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Effect of Different *Anabaena variabilis* (Kütz) Treatments on Some Growth Parameters and Physiological Aspects of *Hordeum vulgare* L. and *Trigonella foenum-graecum* L.

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CYANOBACTERIA are a diverse group of prokaryotes which occupies many environments. In terrestrial habitats, they were famed to benefit both soil and plants through releasing bioactive substances of various biological functions. In the present study four treatments of *Anabaena variabilis* (seeds primed in 1% fresh cyanobacterial extract, seeds sown in soils inoculated with 3g fresh or dry cyanobacterial cells per 1 kg soil and wet seeds primed with 3g dry cyanobacterial powder per 1 kg seeds) were examined for their effect on *Hordeum vulgare* and *Trigonella foenum-graecum* L. plants. All treatments increased the germination percentage, shoot length, fresh and dry weights. The photosynthetic pigments, proteins, glutamic-oxaloacetic and glutamic-pyruvic transaminases (GOT and GPT) activities were also markedly increased specially in seeds primed with cyanobacterium dried cells. The protein profile of *Hordeum vulgare* seedlings revealed the appearance of newly formed protein band with molecular weight of 220 KDa in response to soaking the seeds in fresh cells extract. On the other hand, the protein profile of *Trigonella foenum-graecum* L. shows the induction of low molecular weight protein bands 10-14 KDa with all treatments. The plant growth promotive effect was attributed to the bioactive materials like phytohormones, exopolysaccharides, nitrogen, phosphorus and potassium estimated in the cyanobacterial biomass. The results recommended bio-priming the seeds of the two examined plants in dried cyanobacterial biomass as an economical and safe route fertilizer.

Keywords: Cyanobacteria, Transaminase enzymes, Gel electrophoreses, Biofertilizers.

Introduction

The overuse of synthetic chemicals in agriculture has resulted in great ecological degradation throughout the world, leading to soil infertility, ocean dead zones, and biodiversity loss (Chagnon et al., 2015). Biofertilizers are environmentally friendly, coast effective, alternative to synthetic fertilizers, for they not only enhance agricultural production but also diminish environmental pollution. The use of microalgae and cyanobacteria as biofertilizers has been experienced as possible solution for the defects resulted from the extensive use of chemicals in agriculture (Kawalekar, 2013).

Many researches recommended cyanobacteria as a biofertilizer for many vegetable crops e.g. sorghum, maize, lentil, chickpea, barley, sugar beet, and bean (Adam, 1999; Hegazi et al., 2010 and El-Naggar et al., 2014) . Cyanobacteria can enrich the soil with micro and macronutrients, plant growth regulators and different bioactive secondary metabolite compounds that inhibit the growth of soil pathogenic bacteria and fungi (Karthikeyan et al., 2007). In addition they secrete mucilage and polysaccharide materials that improve soil structure, porosity, aggregation stability and fertility, thus enabling recovery of poor soils and increase plant growth (Osman et al., 2005 and Maqubela et al., 2008) .

Seed treatment is a technique in which several organic, inorganic and biotic materials are added to seed by adhesive agents. This can be performed in two major ways: seed hydration (soaking or priming) treatment and coating seed treatment (Taylor et al., 1998). Seed soaking or priming has been defined as "a process by which seeds can be led to absorb nutrients, protectants, growth regulators, etc. by immersing them in appropriate solutions for extended periods" (Scott, 1998). Seed coating is a general term for the application of finely ground soils or liquids containing dissolved or suspended solids to form a more or less continuous layer covering the natural seed coat and includes pelleting and many other seed treatments. By priming, seeds are soaked in

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different low molecular weight osmoprotectants solutions to activate germination related metabolic processes. This inhibits seeds from imbibing sufficient water for radical projection, thus seeds remain hanging longer time in the lag stage of hydration. Additionally, it is a simple, inexpensive, easily trained treatment that increases germination, harmonize and reduces seedling emergence time, and increases stand formation (Taylor et al., 1998). The present-day management policy for agriculture depends on the keen exploration for new- natural biological means capable of protection against pathogens and stimulate vigorous growth in the same time. In this direction, cyanobacterial species were well-known as natural biofertilizers for many crops especially for rice and wheat cultivations, yet scanty studies reported using cyanobacteria as a bio-priming agents (Haroun & Hussein, 2003; Hegazi et al., 2010 and Osman et al., 2016).The cellular extracts and growth medium of several microalgae species have been found to contain phytohormones like gibberellins and cytokinin which are known to play an important roles in plant growth and development. Several studies have found a relation between better nutrient uptake, higher biomass accumulation, and greater crop yields to the use of microalgae biofertilizers (Selvakumar et al., 2012; El-Naggar et al., 2014 and Mohan et al., 2015). Barley is an annual grass. The juice of barley grass contains beta carotene, vitamins B1, B2, B6, B12, pantothenic acid, and folic acid. Minerals present include potassium, calcium, iron, phosphorus, and magnesium. Barley leaf extract has the ability to scavenge free radicals. *Trigonella* is a winter plant, it is an important medicinal plant and has a great therapeutic effect; it is natural antioxidant source acts as anticancer, decreases serum total lipids and serum cholesterol, enhances insulin sensitivity, and has antidiabetic effect (Prabhu & Krishnamoorthy, 2010).

