Anabaena oryzae*. They also found that application of both *Cylindrospermum muscicola* and *Anabaena oryzae* increased the intensities of protein bands from 20 to 32 KDa.

The appearance of new protein bands in response to cyanbacterium treatment could be indicating a changed pattern of gene expression (Popova *et al.,* 1995). Moreover, the lower molecular weight proteins are known to have a role in the stress tolerance (Waters *et al.,* 1996). Thus, it could be concluded that priming of barley grains in cyanbacterium suspension (1% *Nostoc muscorum*) induced biochemical changes in some amino acids and protein profile which may play an important role for the protection against the adverse action of herbicide granstar on barley plant.

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