Int J Pharm Bio Sci 2013 Oct; 4(4): (B) 446 - 455

Research Article **Biotechnology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

EFFECT OF DIFFERENT CYANOBACTERIAL SPECIES ON GROWTH, PHOTOSYNTHETIC ACTIVITY AND ANTIOXIDANT SYSTEM OF FLAX PLANT

NARESH .L[1*], ALEX B. K.[1] AND KOSHY E.P.[2]

*[*1] Department of Molecular and Cellular Engineering, Jacob School of Biotechnology & Bioengineering, Sam Higginbottom Institute of Agriculture, Technology & Sciences Deemed-to-be University*
[2] Department of Tissue Engineering, Jacob School of Biotechnology & Bioengineering, Sam Higginbottom Institute of Agriculture, Technology & Sciences Deemed-to-be University.

ABSTRACT

Cyanobacteria produce bioactive compounds including plant growth regulators that influence the physiological and biochemical profile of inoculated plants. The objective of the present investigation was to study the effect of different cyanobacterial species viz. *Nostoc commune, Anabaena flos-acquae* and *Westiellopsis* sp. on growth parameters, photosynthetic activity and antioxidant system of flax (*Linum usitatissimum* L.), a recently promoted medicinal plant. Seeds presoaked in the culture extract of these cyanobacterial species enhanced the germination percentage, vegetative growth, chlorophyll content, as well as stimulated the antioxidant system (carotenoid content, peroxidase enzyme and catalase enzyme activity) of the plant and these effects were statistically proved significant. In all the treatments, the germination of flax seeds was faster compared to the control regardless of species employed. 10% extracts of all the selected species was found to be the best for augmenting growth characteristics and antioxidant system.

KEY WORDS: **Flax, Cyanobacteria, Antioxidant system, Photosynthetic activity**

NARESH .L
Department of Molecular and Cellular Engineering, Jacob School of Biotechnology & Bioengineering, Sam Higginbottom Institute of Agriculture, Technology & Sciences Deemed-to-be University

Corresponding author

INTRODUCTION

The well-known correlation between diet and health demonstrates the great possibilities of food to maintain or even improve our health. One of the main areas of research in Food Science and Technology is the extraction and characterization of new natural ingredients with biological activity (e.g., antioxidant, antiviral, antihypertensive activity, etc.) that can contribute to consumer's well-being as part of new functional foods [1]. Although oxidation reactions are crucial for life, they can also be damaging; hence, plants and animals maintain complex systems to protect cellular and subcellular systems from the cytotoxic effects of active oxygen radicals with antioxidative enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and glutathione reductase (GR) as well as metabolites like glutathione, ascorbic acid, α-tocopherol and carotenoids. Cyanobacteria potentially a great source of natural compounds, could be used as ingredients for preparing functional foods. Different compounds with antibacterial, antiviral and antifungal activity can be found in these types of organisms, along with compounds having antioxidant activity[2]. The application of diazotrophic cyanobacteria as biofertilizers in the cultivation of wet-land rice has a beneficial effect on the growth and yield [3, 4]. Algal filtrate of the cyanobacterium *Nostoc muscorum* significantly increased germination of wheat seeds as well as their growth parameters and nitrogen compounds, compared to control [5]. The application of an extract from algae to soil or foliage increased ash, protein and carbohydrate contents of potatoes (*Solanum tuberosum*) [6]. Blue-green algal extract excretes a great number of substances that influence plant growth and development [7]. These microorganisms have been reported to benefit plants by producing growth promoting regulators, vitamins, amino acids, polypeptides, antibacterial and antifungal substances that exert phytopathogen biocontrol and polymers, especially exopolysaccharides, that improve plant growth and productivity [8]. Beneficial effects of cyanobacteria inoculation have also been reported on a number of other crops such as barley, oats, tomato, radish, cotton, sugarcane, maize, chilli and lettuce [9]. Flax or linseed, (*Linum usitatissimum* L.) is a plant that is grown both for its seeds and for its fibers. It belongs to the family Linaceae. It is an erect annual plant growing to 1.2 m tall with slender stems. Flax is nature's richest source of omega-3 fatty acid. The flowers are attractive blue or bright red and the fruits are dry capsules containing several brown, oval-shaped flaxseeds. Their constituents include 30-40% of fatty acids (linseed oil) with esters of linoleic acid (60%), linolenic acid (20%), stearic acid (8%) and oleic acid along with mucilage, proteins and cyanogenic glycosides. The oil also is important in the manufacture of paints, soap and printer's ink [10]. The flaxseed lignan and its mammalian metabolites have been reported to exert protective effects against diet-related chronic diseases through a variety of mechanisms including phytoestrogenic and antioxidant effects from *in vitro* and *in vivo* studies [11, 12, 13].Cyanobacteria produce bioactive compounds including plant growth regulators and enzymes that regulate growth and detoxify oxidative agents and free radicals. The responses and mechanisms employed by cyanobacteria may be implemented to design strategies for understanding their metabolic and genetic plasticity for further development of technologies relevant to enhancing the stress tolerance capacity of living organisms[14].The present study is an attempt to determine antioxidant effects produced by extracts of different cyanobacterial species (*Nostoc commune, Anabaena flos-acquae* and *Westiellopsis* sp.) on growth and antioxidant system of flax plant (*Linum usitatissimum* L).

