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Mohamed El-Anwar H. Osman; Atef M. Abo-Shady and Maysa M. F. El-Nagar
Department of Botany Faculty of Science, Tanta University, Tanta, Egypt

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Original Article

Treatment of broad bean seeds with algal suspensions to study their effects on certain growth and yield parameters

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Department of Botany Faculty o f Science, Tanta University, Tanta, Egypt

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Abstract

In the present study ***chlorella vulgaris and Nostoc muscorum*** were used as priming solutions for broad bean seeds before cultivation. Presoaking caused a significant ($p \leq 0.05$) increase in growth and yield parameters in addition to some metabolites. Treatment with ***Nostoc muscorum*** showed highly significant increase in growth parameters, root length (by 30%), shoot length (by 44%), 2fold increase in root fresh weight and 1.5fold fold increase in shoot fresh weight, 67% increase in root dry weight and about 1.6 fold increase in shoot dry weight, also significant increase in photosynthetic pigments, carbohydrates and protein by 52%, 20% and 1.7 fold, respectively, were also recorded.Yield parameters also increased due to algal treatment, the increase was 7% for weight of 100 seeds, 5% for carbohydrates and 32% for protein.Furthermore, algal treatments caused changes in the activity of different antioxidant enzymes (peroxidase and catalase) accompanied with a reduction in lipid peroxidation in the plant

1. Introduction

Broad bean (Viciafaba L.) is a crop grown primarily for its edible seeds (beans) and is a major legume seed consumed by humans worldwide. The dried seeds are cooked, canned or frozen (Mohiuddin Das and Ghosh, 2000). Micro-algae have multi-functional properties in agriculture, nutrient uptake, improving crops, physiological and tolerance to a biotic stress (Rongaet al., 2019; Renuka et al., 2018).

Algae are considered a rich source for various products that positively affect both growth and yield of most of plant crops (De Morais et al., 2015), Because of their high content of protein, algae have been extensively used as nitrogen rich biofertilizer and also as nutritious animal feed (Gaese, 2012). It has been reported that the gelatinous sheath of some algal species was able to chelate Fe, Cu, Mo, Zn, Co, Mn, and other elements essential for their growth (Lange, 1976). The sheath was also thought to influence the availability of elements to other organisms (Belnap and Harper, 1995).

Exopolysaccharides secreted by many microalgae species provide organic carbon for the growth and development of beneficial microbes, leading to the formation of useful biofilms in the rhizo sphere (Xiao and Zheng, 2016).

In general, soil fertility could be diminished due to soil erosions, loss of nutrients, accumulation of salts and other toxic elements. Organic and bio-fertilizers are the alternative sources to meet the nutrient requirements of different crops. Nowadays, algal have been emerged as a valuable component for integrating nutrient supply system in agriculture.

Several studies have already showed a host of beneficial effects of algal application on plants, such as enhanced seed germination, improved crop and yield, resistance to biotic and abiotic stresses (Mohsen et al., 2016; Kawalekar, 2013; Sengar et al., 2010).

Therefore, this work was designed to study the effect of some algal suspensions as priming solution for seeds before cultivation on growth of some metabolic activities and yield parameters of broad bean plant

2. Materials and Methods

Algal biomass and growth medium

Nostoc muscorum was grown on BG11 medium (Rippka et al., 1979) and Chlorella vulgaris was grown on Khul medium (Kühl, 1962) for 12 days. The culture was incubated under continuous fluorescent light of intensity 55 µ mole photon m-2 s-1and temperature of 25°C± 2°C with constant aeration. The algae were isolated and purified according to Stein, 1973. All cultures were harvested at the beginning of stationary phase; cells were rinsed three times and washed with sterilized distilled water to remove traces of the growth medium (Rogers and Burns, 1994). Experimental design

Seeds of broad bean were selected then sterilized in 1% sodium hypochlorite solution for 15 minutes. The seeds were washed thoroughly with distilled water, and then soaked overnight (12 hours) the following solutions:

a) Distilled water (control).

b) Suspensions of algal samples as soaking solutions each at 1% fresh weight biomass of Chlorella vulgaris and **Nostoc muscorum.Afterwards**, the seeds were sown in pots (35 cm diameter and 30 cm depth) containing equal amounts of loamy soil .Seeds were sown at 2cm depth as 10 seeds per pot. The pots were kept in the green house under normal conditions of light, temperature and irrigated with tap water. Chemical fertilizers applied were ammonium nitrate and super phosphate (as the recommended dose of the Ministry of Agriculture for each plant) added as referred to the surface area of one feddan. Plant samples were taken after 15 days old (germination stage) and yield (fruiting stage).