The aim of this work was to study the effect of different fresh and dry *Anabaena variabilis* (Kutz) treatments as biofertilizer on the growth and some physiological aspects of *Hordeum vulgare* and *Trigonella foenum-graecum* L. plants.

Materials and Methods

Growth conditions of cyanobacterium culture and biochemical characterizations

Cyanobacterial strain, *A. variabilis* (Kutz), was obtained from Phycology Lab., Botany Department, Faculty of Science,TantaUniversity, Egypt. The cyanobacterium was grown on nitrate-free BG_{11} medium (Rippka et al., 1979) for 15 days in 1 L Erlenmeyer flasks. The culture was incubated under continuous illumination (2500 lux) and temperature of 25°C± 2°C with constant stirring and aeration.

Culture parameters as pH value, optical density (OD) and dry weight (DW) were estimated according to (Vonshak, 1986). Chlorophyll pigments were extracted in 90% acetone and measured spectrophotometrically at 663 and 645nm (Metzner et al., 1965); while carotenes were determined at 480nm according to Kirk & Allen (1965). For phycobiliproteins estimation; the harvested cells were grinded in pestle and mortar in the presence of acetic acid- sodium acetate buffer (1M) for rupturing cells. After repeated freezing and thawing, the cells were centrifuged and absorbance was read at 498.5, 614 and 651nm for phycoerythrin, allophycocyanin and c-phycocyanin, respectively (Kursar et al., 1983). Protein and carbohydrates were estimated using Lowry et al. (1951) and Dubios et al. (1956) methods, respectively. To explore the influence of cyanobacterial treatments on seed germination and seedling development of the tested plants, nitrogenase activity and growth hormones of *A. variabilis* cultures were evaluated. Nitrogenase activity was measured as described by Hardy et al. (1973) while growth hormones (Indole acetic acid (IAA), cytokinins and gibberellins) were determined according to Ünyayar et al. (1996).The exopolysaccharides content of the cyanobacterium cultures was estimated according Sudo et al. (1995) and the fertilizer nutrient value as (N, P and K) of the biomass was analyzed according to Allen et al. (1989).

Preparation of cyanobacteria extract and seed treatments

A. variabilis biomass was harvested by centrifugation after growing for 15 days (beginning of stationary phase) and the cells were washed with distilled water. Cell extracts were made by grinding the biomass with a pestle and blender. 1% cells extract was prepared by suspending 5g grinded fresh cyanobacterial cells in 500 ml distilled water (Shariatmadari et al., 2011).

Plant materials and seed treatments

Seeds of *Hordium vulgare* and *Trigonella foenum-graecum* L. were obtained from the Egyptian Ministry of Agriculture, Giza, Egypt. The seeds were surface sterilized with 0.01% $HgCl_2$ for 1 min and then washed several times with distilled water. To evaluate the effect of different application methods of *A. variabilis* on the performance of the

tested seedlings, four seed treatments were made:

Treatment 1: Seeds primed in water for 12 h (control).

Treatment 2: Seeds primed in (1%) fresh cyanobacterial extract for 12 h.

Treatment 3: Seeds directly sown in soils pre-inoculated with fresh cyanobacterial cells (3g fresh cells per 1 kg soil).

Treatment 4: Seeds treated according to the bio-priming technique (Reddy, 2013). Seeds were soaked in water for 12 h, after water discharged,seeds were mixed with dry cyanobacterial powder (3 gm dry powder per 1 kg seeds), arranged as a heap, raped in a jute cloth and kept under this high relative humidity conditions and a temperature of 25-30°C for another 12 h.

Treatment 5: Seeds directly sown in soils pre-inoculated with dry cyanobacterial cells (3g dry cells per 1 kg soil).