MATERIALS AND METHODS

Germination studies and measurement of physiological parameters such as radicle and plumule length were done with the plantlets grown in petridishes layered with cotton base

and 25 seeds were put on each dish to serve the purpose. The petridishes were kept in a plant growth chamber and 5ml of different preparations (1%, 5% and 10%) of each algal extract was given at regular intervals. Plates containing seeds with 5ml distilled water served as control. Germination studies were conducted with respect to time (48, 96, 144 and 192 hours). Radicle and plumule length were measured on the 10th day along with fresh and dry weight estimation. The experimental system comprised of 30 pots in order to sow the flax seeds and they were arranged in rows and each row was assigned for a particular treatment. The periodic assessment of the effects of treatments was carried out after every 10th day up to 40 days. The system was set up in such a way that the seeds were treated with three different preparations (1%, 5% and 10% algal extract) of each cyanobacterial species. The leaf extracts of plants from each row was used to assess the effect of cyanobacterial extract on growth parameters, photosynthetic activity and the antioxidant system of flax plant. Total chlorophyll and carotenoid content were estimated by the procedure of Schopfer[15]. The peroxidase activity and catalase activity were measured by following the methods of Putter[16] and Luck[17] respectively. Total soluble protein content of leaf sample was estimated using Lowery's[18] method. Data are expressed as means of 3 independent replicates and statistically analyzed using ANOVA [19]. The significant difference between the means was tested against the critical difference at 5% probability level.

RESULTS AND DISCUSSION

Seed Germination Studies

The effect of algal extracts on germination percentage of flax seeds was observed at 48, 96, 144 and 192 hours and the data were recorded accordingly (Table 1). Extracts of all the three species namely, *N. commune, A. flos-acquae* and *Westiellopsis* sp. showed stimulatory effects on germination percentage of seeds compared to control. The seeds treated with 10% extract of *Westiellopsis* were found to give maximum germination of 84%.

Table 1
Effect of algal extract on germination percentage of Flax seeds

Species	Conc. of algal extracts (%)	Seed germination (%)			
		48 hrs	96 hrs	144 hrs	192 hrs
N. commune	1	8	28	48	72
	5	12	32	52	76
	10	12	40	56	80
A. flos-acquae	1	8	20	44	68
	5	8	28	52	72
	10	12	32	60	76
Westiellopsis sp.	1	8	28	44	76
	5	12	36	56	80
	10	16*	44*	64*	84*
Control	-	0	12	36	64
CD Value		1.32	1.80	1.48	1.50

**** Significant at 5% P level***

Study of growth parameters of Flax plants

Cyanobacteria play a key role in improving growth of many plants when applied as biofertilizers. This evidence was clearly appeared in growth criteria of flax as represented in Table 2. The growth profile of flax plants, in terms of plant height and biomass (fresh and dry weight) were enhanced by administration of cyanobacterial extracts (Fig 1-4).