1-Growth parameters

Root and shoot length, fresh and dry weights were recorded at the seedling stag.

2-Some metabolic activities

Total pigments were estimated quantitatively Metzner et al. (1965). Carbohydrates in the plant root and shoot were estimated quantitatively Nelson (1944) and Naguib (1963). Borate buffer extract was used to estimate the direct reducing (DRV) and total reducing values (TRV) of carbohydrates. Total protein was estimated Bradford (1976).The activity of some antioxidant enzymes (peroxidase, catalase) were measured Kato and Shimizu (1987). Lipid peroxidation was estimated the concentration of malondialdehyde (Uchiyama and Mihara, 1978).

3-Yield parameters

Some productivity criteria including the number of pods/plant, number of seeds/pod, number of seeds/plant, weight of 100 seeds (g) were estimated at fruiting stage.

Statistical Analysis

Statistical analysis was performed with one-way ANOVA, using SAS program version 6.12 at $p \leq 0.05$ level of significance (Snedecor, 1970).

3. Results

Data present in Table **1**show the effect of the different algal treatments (***Chlorella vulgaris*** and ***Nostoc muscorum***) on some growth parameters. All algal treatments induced an increase in root and shoot length, fresh weight and dry weight of root and shoot. The stimulatory effect induced by algal presoaking on the roots and shoots length of broad bean seedlings show that ***Nostoc muscorum*** maintained higher effect compared to ***Chlorella vulgaris***.

Results show that treatment with ***C. vulgaris*** increased the fresh weight by 33.3% for root and 82.7% for shoot, dry weight by 50% for root and 90% for shoot and water content by 29.2% for root and 81.7% for shoot in compared to control, on the other hand treatment with ***Nostoc muscorum*** showed the highest increase in fresh weight by 2 fold for root and 1.5 fold for shoot, dry weight by 66.7% for root and 1.6 fold for shoot and water content by 1.9 fold for root and 1.5 fold for shoot in compared to control (Table **1**).

Table 1: Effect of seeds priming in algal suspensions on various growth parameters of 15-day old broad bean seedlings

Treatment	Root length **(cm)**	Shoot length (cm)	Fresh weight (g)		Dry weight (g)	
			Root	Shoot	Root	Shoot
(Control H_2O))	0.3 ^{d}±9.17	0.2d± 14.50	0.005d ±0.03	0.05 d±0. 81	0.0001d±0. 006	0.01d±0. 10
Chlorella vulgaris	0.1^{c}±10. 63	0.1c± 17. 27	0.008c ±0.04	0.01 c±1. 48	0.0001c±0. 009	0.01c±0. 19
Nostoc muscorum	12.50±0^{b}	0.2 b±20. 83	0.002a ±0.09	0.01 a±2. 08	0.001a±0.0 1	0.02a±0. 26
F(value)	238.44***	93.41***	255.51 ***	78839.51 ***	929.22***	99999.99***

Each value represents the mean of three readings ± the standard error.

Mean values with the same letters are not significantly different.

Results presented in Table **2** show increase in the contents of chlorophyll a, b and carotenoids in leaves of broad bean seedling produced from algal-treated seeds over control. The highest stimulatory effect was observed in plants presoaked in ***Nostoc muscorum.***

Table 2: Effect of seed priming in algal suspensions on the photosynthetic pigments of broad bean leaves of 15-day old seedlings

Treatment	Chl. a (mg/g D.wt)	Chl. b (mg/g D.wt)	Carotenoids (mg/g D.wt)
Control (H_2o)	0.0 ^{d}±1.00	0.0 ^{d}±0.87	0.05 ^{d}±0.27
Chlorella vulgaris	0.05 ^{c}±1.31	0.08 ^{c}±1.21	0.05 ^{c}±0.40
Nostoc muscorum	0.05 ^{b}±1.37	0.05 ^{b}±1.28	0.005 ^{b}±0.61
F(value)	99999.99***	99999.99***	77469.59***

Each value represents the mean of three readings ± the standard error.

Mean values with the same letters are not significantly different.