After each treatment, the seeds were left to grow in plastic pots (10 seeds / pot) 16 cm height and 13 cm diameter containing 600 gram soil which composed of clay and sand (2:1). Each treatment was replicated 5 times and completely random design was used.Upon germination, the number of germinated seeds was determined and the germination percentage was calculated. After 21 days plants in the vegetative stage were harvested for each treatment. Fresh plants were used for protein gel electrophoresis and fresh leaves were used for assaying of pigments, GOT and GPT enzymes. Shoot length and fresh weight were determined and then dried in an oven at 60°C to a constant weight. Dry samples were used for the determination of soluble proteins.

Estimation of photosynthetic pigments

The pigments in a known weight of fresh leaves were extracted with 85% cold acetone. The absorbance of the acetone extracts was measured at 663, 644 and 452.5 nm by using a spectrophotometer for the determination of chlorophyll a, chlorophyll b and carotenoids contents according to the method of (Metzner et al., 1965).

Estimation of total soluble proteins

The total soluble proteins content was measured quantitatively in the borate buffer extract by using the method of Bradford (1976). The protein content was calculated as mg/g dry weight using a calibration curve of Bovine Serum Albumin protein.

Estimation of transaminases activity

Glutamic-oxaloacetic and glutamic-pyruvic transaminases (GOT and GPT) activities were measured in the cell-free extracts using the method described by Bergmeyer (1974). The number of units (µM keto acid / ml sample) was calculated using the standard curve of pyruvate.

Qualitative characterization of protein using SDS-PAGE

A fresh plant sample was homogenized with 1 ml of extraction buffer (25 mM Na-acetate, pH 4.5 and 1mM phenyl methyl sulphonyl fluoride), vortexed and left for 2 h at 4°C. The extract was centrifuged at 10.000 rpm at 0°C for 15 min. and the clear supernatant was used as the total protein extract. Characterization and molecular mass determination of proteins were carried out using one dimensional SDS-polyacrylamide gel electrophoresis as described by Laemmli (1970).

Statistical analysis

All results were expressed as mean± SD of three replicates. Analysis of variance (ANOVA), a test for significant differences between means at $P \leq 0.05$ and $P \leq 0.01$, was performed to compare the impact of different treatments on growth and performance of the tested plants.All statistical analyses were performed using SPSS 17.0 software.

Results and Discussion

Cyanobacteria organisms are famous of biofertilization ability and their valuable contribution in soil fertility and plant development. Analysis of the used cyanobacterium cells showed a considerable amounts of pigments especially chlorophylls and phycobiliproteins (Table 1). A worthy content of carbohydrates and plant growth hormones were also recorded which could influenced the vigor of the produced seedlings in terms of seed germination and seedling morphological criteria compared to the control. In this study, the increment in germination percentage resulting from different cyanobacteria treatments was insignificant for *Hordeum* seedlings while significant for *Trigonella* ones (Fig. 1). Using different priming techniques improved seedling vigor, growth and yield of tomato, cucumber, chili, cabbage and watermelon crops (Maiti & Pramanik, 2013); although the cultivars showed variation in germination percentage in responses to different treatments.In this respect, different cyanobacterial isolates showed a significant increase in percentage of germination of maize plant (Mohan et al., 2015).

In contrast to the minor increase in germination

TABLE 1. Growth parameters and biochemical composition of *A. variabilis* culture (mean ± SD, n=3). *(µmole C_2H_4 /ml culture/day).

Parameter	Content	Parameter	Content
PH	7.8±0.01	Chl a (mg/g dry wt.)	9.1 ± 0.43
OD (750nm)	1.029±0.06	Carotene (mg/g dry wt.)	1.07± 0.22
Dry weight (g/l)	0.873±0.03	Allphycocyanin (mg/g dry wt.)	47.55± 0.12
Carbohydrates (mg/ml)	0.431± 0.03	Phycocyanin (mg/g dry wt.)	21.42± 0.23
Proteins (mg/ml)	0.163± 0.06	Phycoerythrin (mg/g dry wt.)	6.99 ± 0.32
Lipids (mg/ml)	0.151± 0.1	IAA (µg/g dry wt.)	180.5 ± 1.6
Exopolysaccharides (mg/ g dry wt.)	145.12	Gibberellins (µg/g dry wt.)	337.9± 3.9
Nitrogen N (% dry wt.)	9.863	Cytokinins (µg/g dry wt.)	82.76 ±1.2
Phosphorus P (% dry wt.)	2.12	Nitrogenase Activity*	102.2 ± 7.8
Potassium K (% dry wt.)	2.75		