Treatment with *Nostoc*

Figure 1 Growth of Flax plants treated with extract of *Nostoc*

Treatment with *Anabaena*

Figure 2 Growth of Flax plants treated with extract of *Anabaena*

Treatment with *Westiellopsis*

Figure 3 Growth of Flax plants treated with extract of *Westiellopsis*

Control Plants without treatment

Figure 4 Growth of Flax plants without any Treatment

Data presented in the table 2 indicates the effect of three different concentrations of extracts (1%, 5% and 10%) made from selective micro algal species on growth of flax seedlings in terms of radicle and plumule length. The 10% treatments with *Nostoc* and *Westiellopsis* extracts showed significant stimulatory effect on the length of roots and shoots.

Table 2
Effect of algal extract on growth of Flax plants

Species	Conc. of algal extracts (%)	Radicle length (cm)	Plumule length (cm)	Total plant length (cm)
N. commune	1	1.56	5.46	7.03
	5	2.73	7.96*	10.7
	10	5.36*	8.03*	13.4*
A. flos-acquae	1	1.76	5.43	7.2
	5	2.4	6.23	8.6
	10	2.46	7.1	9.5
Westiellopsis sp.	1	4.6	6.26	10.8
	5	4.86	7.23	12.1
	10	5.43*	7.93*	13.36*
Control	-	1.2	5.56	6.76
CD Value		0.18	0.22	0.36

**** Significant at 5% P level***

The biomass of flax plants was greatly increased with treatments done using cyanobacterial extracts (Table 3). Results obtained showed that treatments of extracts prepared from *Westiellopsis* sp. increased growth parameters apparently.

Table 3
Effect of algal extract on weight of Flax plants

Species	Conc. of extracts (%)	Root		Shoot	
		Fresh wt. (g)	Dry wt. (g)	Fresh wt. (g)	Dry wt. (g)
N. commune	1	0.041	0.010	0.107	0.061
	5	0.046	0.013	0.115	0.072
	10	0.053*	0.014	0.120	0.073
A. flos-acquae	1	0.040	0.010	0.101	0.055
	5	0.047	0.011	0.109	0.063
	10	0.049	0.014	0.112	0.068
Westiellopsis sp.	1	0.043	0.010	0.110	0.074*
	5	0.053*	0.014	0.119	0.072
	10	0.055*	0.017*	0.126*	0.078*
Control	-	0.038	0.013	0.102	0.056
CD Value		0.002	0.001	0.003	0.004

**** Significant at 5% P level***

The present study is in accordance with those of Adam [5] and Wang *et al.* [20] who found that the pre-soaking of seeds of several plants in cyanobacterial cultures enhanced the germination rate. El-Nahas and Abdel-Azeem [21] also stated that pretreatment of *Vicia faba* seeds with the extract of *Anabaena variabilis* induced an increase in germination percentage, root growth, seedling dry weight and soluble proteins as compared with untreated seeds. Gupta [22] found that extract of *Scytonema hofmanni, Fischeralla mucicola* and *Nostoc* sp. enhanced seed germination in rice. Direct evidence for hormonal effects of culture filtrates primarily with pre-soaking of rice seeds, decreasing the losses from sulphate reducing processes attributing to the enhancement of germination and a faster seedling growth was reported by Christopher *et al.* [23]. Similar results were obtained by Ramakant and Yadava [24] who found that water extract of thalli of *Nostoc commune* Vaucher and *Aulosira fertilissima* Ghose stimulated the growth of shoot and root of seedlings of paddy varieties Pusa 33 and Ratna. The concentrated culture filtrates of three cyanobacterial strains *Calothrix ghosei*, *Hapalosiphon intricatus* and *Nostoc* sp. were able to enhance germination percentage, radicle and coleoptile length in studies with wheat seeds. TLC analyses of the filtrates revealed the presence of several amino acids, such as histidine and auxin like compounds. Gibberellin like substances has also been isolated from the cyanobacterium, *Phormidium foveolarum* [25] *Cylindrospermum* sp., *Anabaenopsis* sp. [26] and *Scytonema hofmanni* [27].