Data of carbohydrates and protein content in broad bean seedlings (Table **3**) revealed that different algal treatments had increase in these contents compared with control. Priming of seeds in ***Chlorella vulgaris*** increased both **DRV** and **TRV** of seedling by 5.8% and 9.7% in root, and 8.9% and 2.2% in shoot compared to control, respectively. On the other hand, treatment of seeds by ***Nostoc muscorum*** increased these values in root by 15.3% and 14.7% and by 12.7% and 4.6% for shoot over control, respectively. Protein content also increased in case of

Nostoc muscorum treatment (1.7 fold) and (78%) in case of ***Chlorella vulgaris***.

Table 3: Effect of seed priming in algal suspensions on carbohydrates and protein content of broad bean leaves of 15-day old seedlings

Treatment	DRV (mg/g D.wt)		TRV (mg/g D.wt)		Protein (mg/g D.wt)
	Root	Shoot	Root	Shoot	
(Control H_2o)	±0.005^{d} 21.43	±0.05^{d} 32.73	0.0001^{d} 26.77±	0.01^{d} ± 40.53	0.1 13.75±^{a}
Chlorella vulgaris	±0.008^{c} 22.67	±0.01^{c} 35.63	0.0001^{c} 29.37±	0.01^{c} ± 41.43	0.01 24.49±^{b}
Nostoc muscorum	24.7±0.002^{a}	36.9±0.01^{a}	±0.001^{a} 30.7	0.02^{a} 42.4±	0.1 37.5±^{c}
F(value)	260.24***	139.38***	400.91***	196.56***	20778.27 ***

Each value represents the mean of three readings ± the standard error.
Mean values with the same letters are not significantly different.

Results presented in Table **4** show that the activities of peroxidase, catalase (µM /g F.wt) and the values of lipid peroxidation measured as Malondialdehyde (µmols MDA g^{-1} F. wt) in leaves of broad bean seedlings were also reduced in response to both algal treatments. The reduction in lipid peroxidation was more intense in case of ***Nostoc muscorum*** than ***Chlorella vulgaris*** treatment.

Table 4: Effect of seed priming in algal suspensions on antioxidant enzymes and lipid peroxidation content of Broad bean leaves of 15-day old seedlings

Treatment	Peroxidase (µmols MDA g^{-1} F. wt)	Catalase (µmols MDA g^{-1} F. wt)	Lipid peroxidation (mg/g D.wt)
Control (H_2o)	0.05 ^{a}±7.71	0.002 ^{a}±0.15	0.003 ^{a}±0.6
Chlorella vulgaris	0.06 ^{b}±2.1	0.005 ^{b}±0.12	0.005 ^{b}±0.5
Nostoc muscorum	0.05 ^{c}±1.84	0.005 ^{c}±0.08	0.005 ^{c}±0.5
F(value)	26343.98***	731.10***	733.70***

Each value represents the mean of three readings ± the standard error.
Mean values with the same letters are not significantly different.

Table **5** shows that the highest increase in yield parameters was recorded in case of ***Nostoc muscorum*** treated seeds, which reaching 6%, 11%, 6% and 7% for the number of pods/plant, number of seeds/pod, number of seeds/plant, weight of 100 seeds (g), respectively. On the other hand, ***C. vulgaris*** treatment showed lower values.

Table 5: Effect of seed priming in algal suspensions on various yield parameters of broad bean plant

Treatment	No of pods / plant	No of seeds / pods	No of seeds / plant	Weight of 100 seeds (g)
Control (H_2o)	0.3 ^{c} 6.00±	0.05 3.0±^{b} 0	0.1 ^{b} 18.00±	0 53.7±^{g}
Chlorella vulgaris	0 ^{c} 6.0±	0.05 3.0±^{b} 0	0.5^{b} 18.00±	0^{f} 57.0±
Nostoc muscorum	0 ^{c} 6.33±	0.08 3.3±^{b} 3	0.005 19.00±^{b}	0 57.3±^{e}
F(value)	4.25**	1.57*	3.66**	353.30 ***

Each value represents the mean of three readings ± the standard error.
Mean values with the same letters are not significantly different.

The results showed that both treatments **(*Nostoc muscorum*** and ***Chlorella vulgaris*)** caused increases in DRV, sucrose, starch levels and protein content. However, the values recorded in case ***Nostoc muscorum*** treatment were higher than those recorded in case of ***Chlorella vulgaris*** treatment (Table **6**).