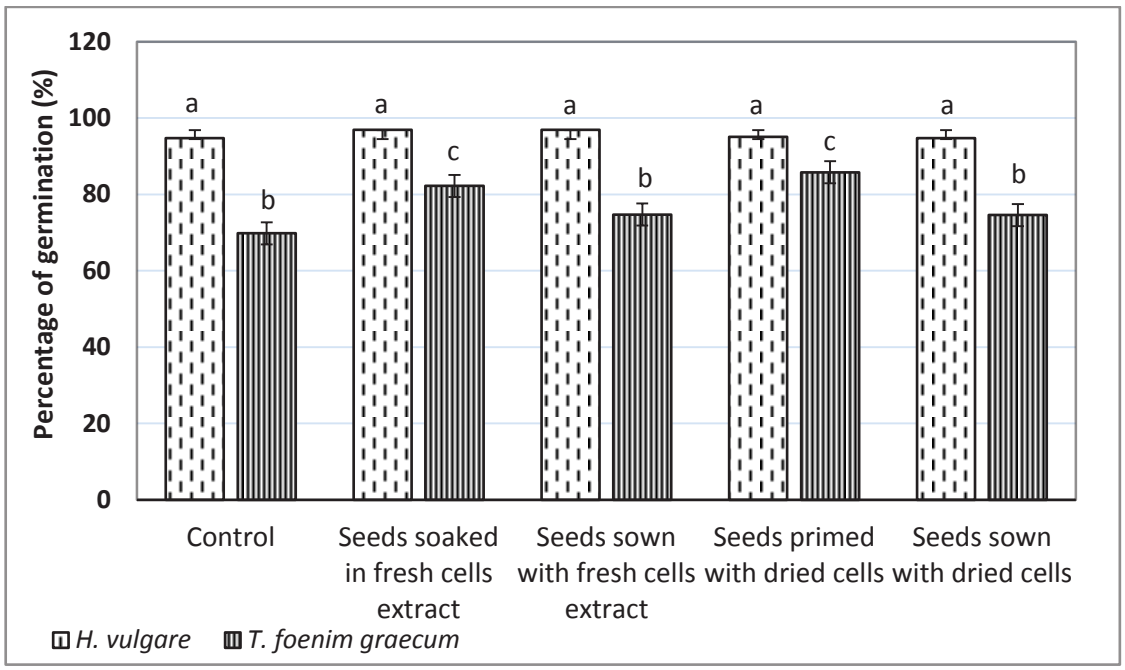

Fig. 1. Effect of *A. variabilis* on percentage of germination of 21 days old *Hordeum vulgare* and *Trigonella foenum-graecum* L. Similar superscript letters are not significant at 0.05 level of significance.

percentage, the growth and performance of *Hordeum sp*and *Trigonella sp.* seedlings in terms of shoot length, shoot fresh and dry weight was highly significant enhanced, especially for barely seedlings, using different treatments of *A. variabilis* (Table 2).

Generally, the bio-priming treatment of seeds (seeds primed with dried cells) was superior in almost all the estimated morphological criteria followed by sowing seeds with dried cells. The promoting influence of cyanobacteria treatments affected seed quality causing an increase in shoots criteria in both studied plants. The bioactive substances such as auxins, gibberellins, cytokinins, proteins and lipids found in cyanobacteria cells were recorded to promote plant growth and development and increase the crop protection ability against soil harmful

bacteria and fungi (Shariatmadari et al., 2013 and Gheda & Ahmed, 2015). In addition, the presence of valuable carbohydrates, polysaccharides, N, P and K in *A. variabilis* cells (Table 1) contributed to their plant nutritive ability and soil fertility (Osman et al., 2005; El-Naggar et al., 2014 and Ismail & El-Shenody, 2015).

TABLE 2. Effect of *A. variabilis* on shoot growth parameters of 21 days old *Hordeum vulgare* and *Trigonella foenum-graecum* L.

Treatment	Shoot length (cm)		Shoot fresh weight (mg)		Shoot dry weight (mg)	
	Hordeum	*Trigonella*	*Hordeum*	*Trigonella*	*Hordeum*	*Trigonella*
Control	14.5± 0.6	9.9± 0.9	208± 16.4	196± 11.4	16± 1.2	11.2± 1.1
Seeds soaked in fresh cells extract	26.2± 0.5**	10.9± 0.8*	420± 7.1**	200± 24.5	20± 1.6**	12± 1.2
Seeds sown with fresh cells extract	24.4± 0.5**	11.02± 0.1*	340±14.1**	248±35.6**	20± 1.6**	12± 0.7
Seeds primed with dried cells	31.1± 1.1**	15.02± 0.4**	396±21.9**	380± 20**	28± 2.2**	20± 1.6**
Seeds sown with dried cells	28.1± 1.3**	12.1± 0.8**	420± 7.1**	232± 17.9*	28± 1.6**	16± 0.7**

Mean ± SD, n=5. *Significant at 0.05 level and **Significant at 0.01 level of significance.