Photosynthetic activity

The results tabulated in Table 4 shows the effect of algal extracts (1%, 5% and 10%) on chlorophyll-a (chl-a) and chl-b of Flax leaves assayed on 10^{th}, 20^{th}, 30^{th} and 40^{th} day of plantation with respect to control. It was observed that plants treated with 10% extract of *Nostoc* and *Westiellopsis* possess the highest amount of chlorophyll on 10^{th} and 20^{th} day assays; while plants treated with 10% extract of *A. flos-acquae* had a significant effect on 30^{th} and 40^{th} day estimation with reference to control value.

Table 4
Effect of cyanobacterial extract on Total Chlorophyll content of Flax plants

Species	Concentration of algal extracts (%)	Chlorophyll (mg/g fresh wt.)			
		10th Day	20th Day	30th Day	40th Day
N. commune	1	0.319	0.746	1.61	2.09
	5	0.353	0.806	1.73	2.31
	10	0.385*	1.004*	1.83	2.43
A. flos-acquae	1	0.337	0.717	1.83	2.40
	5	0.359	0.766	2.29	3.08
	10	0.374*	0.818	2.41*	3.27*
Westiellopsis sp.	1	0.328	0.782	1.20	1.89
	5	0.358	0.813	1.23	2.16
	10	0.373*	0.985*	1.29	2.26
Control	-	0.304	0.543	1.03	1.21
CD Value		0.008	0.003	0.014	0.49

**** Significant at 5% P Level***

Das [28] investigated the effect of cyanobacterial fertilizers from four species viz., *Anabaena circinalis, Scytonema coactail, Lyngbya mucicola* and *Oscillatoria princeps* on the growth and biochemical characteristics of *Vinca rosea* L. and reported that plants treated with 10% extract of the heterocystous algae *A. circinalis* possesses the highest amount of chlorophyll, while least in plants treated with 1% extract of the non-heterocystous algae *L. mucicola* with reference to control.

Protein estimation

Results obtained in the analysis of protein content of treated flax plants revealed that it was greatly enhanced by algal extract when compared to control (Table 5). Higher the concentration of extract treatment, greater was the protein content in plants. Although all the extracts gave positive results, maximum enhancement was observed by the treatment of *Westiellopsis* sp. and *N. commune*. Protein content of flax plants was significantly increased with 10% extracts of *N. commune* and *Westiellopsis* sp.

Table 5
Effect of cyanobacterial extract on Protein content of Flax plants

Species	Conc. of algal extracts (%)	Protein (mg/g)			
		10th Day	20th Day	30th Day	40th Day
N. commune	1	0.32	0.44	0.73	0.83
	5	0.44	0.58	0.89	1.01
	10	0.6*	0.7*	1.01	1.2*
A. flos-acquae	1	0.27	0.64	0.69	0.76
	5	0.41	0.53	0.73	0.97
	10	0.51	0.63	0.8	1.1
Westiellopsis sp.	1	0.34	0.49	0.78	0.92
	5	0.46	0.6	0.96	1.13
	10	0.65*	0.72*	1.34*	1.25*
Control	-	0.18	0.25	0.50	0.65
CD Value		0.039	0.033	0.137	0.186

**** Significant at 5% P level***

Mahmoud and Abdel-Rahman [29] observed a general stimulatory effect on growth, pigments, carbohydrates, proteins and amino acid biosynthesis of macroalgae with the crude extracts of *Nostoc* and *Lyngbya* at concentrations ranging from 5 to 15 g/ml, which is in accordance with the observations of the present study. Antioxidant system of Flax plants after treating with algal extracts

Carotenoid assay

Elevated level of carotenoid content was observed in flax plants treated with cyanobacterial extracts. The estimation carried out on different days revealed that the treatments with *Westiellopsis* and *Nostoc* extract had a significant effect on flax (Table 6). Pitchai *et al.* [30] analyzed biochemical constituents of cow pea plants treated with 5% concentration of cyanobacterial extract and coir waste and reported significant increment in all the components when compared to control.