Table 6: Effect of seed priming in algal suspensions on carbohydrates and protein content of harvested seeds

Treatment	DRV (mg/g D.wt)	Starch (mg/g D.wt)	Sucrose (mg/g D.wt)	Total protein (mg/g D.wt)
Control (H_2o)	39.02±0.8 ^{g}	100.02±1 ^{g}	45.2±0.5^{g}	83.1±1^{h}
Chlorella vulgaris	40.07±0.8^{f}	102.33±1^{f}	45.73±0.1^{f}	103.1±1.1 ^{f}
Nostoc muscorum	41.33±0.8^{e}	104.770.7 ±^{e}	46.47±1.1^{e}	110.1±0.8 ^{e}
F(value)	97.25***	1239.35***	1860.33***	16291.99 ***

Each value represents the mean of three readings ± the standard error.

Mean values with the same letters are not significantly different.

4. Discussion

In the present work soaking of broad bean seeds in different algal suspensions (Chlorella vulgaris and Nostoc muscorum) caused a marked increase in the measured growth parameters of the produced root and shoot (length, fresh and dry weight) in compared to control (Table 1).

Other Experimental studies tested the action of microalgae under open-field and greenhouse conditions, have demonstrated that they stimulate germination, seedling growth, shoot, and root biomass in several crops such as lettuce, red amaranth, tomato and pepper (Garcia-Gonzalez and Sommerfeld, 2016; Barone et al., 2018; El Arroussiet al., 2018).

Furthermore, Likhitkar and Tarar (1995) found that soaking of cotton seeds in extract of Nostoc muscorum increased germination, total length of seedlings and length of radical after 10 days.

Results of the current study showed that all pigment contents (chl. a, b and carotenoids) of leaves of broad bean were significantly increased in response to treatments with both algal suspensions (Table2) in compared to control, which agreed with the studies of Abd–Allah et al.(1994); Abou-Khadrah et al. (2000) who reported that the cyanobacterial extract enhanced chlorophyll bio-synthesis. Such results could be attributed to some bioactive substances which have been produced by some algae and cyanobacteria, as gibberellin, auxin [including indole-3-acetic acid, indole-3-propionic acid, indole-3-butyric acid and 1-naphthalene acetic acid], cytokinins (Hashtroudi et al., 2011),vitamins, amino acids and exopolysaccharides (Osman et al., 2005), thus simulating growth and chlorophyll biosynthesis. The obtained data concerning carbohydrates content (Table 3) in broad bean plants showed increase values in response to different algal treatments in compared with control in both stages, indicating a stimulation of the photosynthetic process in response to algal pretreatment of seeds.

These results are in accordance with Haroun and Hussein (2003), who showed that the increase in pigments production in Lupinus leaves pretreated with the algal filtrate of Cylindrospermum led to an increase in the photosynthetic activity and carbohydrates content of plant tissue.

Results (Table 3) also showed that protein of broad bean plants has increased markedly in response to treatment with both algal suspensions in comparison with control; More or less similar results were reported by Gururag and Mallikarjunaiah (1995).

It is worth to attribute the increase in protein content to the increase of nitrogen uptake and consequently amino acid level contents which play a prominent role in the building of protein structure (Haroun and Hussein, 2003). However, the increase in total protein level content may be attributed to increased respiration, resulting in increasedα-keto acids (Krebs cycle) which are the main precursor for amino acid biosynthesis. On the other hand, Cytokinin present in the algae could stimulate also protein biosynthesis in the treated plants (Norrie and Hiltz, 1999; El-Sheekh and El-Saied, 2000). Ghallab and Salem (2001) indicated that blue green treatment of wheat plant increased growth criteria, nutrients, sugars, amino acids and growth regulators as well as crude protein content.

In addition, pre-soaking of broad bean seeds in different algal suspensions caused different changes in antioxidant enzymes and lipid peroxidation in seedlings (Table 4). Broad bean peroxidase activity, catalase and Lipid peroxidation have reduced in broad bean seedlings in response to seeds presoaking in both algal suspensions.

Carocho and Ferreira (2013) stated that antioxidant enzymes can act as scavenging chain initiating radicals like hydroxyl, alkoxyl, or peroxyl, quenching singlet oxygen, decomposing hydro peroxides, and chelating prooxidative metal ions.

It can be concluded that pre-soaking of seeds in both algal suspensions has brought about changes in antioxidant enzymes and lipid peroxidation causing improvement and accelerating the different metabolic process of plants through some activates (Chen et al., 2010; Barron, 2010; Wu et al., 2010).

Yield parameters of broad bean plant, carbohydrates and protein content of the harvested seeds have significantly increased (Table 5 and Table 6) in response to both algal treatments.