Results shown in Table 3 revealed fluctuated response of the photosynthetic pigments content between the two tested crop plants due to different cyanobacterial applications. A significant increase in Chl a, Chl b and total pigments was recorded; being more pronounced with barley than fenugreek seedlings. However, the bio-priming treatment with dried cells followed by sowing seeds with cyanobacteria dry cells proved to give higher pigment content over the other tested treatments and the untreated control. Oppositely, insignificant increase in carotenoids content was recorded with nearly all the examined treatments for both studied plants. The results are in agreement with those obtained by Hegazi et al. (2010) who proved that addition of cyanobacteria increased chlorophyll and carotenoid contents of bean plant. The promotive effect of the different cyanobacterial treatments may be due to high levels of GA3 in these treatments which can inhibit chlorophyllase activity (Haroun & Hussein, 2003). In this concern, *A. variabilis* cells contained elevated levels of plant growth regulating hormones as IAA, gibberellins and cytokinins (Table 1) which may induce pigments biosynthesis and increase photosynthetic activities of the emerging seedlings. Shariatmadari et al. (2013) reported that cyanobacterial chemical composition (e.g. N, P& K), nitrogenase enzyme activity and phytohormones stimulated rice plants growth. (Mazhar et al., 2013) suggested that wheat plants might release some signals leading to higher auxins production by the in-supplemented cyanobacterial cultures which stimulated wheat growth and biosynthesis of pigments.

As shown in Fig. 2, the soluble protein content of the produced seedlings was varied. Insignificant increase in soluble protein was recorded for *Hordeum* seedlings except in seeds primed with dry alga; while significant increase was resulted for *Trigonella* seedlings except in seeds sown with dry alga compared to control. This difference between the two tested plants in relation to the tested cyanobacterial treatments was probably due to their different proteolytic enzymes activities which normally degrade proteins to soluble nitrogenous compounds available for seedlings nutrition (Haroun & Hussein, 2003). Being capable of nitrogen fixation, this stimulative effect may be also due to nitrogenase activity (Table1), nitrogenase reductase and amino acids or peptides produced from cyanobacterium cells and associated with seeds during different treatments leading to growth promotion and a pronounced increase in protein content. El-Nahas & Abd El-Azeem (1999) reported that priming of *Vicia faba* seeds with *Anabaena variabilis* extract increased germination percentage, seedlings dry weight and soluble proteins compared to control seeds. Depending on species, presoaking of seeds

may change the mobilization of inorganic and organic nutrients from the storage cells to the emerging embryo and thus affecting plant growth and developments. The presented findings were also in conformity with Adam (1999), Shariatmadari et al. (2011) and Osman et al. (2016).

TABLE 3. Effect of *A. variabilis* on pigments content (mg/g fresh wt.) of 21 days old *Hordeum vulgare* and *Trigonella foenum-graecum* L.

Treatment	Chlorophyll a		Chlorophyll b		Carotenes		Total	
	Hordeum	*Trigonella*	*Hordeum*	*Trigonella*	*Hordeum*	*Trigonella*	*Hordeum*	*Trigonella*
Control	0.555 ±0.06	0.538 ±0.005	0.237 ±02	0.272 ±0.003	0.152 ±0.03	0.138 ±0.03	0.944 ±0.06	0.948 ±0.02
Seeds soaked in fresh cells extract	0.626 ±0.04	**0.426** ±0.03*	0.281 ±0.05	**0.192** ±0.04*	0.175 ±0.02	**0.112** ±0.01	1.082 ±0.1	**0.730** ±0.05
Seeds sown with fresh cells extract	0.740 ±0.04**	0.548 ±0.003	0.349 ±0.05**	**0.252** ±0.03	0.217 ±0.003**	0.147 ±0.02	1.304 ±0.04**	0.948 ±0.01
Seeds primed with dried cells	0.788 ±0.02**	0.741 ±0.01**	0.434 ±0.06**	0.421 ±0.02**	0.164 ±0.005	0.150 ±0.005	1.387 ±0.07**	1.311 ±0.006**
Seeds sown with dried cells	0.694 ±0.06**	0.643 ±0.12*	0.362 ±0.04*	0.394 ±0.07**	0.161 ±0.01	0.142 ±0.05	1.217 ±0.12**	1.179 ±0.24*

Mean ± SD, n=3. *Significant at 0.05 level and **Significant at 0.01 level of significance. (In bold, -ve insignificant values).