Table 6
Effect of Cyanobacterial extract on Carotenoid content of Flax plants

Species	Conc. of algal extracts (%)	Carotenoid (mg/g fresh wt.)			
		10th Day	20th Day	30th Day	40th Day
N. commune	1	0.112	0.14	0.287	0.554
	5	0.125	0.172	0.308	0.578
	10	0.133*	0.176*	0.317*	0.626
A. flos-acquae	1	0.089	0.134	0.291	0.567
	5	0.098	0.151	0.294	0.577
	10	0.107	0.168	0.297	0.586
Westiellopsis sp.	1	0.06	0.152	0.31*	0.602
	5	0.065	0.174*	0.317*	0.631*
	10	0.077	0.184*	0.318*	0.635*
Control	-	0.04	0.112	0.265	0.542
CD Value		0.002	0.011	0.002	0.005

**** Significant at 5% P level***

Peroxidase assay

It was observed that the treatment of flax with cyanobacterial species enhanced the peroxidase content. The increase in concentrations of extracts resulted in elevated levels of peroxidase content. The estimations were carried out for a period of 40 days with 10 days interval and data showed that 10% extracts of *Westiellopsis* treatment was highly significant over the control plants (Table 7).

Table 7
Effect of cyanobacterial extract on Peroxidase activity of Flax plants

Species	Conc. of algal extracts (%)	Peroxidase (Units/l)			
		10th Day	20th Day	30th Day	40th Day
N. commune	1	108.23	119.79	122.14	130.93
	5	114.08	136.02	154.17	191.63
	10	118.68	149.52*	162.57	208.81
A. flos-acquae	1	103.71	108.23	123.11	110.63
	5	105.92	116.12	119.48	132.14
	10	108.23	130.93	134.95	137.14
Westiellopsis sp.	1	109.80	132.27	142.2	147.7
	5	118.56	142.20	166.03	199.33
	10	124.47*	151.22*	184.49*	237.42*
Control	-	103.71	105.78	108.23	121.52
CD Value		2.30	4.40	4.89	6.72

**** Significant at 5% P level***

Catalase assay

There was a noticeable increase in catalase enzyme levels according to the increase in the concentration of the treatment extracts. *Westiellopsis* sp. registered appreciable increase in concentration of the enzyme when compared to *Nostoc* and *Anabaena* (Table 8). The 5% and 1% extracts of all species also had beneficial results over the control plants. Gradual elevation of catalase content was also observed according to the progression of the time in both treatments and control. All the assays showed significant results with *Westiellopsis* treatment.

Table 8
Effect of cyanobacterial extract on Catalase activity of Flax plants

Species	Conc. of algal extracts (%)	Catalase (Units/ml)			
		10th Day	20th Day	30th Day	40th Day
N. commune	1	305.54	323.11	333.67	356.88
	5	314.88	329.38	344.62	383.81
	10	333.41*	340.08	354.86	405.37
A. flos-acquae	1	293.15	307.38	325.02	345.40
	5	301.79	313.04	335.72	359.62
	10	309.15	318.92	347.63	386.89
Westiellopsis sp.	1	307.25	333.41	342.39	421.88
	5	316.79	340.08	361.80	447.56
	10	335.64*	349.54*	383.75*	464.17*
Control	-	291.50	296.64	309.15	333.41
CD Value		4.65	6.94	10.26	11.81

**** Significant at 5% P level***

Cyanobacteria contain a wide range of antioxidants in the form of specific trace minerals, amino acids, vitamins and pigments. *Phormidium tenue* and *P. fragile* showed high potent antioxidants catalase and peroxidase activity that is in accordance with the present result [31]. A number of studies have demonstrated the growth promotion activity of cyanobacterial extracts on plant regeneration and plantlet formation. Cyanobacteria have potential to produce many metabolites which includes the phytohormones (IAAs, cytokinin and gibberillin-like compounds) and iron-chelators (schizokinen, anachelin and synechobactins) and exhibit profound effect on the productivity of the ecosystem [32]. As plant growth promoting agents, advantages of cyanobacteria over expensive synthetic phytohormones include broader spectrum of activity and optimum levels of biologically active molecules, which are needed for normal plant development *in-vivo* or *in-vitro* [33, 34].

