The Bioscan 8(3): 865-869, 2013
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

PHOTOSYNTHETIC AND ANTIOXIDANT ACTIVITY OF OKRA (*ABELMOSCHUS ESCULENTUS*) SEEDS GERMINATEDON AQUEOUS EXTRACTS OF SELECTED CYANOBACTERIAL SPECIES

ABHISHEK KUMAR PANDEY[1]*, BLESSYMOLE K. ALEX[1], EAPEN P. KOSHY[2] AND GEORGE THOMAS[1]
[1]Department of Molecular and Cellular Engineering, Jacob School of Biotechnology and Bioengineering,
Sam Higginbottom Institute of Agriculture Technology & Sciences, Deemed-to-be-University, Allahabad - 211 007, UP
[2]Department of Tissue Engineering, Jacob School of Biotechnology and Bioengineering,
Sam Higginbottom Institute of Agriculture Technology & Sciences, Deemed-to-be-University, Allahabad - 211 007, UP
e-mail: abhi73025@gmail.com

KEYWORDS
Cyanobacteria
Photosynthetic activity
Antioxidant activity
Okra

Received on :
24.01.2013

Accepted on :
29.04.2013

***Corresponding author**

ABSTRACT
Okra is a vegetable crop which is having high medicinal properties. The objective of the present investigation was to study the effect of selected cyanobacterial species on growth parameters, photosynthesis and antioxidant system of Okra plant. As compared to control plants, seeds presoaked in the culture extract of *Spirulina*sp., *Chroococcus*sp. and *Anabaena* sp. *enhanced* the germination percentage, vegetative growth, chlorophyll content andantioxidant system (carotenoid content, total phenol content and peroxidase enzyme activity) of Okra (*Abelmoschusesculentus*). 5% extracts of the selected cyanobacterialspecies showed maximum effect in enhancing growth, biochemical constituents and peroxidase enzyme activity of the plant. Cyanobacterial treatment can be used to increase the photosynthetic and antioxidant activity of other vegetable crops and also as a growth enhancer.

INTRODUCTION

Biological nitrogen fixation is a vital component of agricultural sustainability. The cyanobacterial biofertilizer is an easily manageable, self re-generating system, which not only contributes valuable nutrients to plants in terms of nitrogen, amino acids and growth promoting substances but also improves the soil health and textures (Sophiya*et al.,* 2009). Cynobacteria are used as biofertilizers which improves the growth of higher plants like wheat, rice, maize, vegetables and certain medicinal plants. The distinct compounds present in cynobacteria help in creating an environment that enhances the growth and improvement of plants.

Okra is a widely used vegetable plant having many industrial and medicinal properties. It is an incredibly valuable vegetable which not only binds excess cholesterol and toxins (in bile acids) but also assures their easy passage from the body (Nadkarni, 1998). Okra plants are used in manufacture of jaggery, ripe seeds are used as a substitute for coffee, matured fruits and stems containing crude fibre are used in the paper industry and theroots and stems of okra are used for clearing canejuice(Mehta, 1959). Okra is very useful against genito-urinary disorders, dysuria, chronic dysentery, spermatorrhoea, ulcers and relief from hemorrhoids (Adams, 1975). The barkfiber is suitable for spinning into rope and for paper and cardboard manufacture.Mucilage is used as a plasma replacementor blood volume expander. Leaves are sometimes used as a base for poultices, as an emollient, sudorific or antiscorbutic and to treat dysuria(Jambhale andNerkar, 2010).

To the best our knowledge, there is no report regarding the effect of cyanobacterial extract on the physiological and biochemical profile of Okra. So the present study is an attempt to study the influence of aqueous extract of selected cynobacterial species (*Spirulina*sp., *Chroococcus*sp. and *Anabaena* sp.) on photosynthesis and growth characteristics of Okra (*Abelmoschusesculentus*) along with its antioxidant profile.

MATERIALS AND METHODS

The cyanobacterial samples were collected from Indian Agriculture Research Institute (IARI), PUSA, New Delhi and was grown in a thermostatically controlled culture room at 25 ± 2^{o}C with an illumination of 4000 lux in BG-11 medium.