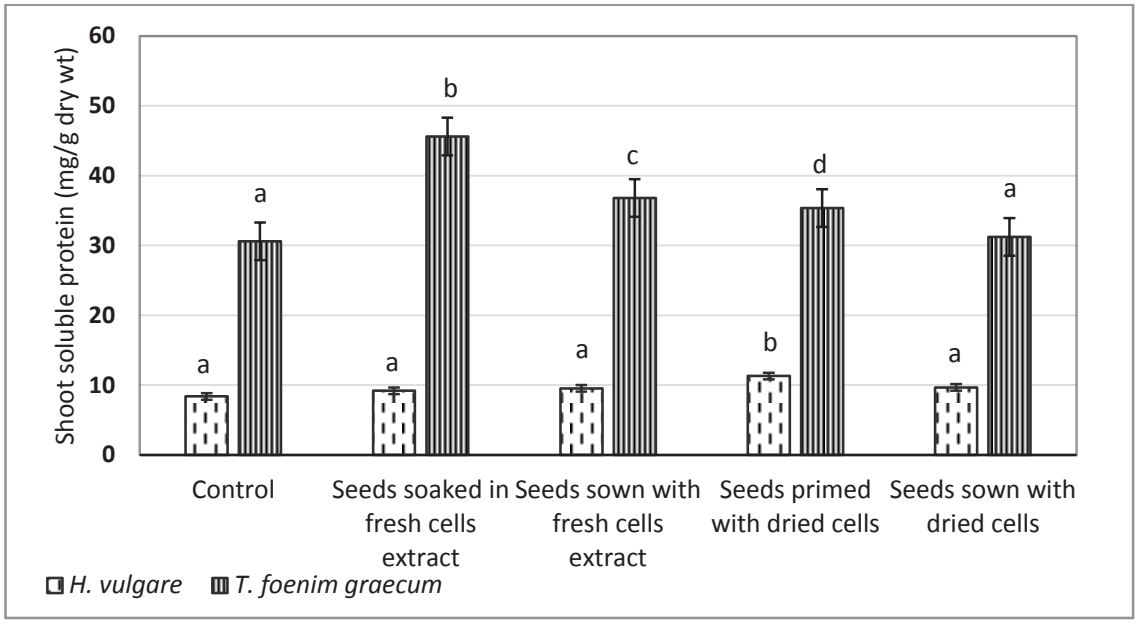

Fig. 2. Effect of *A. variabilis* on shoot soluble proteins (mg/g dry wt) of 21 days old *Hordeum vulgare* and *Trigonella foenum-graecum* L. Different superscript letters are significant at 0.01 level of significance.

The protein profile of *Hordeum* seedlings (Fig. 3) indicated the appearance of newly formed protein band with molecular weight of 220 KDa in response to soaking the seeds in fresh cells extract compared with the control. It could be considered as treatment specific proteins (King, 1991) or revealed a changed pattern of gene expression after cyanobacteria treatments (Haroun & Hussein, 2003). On the other hand, the protein profile of *Trigonella* (Fig. 3) shows the induction of low molecular weight protein bands ranging from 10-14 KDa with all treatments compared with the control. These low molecular weight proteins might represent phytohormones receptors (Davies, 1995). Moreover, such proteins may be used as an adaptive mechanism for application of a biofertilizers to give a maximum yield (Selvakumar et al., 2012). Depending on cluster analysis dendrogram, the protein patterns of *Hordeum* seedlings showed similarity above (90%) under cyanobacterial treatments 2,3,4 and 6 and above (70%) under treatment 5. On the other hand, different treatments affected the protein patterns in *Trigonella* seedlings more obviously with a homology percentage of only (40%). However, cyanobacterial treatments 9, 10 and 11 were closely similar (> 60%). Generally, seeds primed with dried cells (treatments 5 and 10) revealed a pronounced effect on the protein profile of both studied plants (Fig. 3).