SUMMARY AND CONCLUSION

The occurrence of biologically active substances in algae which promote the growth of other plants had been reported by many investigators. Cyanobacteria produce bioactive compounds including plant growth regulators and enzymes that detoxify oxidative and free radicals. The present study was designed to understand the effect of cyanobacterial extracts on the physiological and biochemical profile of the medicinal plant flax (*Linum usitatissimum* L.). Cyanobacterial treatment resulted in a significant increment on percentage germination, vegetative growth, chlorophyll content and antioxidant system (carotenoid content, peroxidase enzyme and catalase enzyme activity) flax plants. Even though all the three species had a stimulatory effect on growth and antioxidant profile, *Westiellopsis* sp. treatment resulted in apparent enhancement in all physiological parameters and biochemical constituents including antioxidant system of Flax plants. From the study it can be inferred that the method of treating flax plants with cyanobacteria can be adopted to improve the growth profile and antioxidant activity of the plant. The results obtained suggest that selected cyanobacterial species contain growth promoting substances that enhanced growth and biochemical constituents of flax plant. However, since it is not clear whether the results would hold for other agricultural crops, future investigations are needed in this regard.

REFERENCES

1. Mendiola J A, Rodríguez-Meizoso I, Senorans F J, Reglero G, Cifuentes A and Ibanezl E, Antioxidants in plant foods and microalgae extracted using compressed fluids. EJEAFChe, 7(8), 3301-3309, (2008).
2. Molina Grima E, Belarbi E H, Acien Fernandez F G, Robles Medina A and Chisti Y, Biotechnol. Adv. 20: 491, (2003).
3. Swaminathan M S, Biotechnology research in third world agriculture. Sci. 218: 967-972, (1982).
4. Grant I F, Roger E A and Watanabae I, Ecosystem manipulation for increasing biological N fixation by blue-green algae (cyanobacteria) on lowland rice fields. Biol. Agr. Horticul. 3: 299-315, (1986).
5. Adam M S, The promotive effect of the cyanobacterium *Nostoc muscorum* on the growth of some crop plants. Acta Microbiol. Polonica. 48: 163-171, (1999).
6. Lozano M S, Star Verde J, Maiti R K, Oranday C A, Gaona R H, Aranda H E and Rojas G M, Effect of an algal extract and several plant growth regulators on the nutritive value of potatoes (*Solanum tuberosum* L. var. gigant). Archives Latino americanos de Nutricion. 49: 166- 170, (1999).
7. V. Ordog, Beneficial effects of microalgale and cyanobacteria in plant/soil-systems, with special regard to their auxin- and cytokinin-like activity. *International workshop and training course on microalgal biology and biotechnology Mosonmagyarovar, Hungary*, pp:13-26, (1999).
8. Zaccaro de Mule M C, Caire G, Cano M, Palma M and Colombo K, Effect of cyanobacteiral inoculation and fertilizers on rice seedlings and post-harvest soil structure. Comm. Soil Sci. Plant Anal. 30: 97-107, (1999).
9. Kaushik P and Garg V K, Vermicomposting of mixed solid textile mill sludge and cow dung with the epigeic earthworm *Eiseniafoetida biores.* Technol. 90: 311-316, (2003).
10. Safaa R, El-Leathy, Hasnaa S A and Imran M T, Phiosiological effect of some Antioxidants on Flax plant (*Linum usitassimum* L.). World J. Agric. Sci. 6 (5): 622-629, (2010).
11. Pool-Zobel B L, Adlercreutz H, Glei M, Liegibel U M, Sittlingon J, Rowland I, Wahala K and Rechkemmer G, Isoflavonoids and lignans have different potentials to modulate oxidative genetic damage in human colon cells. Carcinogenesis. 21: 1247-1252, (2000).
12. Prasad K, Antioxidant activity of secoisolariciresinol diglucoside-derived etabolites, secoisolariciresinol, enterodiol, and enterolactone. Int. J. Angiol. 9: 220-225, (2000).
13. L. U. Thompson, Flaxseed, lignans and cancer. In: Thompson, L. U, Cunnane, S. C. (Eds.), *Flaxseed in Human Nutrition*, Second ed. AOCS Press, Champaign, IL, 2003, pp.194-222.
14. Kesheri M, Richa and Sinha R. P, Antioxidants as natural arsenal against multiple stresses in cyanobacteria. Intr. J. of Pharma and Bio Sciences. 2: 168-187, (2011)
15. Schopfer P, Experimentelle Pflanzenphysiologie-Einfu hrung in die Anwendung B and 2. Springer, Berlin, (1989).
16. J. Putter, In: *Methods of Enzymatic Analysis (2 ed Bergmeyer).* Academic Press New York, 1974, pp. 685.
17. H. Luck, In: *Methods in Enzymatic Analysis 2 (Ed Bergmeyer).* Academic Press New York, 1974, pp.885.
18. Lowry O. H, Rosebrough N J, Farr A L and Randal R L, Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265-275, (1951).
19. R. A. Fischer, *Contributions to mathematical statistics*. Philosophical