This increase could be related to the significant increase in photosynthetic activity of plant in response to algal treatment.

Conclusion

Priming of broad bean seeds before cultivation in different algal suspensions (Chlorella vulgaris and Nostoc muscorum) stimulated growth parameters and some metabolite biosynthesis in the seedling causing a marked increase in the yield of broad bean plant. We may recommend the use of this method (seeds priming in some tested algal suspensions) as alternative method than using chemicals for the improvement of crop yield and quality.

5. References:

Abd–Allah, M.H.; Mahmoud A.L.E. and A.A. Issa. (1994): Cyanobacterial biofertilizers improved growth of wheat. Phyton (Horn), 34 (1): 11–18.

Abou-Khadrah, S.H.; Mohamed, A.A.; Tabl M.A. and Demian. K.R. (2000): Effect of nitrogen fertilization on growth and yield of some sunflower cultivars grown in calcareous soil. Proc. 9th Conf. Agron Minufiya Univ, 1 (2): 483–493.

Barone, V.; Puglisi I.; Fragalà F.; Lo Piero A. R.; Giuffrida F. and Baglieri A. (2018): Novel bioprocess for the cultivation of microalgae in hydroponic growing system of tomato plants. Journal of Applied Phycology, 9:1-6.

Barron, J. (2010): Organic vs. Conventional. Natural Health Newsletter, 9 (17):1-11.

Belnap J. and Harper K.T. (1995): Influence of crypto biotic soil crusts on element content of two desert seed plants. Arid Soil Research Rehabilitation, 9: 197–115.

Bradford, M.M. (1976): A rapid and sensitive method for quantification of microgram quantities of protein utilization the principle of protein-dye binding. Analytical Biochemistry, 72: 248–254.

Carocho, M. and Ferreira, I. C. F. R. (2013): A review on antioxidants, prooxidants and related controversy: natural and synthetic compounds, screening and analysismethodologies and future perspectives. Food and Chemical Toxicology, 51: 15–25.

Chen, Q.; Zhang, M. and Shen, S. (2010): Effect of salt on malondialdehyde and antioxidant enzymes in seedling roots of Jerusalem artichoke (Helianthus tuberosus L.). Acta Physiologiae. Plantarum, 33: 273–278.

De Morais, M. G.; da Silva Vaz B.; De Morais, E. G. and Costa, J. A. V. (2015): Biologically active metabolites synthesized by microalgae. BioMed Research International, 83:57-61.

El Arroussi, H.; Benhima, R.; Elbaouchi, A.; Sijilmassi, B.; El Mernissi, N.; Aafsar, A.; Meftah-Kadmiri,I.; Bendaou, N. and Smouni,A.(2018):Dunaliellasalina exopolysaccharides: a promising biostimulant for salt stress tolerance in tomato (Solanum lycopersicum). Journal of Applied Phycology, 30: 2929–2941.

El-Sheekh, M.M. and El-Saied, A.E. (2000): Effect of crude seaweed extracts on seed germination, seedlings growth and some metabolic processes of Viciafaba L.Cytobios, 101:23–35.

Gaese, H. (2012): Chemical Composition and Potential Application of Spirulina platensis biomass. InternationalAgriculture Environment, 2307-2652.

Garcia-Gonzalez, J. and Sommerfeld. M. (2016): Biofertilizer and biostimulant properties of the microalgae Acutodesmusdimorphus. Journal of Applied Phycology,28: 1051–1061.

Ghallab, A.M. and Salem S.A. (2001): Effect of biofertilizer treatments on growth, chemical composition and productivity of wheat plants grown under different levels of NPK fertilization. Annals of Agriculture. Research(Cairo), 46: 485–509.

Gururag, J.R. and Mallikarjunaiah, R.R. (1995):Interaction among Azotobacter, Chlorococcum, Penicillium glaucum and Glomus and their effect on the growth and yield of sunflower (Helianthus fasciculatum). Helia, 18 (23): 73–84.

Haroun, S.A. and Hussein, M.H. (2000): The promotive effect of algal biofertilizers on growth, protein pattern and some metabolic activities of Lupinustermis plants grown in siliceous soil. Asian Journal of PlantScience, 2 (13): 944–951.

Hashtroudi, M.S.; Shariatmadari, Z.; Riahi H. and Ghassempour, A. (2011): Simultaneous determination of four auxins in cyanobacterial extracts using HPLC-ESI-MS. Planta Medicine, 77: 198.