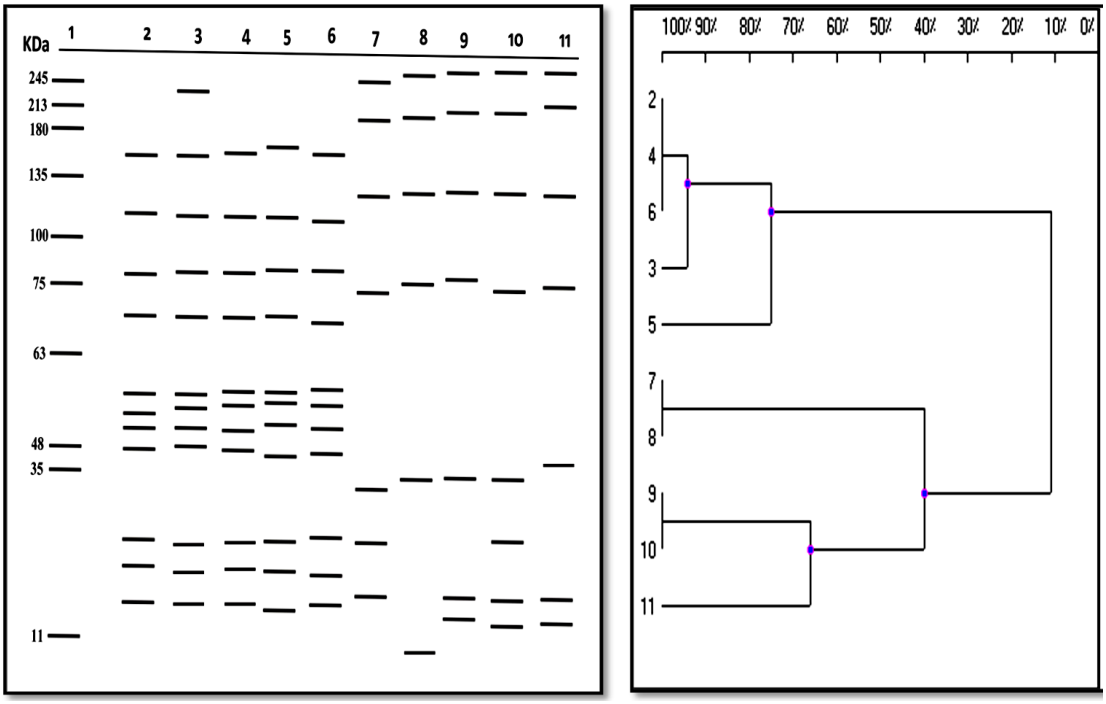

Fig. 3. Computer generated banding for SDS- PAGE protein patternof 21 days old *Hordeum vulgare* and *Trigonella foenum-graecum* L. seedlings under different *A. variabilis* treatments. Banding patterns were used to calculate a cluster analysis similarity dendrogram at 3.0% coefficient (UPGMA). (1= marker; 2&7= *Hordeum* and *Trigonella* controls; 3&8= seeds soaked in fresh cells extract; 4&9= seeds sown with fresh cells; 5&10= seeds primed with dried cells; 6&11= seeds sown with dried cells.

The effect of *A. variabilis* on glutamate-oxaloacetate transaminase (GOT) and glutamate-pyruvate transaminase (GPT) activities of *Hordeum* and *Trigonella* leaves was shown in Fig. 4.The activity of GOT and GPT enzymes was significantly increased in most cyanobacterial treatments of the two tested plants. There was a pronounced increase in GOT activity in both *Hordeum* and *Trigonella* primed with dry alga compared with the control and the other treatments. In contrast, GPT activity was superior in *Hordeum* and *Trigonella* in which the seeds was sown with cyanobacteria cells extract (Fig. 4). GOT and GPT are among the key enzymes participating in nitrogen metabolism. The fluctuations in GOT and GPT activities reflect themselves on the biosynthesis of pyruvate, glutamate and oxaloacetate families of amino acids as well as the tricarboxylic acid cycle where pyruvic, oxaloacetic and α-ketoglutaric acids are important for the cycle.

Phytohormones present in *A. variabilis* (Table 1) may play an important role in enhancement of the biosynthesis of enzyme protein and / or enzyme activation. In this respect, Haroun & Hussein (2003) found that the activities of protease and aminotransferase which are also important enzymes in nitrogen metabolism were increased in *Lupinus termis* plant as a result of seed priming in algal filtrates.Similar results were reported by Prasad et al. (2000) who noticed an increase in the enzyme activity (amylase, proteinase and starch phosphorylase) after presoaking of maize and cowpea plants seeds in a biofertilizer (whey) for 18 h.