For conducting the experiment, a system consisting of 30 pots was designed in three rows which determined the day of assay (day 10, day 20 and day 30). Each pot comprised of 8 seeds that were pre-soaked in the extracts of different cyanobacterial species (*Spirulina*sp., *Chroococcus*sp. and *Anabaena* sp.) and a control i.e. the seeds were soaked in distilled water without any extract for 24 hrs. Each treatment was done in triplicates. After pot preparation, the soaked seeds were transferred to individual pots according to their treatments in natural conditions in the experimental field.

The study was carried out by spreading 8 seeds on different petriplates with filter paper and cotton base. Five ml of various concentrations of algal extractand distilled water was provided under sterile conditions for the test system and control system respectively. Germination studies were conducted on 2nd, 4th, 6th and 8th day. Radicle and plumule length of germinated plantlets were measured on 10th day and biomass estimation was also done.

The leaf sample was prepared andthe supernatant was measured according to procedure given by Schopfer (1989) and concentration of pigments was determined according to the formula of Witham *et al*. (1971).

The estimation of peroxidase activity was carried out according to Boyarkin (1989). The enzyme activity was expressed in terms of absorbance per gram fresh weight. The estimation of total phenols of the plant sample was carried out by using the protocol of Malic and Singh (1980) and the concentration of total phenol was expressed as mg/g fresh weight. The estimation of protein content of the plant sample was carried out and the concentration of protein content was expressed as mg/g fresh weight (Lowry *et al*., 1951). Data were statistically analyzed according to Fisher (1950) against the critical difference at 5% probability level.

RESULTS AND DISCUSSION

The effect of algal extracts on germination percentage of Okra seeds was observed and the data was recorded (Table 1). The seeds treated with 5% extract of *Chroococcus*sp. gave maximum germination showing significant treatment effect.Kumar and Anand (2010) reported the positive effect of algal extract on germination percentage of *Vinca* seeds at various incubation periods and all the treatments with *Anabaena, Scytonema, Oscillatoria*and *Lyngbya* extracts at 10% level showed enhanced growth over the control with time.

All treatments showed significant stimulating effect on the weight and lengthof roots and shoots. Okra seeds treated with 5% extract of *Chroococcus*sp.was observed to show maximum growth in both length and weight of root and shoot (Table 2-3). Sadaatina and Riahi (2010) found similar results on germination of rice seeds treated with cyanobacteria. They noticed an increase of 53% in plant height, 66% in root length, 58% in fresh leaf and stem weight, 80% in fresh root weight, 125% in dry leaf and stem weight.

The estimation carried out revealed that treatments with *Spirulina*sp (10%) showed maximum increase in carotenoid content. All the treatments showed a progression in the amount of carotenoid content (Table 4). Bhuvaneshwari *et al*. (2011) investigated the combined application of cyanopith and cyanospray and showed improvement in morphological, biochemical, chlorophyll and carotenoid content of *Helianthus annus*.

The results tabulated in Table 5 shows that plants treated with 5% extract of *Anabaena* sp. possess the highest amount of chlorophyll. However all treated plants showed increased chlorophyll content against the control. This finding is corroborative to the report of Anandraj (2008) which states that the plants (*Aliumcepa* and *Oryza sativa*) leaves showed

Table 1:Effect of algal extract on germination percentage of Okra seeds

Algaltreatment	Concentrationsof algal extracts(%)	Percentage of seed germination			
		48 hrs	96hrs	144 hrs	192 hrs
*Spirulina*sp.	1	15±2.94	34±3.74	42±2.16	55±1.63
	5	14±1.63	32±2.44	45±1.63	58±2.16
	10	22±2.16*	47±2.94*	60±4.54	78±4.54
*Chroococcus*sp.	1	20±2.16*	39±1.63	58±1.41	85±2.16*
	5	22±1.41*	48±2.82*	62±2.94	87±2.16*
	10	15±2.16	34±1.63	45±1.63	60±2.16
*Anabaena*sp.	1	22±1.63*	47±2.16*	65±1.41*	84±1.41
	5	12±1.41	32±2.94	41±2.44	60±3.55
	10	15±2.16	35±2.16	47±3.55	68±3.55
Control	-	12±0.81	30±0.82	40±2.16	52±2.16
CD value		2.03	2.53	2.72	2.87