Kato, M. and Shimizu, S. (1987): Chlorophyll metabolism in higher plants. VII. Chlorophyll degradation in senescing tobacco leaves: phenolic-dependent peroxidative degradation. Canadian Journal of Botany, 65: 729-735.

Kawalekar, S.J. (2013): Role or biofertilizers and biopesticides for sustainable agriculture. Journal of Bioinnovation, 2: 73-78.

Kühl, A. (1962): Zurphysiologie der speicherungkondersierteranorganischer phosphate in Chlorella. Vortr. Botan.HrsgDeutBotanGes (NF), 1:157–166.

Lange, W. (1976): Speculations on a possible essential function of the gelatinous sheath of blue-green algae. Canadian Journal of Microbiology, 22: 1181–1185.

Likhitkar, V.S. and Tarar, J.L. (1995): Effect of presoaking seed treatment with Nostocmuscorum extracts on cotton. Annals. PlantPhysiology, 9 (2): 113–116.

Metzner, H.; Rau H. and Senger, H. (1965): Untersuchunger, zursynchronisierbarkeieinzelnerpigmentmangelmutanten von Chlorella. Planta, 65: 186–194.

Mohiuddin, M.; Das A.K. and Ghosh, D.C. (2000): Growth and productivity of wheat as influenced by integrated use of chemical fertilizer, biofertilizer and growth regulator. Indian Journal of Plant Physiology, 5: 334–338.

Mohsen, A. A. M.; Salama, A. S. A. and El-Saadony, F. M. A. (2016): The Effect of Foliar Spray with Cyanobacterial Extracts on Growth, Yield and Quality of Lettuce Plants (Lactuca sativa L.). Middle EastJournal of Agriculture Research, 5: 60-96.

Naguib, M.I. (1963): Colorimetric estimation of plant polysaccharides. Zucker,16: 15.

Nelson, N. (1944): A photometric adaptation of somagi method for the determination of glucose. Journal of BiologicalChemistry, 153: 275.

Norrie, J. and Hiltz, D.A. (1999):Seaweed extract research and applications in agriculture. Agron Food Industrial, 10 (2): 15-18.

Osman, M.E.; El-Naggar A.H.; Omar, H.H. and Esmail, G.A. (2005): Effect of cyanobacterial biomass and exopolysaccharides inoculations on soil enzyme activities. El-Minia Science Bulletin, 16 (1): 73–86.

Renuka, N.; Guldhe, A.; Prasanna, R.; Singh, P. and Bux, F. (2018): Microalgae as multi-functional options in modern agriculture: current trends, prospects and challenges. Biotechnology Advances, 36: 1255–1273.

Rippka, R. J.; Demelles, J. B.; Waterbury, M. and Stanier, R. Y. (1979): Generic assignments, strain histories and properties of pure cultures of cyanobacteria. Journal of General Microbiology, 111: 1-61.

Rogers, S.L. and Burns, R.G. (1994):Changes in aggregate stability, nutrient status, indigenous microbial populations and seedlings emergence following inoculation of soil. Biology and Fertility of soils, 18: 209–215.

Ronga , D.; Biazzi, E.; Parati, K.; Carminati, D.; Carminati E. and Tava, A. (2019):Microalgal biostimulants and biofertilisers in crop productions. Agronomy, 1-22.

Sengar, R.M.S.; Bhadauria S. and Sharma, P. (2010): The Effect Of Cyanobacterial Toxin On Seed Germination. Indian Journal of Scienceresearch, 1(2) : 41-45.

Snedecor, J.W. (1970): Statistical Methods, Amis, Lowa, USA. Lowa State University Press.

Stein, J.R. (1973): Handbook of Phycological Methods: Culture Methods and Growth Measurements. Cambridge University Press, Cambridge.

Uchiyama, M. and Mihara, M. (1978): Determination of malondyaldehyde precursor in tissues by thiobarbituric acid test. Analytical Biochemistry, 86: 271-278.

Wu, J.; Yang, Q. W. G.; Fan, Miao, Y.; Liang, C.; Xu, W. and Shen, Q. (2010): Suppression of Fusarium wilt of watermelon by a bio-organic fertilizer containing combinations of antagonistic microorganisms.BioCont, 54: 287-300.

Xiao, R. and Zheng, Y. (2016): Overview of microalgal extracellular polymeric substances (EPS) and their applications. Biotechnology Advanced, 34: 1225–1244.