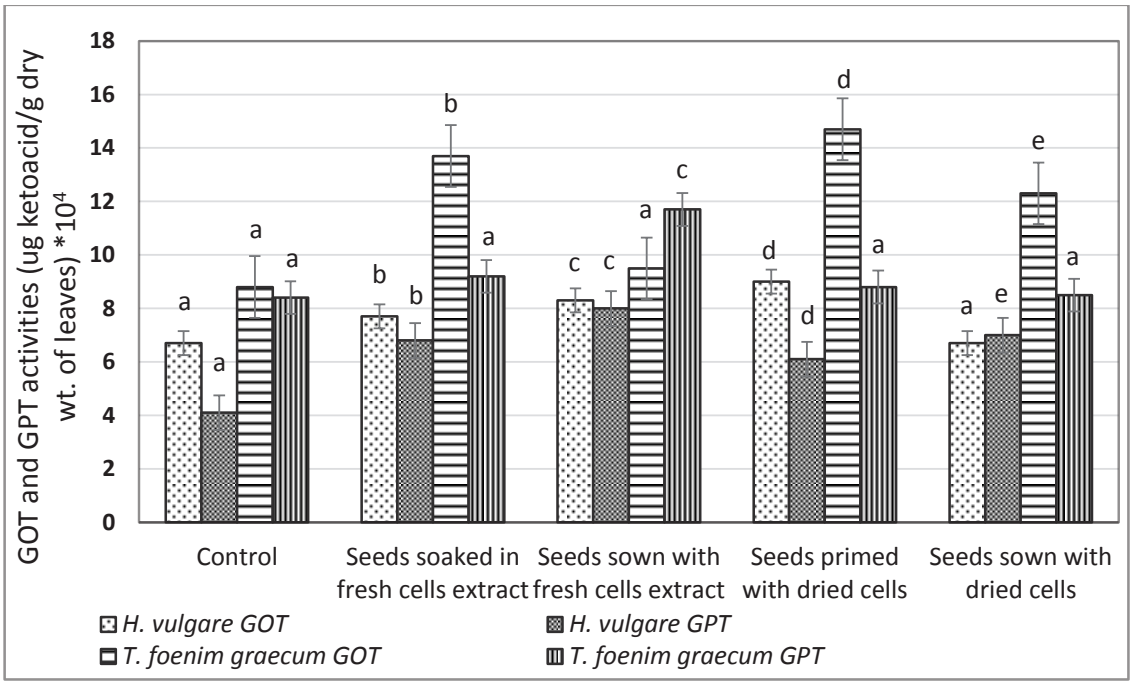

Fig.4. Effect of *A. variabilis* on glutamate-oxaloacetate transaminase (GOT) and glutamate-pyruvate transaminase (GPT) activities (µg ketoacid/g dry wt. of leaves) of 21 days old *Hordeum vulgare* and *Trigonella foenum-graecum* L. Different superscript letters are significant at 0.01 level of significance.

Conclusion

It can be concluded that fresh or dry cyanobacteria as soil additives or as seed pretreatment improved plant nutrients which, in turn, enhances the biochemical routes that lead to a more rapid and proliferated plant growth. The bio-priming treatment of seeds with dried cyanobacterial cells was superior in almost all the estimated growth parameters and physiological processes of *Hordeum* and *Trigonella* studied plants. The results of the study recommended this technique as a promising tool for using cyanobacteria and algae as biofertilizers.

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تأثير المعاملات المختلفة بالانابينا فاريابيليس على بعض مقاييس النمو و الجوانب الفسيولوجية للشعير والحلبة

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تعتبر السيانوبكتريا مجموعه من بدائيات النواة التي تعيش في بيئات متنوعة. تستطيع هذه البكتريا أن تضيف للتربة والنباتات النامية فيها العديد من المواد ذات الوظائف البيولوجية المختلفة. في هذا البحث تم دراسة تأثير أربع معاملات من سيانوبكتيريا **أنابينا فاريابيليس** (المثبت للنيتروجين) على نباتي الشعير والحلبة كالتالي: البذور بعد غمرها في 1% من الخلايا الحية للسيانوبكتريا، زراعة البذور في تربة تحتوى على 3 جم من خلايا السيانوبكتريا الحية أو المجففة لكل كيلوجرام من التربة والبذور الرطبة المغطاة ب 3 جم من السيانوبكتريا الجافة لكل كيلوجرام من البذور. أدت كل المعاملات إلى زيادة في نسبة الإنبات, وطول الساق, والوزن الطازج, والجاف له. أحدثت المعاملات أيضا زيادة في صبغات البناء الضوئي والبروتينات ونشاط إنزيمات النقل الأميني (GOT،GPT) خاصة في البذور المغطاة بالخلايا الجافة للسيانوبكتريا قبل الزراعة. أوضحت نتائج تحليل البروتين أن معاملة البذور بمستخلص الخلايا الطازجة استحثت ظهور بروتين جديد ذو وزن جزيئي 220 KDa لبادرات الشعير. كذلك أدت كل المعاملات إلى ظهور بروتينات جديدة ذات أوزان جزيئية صغيرةبين 14-10 KDaفي نبات الحلبة. بينت الدراسة أن الزيادة في نمو النباتات يرجع إلى احتواء السيانوبكتريا على العديد من الهرمونات و السكريات والعناصر المعدنية حيث توصى النتائج بمعاملة بذور الشعير والحلبة بالسيانوبكتريا المجففة قبل الزراعة كمخصب طبيعي وآمن للحصول على أفضل نمو.