* Significant at p= 5% p level

Table 2: Effect of algal extract on growth of Okra plants

Algaltreatment	Concentrationsof algalextracts (%)	Root length(cm)	Shoot length(cm)	Total plant length(cm)
*Spirulina*sp.	1	3.20±0.25	6.52±0. 16	10.23±0.30
	5	2.98±0.09	6.21±0.17	10.09±0.23
	10	2.90±0.14	6.13±0.12	10.03±0.96
*Chroococcus*sp.	1	3.04±0.07	6.33±0.16	10.37±0.65
	5	3.39±0.02*	6.84±0.33*	11.23±1.62*
	10	3.07±0.26	6.32±0.26	10.33±0.88
*Anabaena*sp.	1	2.94±0.14	6.01±0.11	9.95±0.89
	5	2.54±0.12	5.58±0.34	9.05±0.48
	10	2.68±0.08	5.83±0.14	9.51±0.51
Control	-	2.02±0.22	4.62±0.34	7.64±1.07
CD value		0.17	0.22	0.77

* Significant at p= 5% p level

Table 3: Effect of algal extract on biomass of Okra plant

Algaltreatment	Concentrationsof algalextracts (%)	Root weight (g) Fresh	Dry	Shoot weight (g) Fresh	Dry
*Spirulina*sp.	1	0.07±0.003*	0.02±0.005*	0.23±0.02	0.06±0.007*
	5	0.06±0.009*	0.02±0.002*	0.21±0.02	0.05±0.008
	10	0.06±0.008*	0.01±0.004	0.20±0.007	0.05±0.006
*Chroococcus*sp.	1	0.06±0.011*	0.02±0.005*	0.22±0.01	0.05±0.005
	5	0.07±0.002*	0.02±0.004*	0.29±0.006*	0.07±0.008*
	10	0.06±0.0008*	0.02±0.004*	0.22±0.005	0.05±0.006
Anabaena sp.	1	0.06±0.002*	0.02±0.002*	0.19±0.01	0.05±0.007
	5	0.05±0.008	0.01±0.002	0.18±0.01	0.04±0.007
	10	0.05±0.008	0.01±0.002	0.18±0.008	0.05±0.007
Control	-	0.05±0.008	0.01±0.002	0.16±0.008	0.03±0.009
CD value		0.01	0.00	0.01	0.01

* Significant at p= 5% p level

Table 4: Effect of cyanobacterial extract on carotenoid content of Okra plant

Algal treatment	Concentrationsof algalextracts (%)	10^{th} day(mg/g fresh wt.)	20^{th} day(mg/g freshwt.)	30^{th} day(mg/g freshwt.)
*Spirulina*sp.	1	10.70±0.16*	10.74±0.37*	10.79±0.56*
	5	10.53±0.21*	10.58±0.18*	10.64±0.45*
	10	10.72±0.05*	10.77±0.09*	10.82±0.05*
*Chroococcus*sp.	1	10.54±0.19*	10.58±0.13*	10.53±0.05*
	5	10.59±0.21*	10.62±0.14*	10.78±0.11*
	10	10.42±0.33	10.45±0.40	10.53±0.24*
*Anabaena*sp.	1	10.68±0.30*	10.73±0.23*	10.77±0.26*
	5	10.62±0.39*	10.66±0.41*	10.70±0.28*
	10	10.45±0.18	10.49±0.19	10.54±0.24*
Control	-	9.92±0.09	9.82±0.12	9.76±0.10
CD value		0.21	0.25	0.43