transactions of the Royal Society London. Series A. 222: 309-368, (1950).
20. Wang S M, Wang Q L, Li S H and Zhang J R, A study of treatment of spring wheat with growth promoting substances from nitrogen-fixing blue green algae. Acta Hydrobiol. Sin. 15: 45-52 (1991).
21. El-Nahas A I and Abd El-Azeem E A, *Anabaena variabilis* as biocontrol agent for salt stressed *Vicia faba* seedlings. J. union Arab Biol. Cairo, Physiology Algae. 7 (B): 169-178, (1999).
22. Gupta A B, Algal flora and its importance in the economy of rice field. Hydrobiologia. 28: 213-222, (1966).
23. Christopher P A, Viswajith, V, Prabha S, Sundhar K and Malhga P, Effect of coir pith based cyanobacterial basal and foliar biofertilizer on *Basella rubra* L. Acta Agriculturae Slovenica. 89 (1): 59-63, (2007).
24. Ramakant and Yadava R N, Effect of *Nostoc commune* Voucher and *Aulosira fertilissima* Ghose on growth and development of rice seedlings. Biol. Bull. India. 6(3): 217-221, (1984).
25. Gupta A B and Gupta K K, Effect of Phormidium extract on growth and yield of Vigna catjang (Cowpea) T 5269. Hydrobiologia, 41: 127-132, (1972).
26. Mohan M and Mukerji K G, Some biologically active extracellular products of blue-green algae. Phykos, 18: 73-82, (1979).
27. Rodriguez A A, Stella A M, Storni M M, Zulpa G and Zaccaro M C, Effects of cyanobacterial extracellular products and gibberellic acid on salinity tolerance in *Oryza sativa. L*. Saline Systems, 2: 7, (2006).
28. Das P K, Effect of cyanobacterial biofertilizer on growth and biochemical characteristics of *Vinca rosea* Linn. Res. J. Biotechnol. 4: 76-79, (2010).
29. Mahmoud H M and Abdel-Rahman, Effects of Cyanobacteria crude extracts on growth and related physiological activities of *Chlorococcum humicola* and *Chlorella vulgaris.* Arab. J. Biotech. 8(1): 1-18, (2005).
30. Pitchai P, Perumal M, Solamuthu M, Sivaperumal S K, Munusamy M and Tongmin S, Plant Growth Promontory Effect on Cow Pea (*Vigna unguiculata* L.) Using Coir Pith Aqueous Extract Formulation of Cyanobacterium *Phormidium*. *American-Eurasian* J. Agric. Environ. Sci. 8 (2): 178-184, (2010).
31. Nagasathya A and Thajuddin N, Antioxidant property of hypersaline Cyanobacterium (*Phormidium tenue* (KMD 33). Int. J. Pharmocol. 35: 363-372, (2008).
32. Yadav S, Sinha R P, Tyagi M B, and Kumar A, Cyanobacterial secondary metabolites. Intr. J. of Pharma and Bio Sciences. 2: 144-167, (2011).
33. Sergeeva E, Liaimer A and Bergman B, Evidence for production of the phytohormone indole-3-acetic acid by cyanobacteria. Planta, 215: 229-238, (2002).
34. Prasanna R, Sood A, Jaiswal P, Nayak S, Gupta V, Chaudhary V, Joshi M and Natarajan C, Rediscovering Cyanobacteria as valuable sources of bioactive compounds. Appl. Biochem Micro. 46: 119-134, (2010).