* Significant at p= 5% p level

Table 5: Effect of cyanobacterial extract on chlorophyll content of Okra plant

Algaltreatment	Treatments (algal extracts %)	10^{th} day Chl-a	Chl-b	20^{th} day Chl-a	Chl-b	30^{th} day Chl-a	Chl-b
*Spirulina*sp.	1	11.72±0.22	5.33±0.58	11.88±0.56	6.09±0.45	12.01±0.23	7.29±0.34
	5	12.03±0.26	6.86±0.83	12.2±0.31	7.82±0.40	12.42±0.23	8.77±0.35
	10	11.8±0.53	5.71±0.93	11.86±0.54	6.31±0.56	12.10±0.19	7.45±0.48
*Chroococcus*sp.	1	11.71±0.45	5.55±0.90	11.78±0.48	5.93±0.28	11.95±0.49	6.48±0.17
	5	12.12±0.34	7.41±1.05*	12.54±0.43*	8.08±0.47	11.89±0.50	9.43±0.36*
	10	11.61±0.21	5.59±0.43	11.68±0.19	5.97±0.37	11.89±0.12	6.10±0.25
*Anabaena*sp.	1	12.12±0.62	7.02±0.59	12.24±0.48	8.43±0.88*	12.51±0.24	8.93±0.72
	5	12.56±0.18*	7.93±0.73*	12.80±0.62*	8.8±0.78*	13.15±0.20*	9.47±0.49*
	10	12.01±0.59	7.07±1.51*	12.23±0.38	8.0±0.36	12.51±0.91	8.93±0.52
Control	-	10.89±0.05	4.56±0.63	11.29±0.11	5.16±0.43	11.46±0.26	5.71±0.79
CD value		0.38	0.87	0.39	0.48	0.41	0.39

* Significant at p= 5% p level

6: Effect of cyanobacterial extract on peroxidase activity of Okra plant

Algaltreatment	Concentrationsof algalextracts (%)	10^{th} day(OD/g fresh wt.)	20^{th} day(OD/g freshwt.)	30^{th} day(OD/g freshwt.)
*Spirulina*sp.	1	1.36±0.15	1.58±0.19	1.72±0.08
	5	1.25±0.09	1.46±0.16	1.62±0.19
	10	1.54±0.06*	1.80±0.10	1.04±0.10
*Chroococcus*sp.	1	1.30±0.05	1.50±0.13	1.68±0.08
	5	1.46±0.13	1.72±0.14	1.96±0.20*
	10	1.34±0.05	1.58±0.17	1.76±0.06
*Anabaena*sp.	1	1.42±0.07	1.78±0.16	1.90±0.46
	5	1.22±0.10	1.98±0.9*	2.16±0.41*
	10	1.56±0.10*	1.86±0.08*	2.02±0.03*
Control	-	1.18±0.02	1.36±0.10	1.56±0.25
CD value		0.09	0.13	0.20

* Significant at p= 5% p level

Table 7: Effect of cyanobacterial extract on total phenolic content of Okra plant

Algaltreatment	Concentrationsof algalextracts (%)	10th day(mg/g fresh wt.)	20th day(mg/g freshwt.)	30th day(mg/g freshwt.)
*Spirulina*sp.	1	1.51 ± 0.11	1.65 ± 0.23	1.77 ± 0.16
	5	1.61 ± 0.18	1.73 ± 0.19	1.85 ± 0.19
	10	2.06 ± 0.09*	2.18 ± 0.14*	2.32 ± 0.25*
*Chroococcus*sp.	1	1.70 ± 0.23	1.81 ± 0.06	1.93 ± 0.06
	5	1.62 ± 0.24	1.75 ± 0.08	1.88 ± 0.07
	10	1.82 ± 0.21	1.95 ± 0.14	2.08 ± 0.21
*Anabaena*sp.	1	1.58 ± 0.10	1.66 ± 0.20	1.79 ± 0.10
	5	1.54 ± 0.09	1.70 ± 0.05	1.83 ± 0.08
	10	2.01 ± 0.13*	2.10 ± 0.09*	2.23 ± 0.35*
Control	-	1.44 ± 0.10	1.58 ± 0.14	1.72 ± 0.15
CD value		0.16	0.15	0.18

* Significant at p = 5% p level

Table 8: Effect of cyanobacterial extract on total protein content of Okra plant

AlgalTreatment	Concentrationsof algalextracts (%)	10th day(mg/g fresh wt.)	20th day(mg/g freshwt.)	30th day(mg/g freshwt.)
*Spirulina*sp.	1	0.98 ± 0.02	1.02 ± 0.03	1.08 ± 0.07
	5	0.86 ± 0.10	0.90 ± 0.05	0.97 ± 0.03
	10	0.90 ± 0.10	0.93 ± 0.02	1.01 ± 0.07
*Chroococcus*sp.	1	1.0 ± 0.04	1.04 ± 0.04	1.10 ± 0.03
	5	1.12 ± 0.09*	1.16 ± 0.05*	1.25 ± 0.05*
	10	0.92 ± 0.10	0.95 ± 0.03	1.04 ± 0.04
*Anabaena*sp.	1	0.91 ± 0.0	0.94 ± 0.04	1.02 ± 0.04
	5	0.94 ± 0.0	0.98 ± 0.01	1.05 ± 0.05
	10	0.84 ± 0.0	0.91 ± 0.07	0.99 ± 0.03
Control	-	0.81 ± 0.0	0.86 ± 0.07	0.92 ± 0.04
CD value		0.07	0.05	0.05

* Significant at p = 5% p level

higher chlorophyll content than control when cyanobacterial treatment was given.

It was observed that treatment of Okra with cyanobacterial species enhanced the peroxidase activity in the Okra plant (Table 6). The 5% extract of *Anabaena* sp. treatment showed maximum peroxidase activity. Pietsch and Wiegand (2001) reported significant elevation in the activity of the peroxidase after exposure of *Scenedesmusarmatus*to the cyanobacterial crude extract for 1 hr, when compared to control.

A continuous increase was observed in total phenolic content, when treated with cyanobacterial species. Mainly the 10% extracts of all selected species (*Spirulina* sp., *Chroococcus*sp. and *Anabaena* sp.) showed appreciable increase in amount of total phenol. The samples treated with 10% extract of *Spirulina* sp. showed highest phenol content (Table 7). Suhail*et al*. (2011) reportedthe maximum antioxidant potential of *Spirulina* sp. followed by *Plectonema*sp., *Scytonema* sp. and *Nostoc* sp. through the free radical scavenging activity by using 2, 2-diphenyl-1-picrylhydrazyl(DPPH) method.

All the treatments gave increased amount of protein than the control plants (Table 8). Maximum enhancement was observed in samples treated with 5% extract of *Chroococcus*sp. Uma and Kannaiyan (1995) stated that inoculation of cyanobacterial strains improved the growth, total carbohydrate, protein and chlorophyll content of seedlings significantly which justify the results of the present study.

In 1995, Gauter*et al*. reported that the stimulatory effect of cyanobacterial biofertilizers on Relative Growth Rate (RGR) may be ascribed to an increase in LAR (Leaf area ratio) and NAR (Net assimilation rate). They opined that increase in LAR indicates consistent increase in plant photosynthetic efficiency.

Biochemical and bioassay of plant and algal extracts reveal the presence of gibberellin, auxin, kinetin and macromolecules like proteins, carbohydrates, amino acids and phenolic compounds (Mini *et al*., 1999). The importance of cyanobacterial biofertilizers as a source of nitrogen has been well documented by many researchers. In addition to contributing to nitrogen content cyanobacteria also benefit crop plants by producing various growth promoting substances. Free amino acids like serine, arginine, glycine, aspartic acid, threonine, glutamic acid, cysteine, proline, valine, ornithine, lysine, histidine, isoleucine, the extra- and intra-cellular polysaccharides composed of sugars like xylose, galactose, fructose and several others have been reported in the external medium of cyanobacteria (Kaushik, 2009).

The mechanism of cyanobacterial plant growth promoting action has not yet been understood in detail. It may be due to auxins, gibberlins, cytokinninsand other growth promoting substances present in cyanobacteria.Further studies are needed in this regard to study the biomolecular interaction between cyanobacteria and higher plants through the use of radio labeled compounds and fluorescent microscopic studies.Since all the treatments had shown better germination percentage and growth charecteristics than control plants, it can be concluded that treatment with these cyanobacterialextractscan enhance the growth characteristics of plants.

REFERENCES

Adams, C.F. (1975). Nutritive value of American foods in common units, U.S. Department of Agriculture, *Agriculture Handbook*. **425**: 29.

Anandraj, B. (2008). Studies on coir pith based cyanobacterial biofertilizer for field cultivation. Ph.D, Dissertation, Bharatidasan University, Tiruchirappalli, Tamilnadu, India.

Bhuvaneshwari, B., Subramaniyam, V. and Malliga, P. (2011). Comparative studies on cyanopith and cyanospray biofertilizers with chemical fertilizer on Sunflower (*Helianthus annus*).*Journal of Applied Phycolo*gy.**5**: 235-241.

Boyarkin, O.M. (1989). Total phenol measurement with the Follin phenol reagent. *Journal of Biological Chemistry*. **32**: 828.

Fisher, R.A. (1950). Contributions to Mathematical Statistics. *Philosophical Transactions of the Royal Society London Series A*. **222**: 309-368.

Gauter, M., Kerby, N.W., Rowell, P., Obrent, C. and Scrimgeour, C. (1995). Colonization of wheat (*Triticumvulgare* L.) by nitrogen fixing cyanobacteria; IV dark nitrogenase activity and effect of Cyanobacteria on natural super ^{15}N abundance in plants.*New Phytol*. **129:** 337-343.

Jambhale, N.D. and Nerkar, Y.S. (2010). Okra In: Handbook of Vegetable Science and Technology, Mahatma Phule Agricultural University Rahuri.

Kaushik, B. D. (2009). Developments in cyanobacterial Biofertilizer. In: Khattar, J.I.S., Singh, D.P. and Kaur, G. (eds.) Algal Biology and Biotechnology. IK International Publishing House Pvt. Ltd. pp. 97-108.

Kumar, A.P. and Anand, N. (2010). Studies on phycobilin pigments of the cyanobacterium *Wetiellopsissiyengarii*. *International Journal of Biotechnology and Biochemestry*. **6**: 315-323.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randal, A.L. (1951). Protein measurement with the Follin phenol reagent. *Journal of Biological Chemistry***. 193:** 265-275.

Malic, C.P. and Singh, M.B. (1980). Plant enzymology and Histoenzymology .Kalyani Publishers, New Delhi. **286.**

Mehta, Y.R. (1959). Vegetable growing in Uttar Pradesh. *Bureau of Agricultural research*. **31**: 215-218.

Mini, S. N., Mehta, P. M. and Gajaria, S. C. 1999. Effect of algal and higher plant extracts on carbohydrate metabolism in rice seedlings. In: Fatma, T. (ed.) Cyanobacterial and algal metabolism and Environmental Biotechnology. *Narosa Publishing House,* New Delhi. pp. 81-89.

Nadkarni, K.M. (1998). Indian Medicinal Plants and Drugs with their Medicinal Properties and Uses.Asiatic Publishing House New Delhi. **450**.

Pietsch, C. and Wiegand, C. (2001). The effects of a cyanobacterial crude extract on different aquatic organisms: Evidence for cyanobacterial toxin modulating factors. Institute of Freshwater Ecology and Inland Fisheries, Müggelseedamm, Berlin, Germany.

Sadaatina, H. and Riahi, H. (2010). Cyanobacteria in paddy fields in iron as a biofertilizer in rice fields. *Plant Soil Environment*.**31**: 701-704.

Schopfer, P. (1989). Experimentelle Pflanzenphysiologie Einfuhrung in die Anwendung B and 2. Springer, Berlin.

Sophiya, L., Oinam, G., Leingaklemba, M.K., Singh, H. B., Tiwari, O.N. and Singh, M. R. (2009). Distribution, preservation and maintenance of cyanobacterial diversity and their application as biofertlizer. In: Khattar, J.I.S., Singh, D.P. and Kaur, G. (eds.) Algal Biology and Biotechnology. IK International Publishing House Pvt. Ltd. pp. 109-120.

Suhail, S., Biswas, D., Farooqui, A., Arif, J.M. and Zeeshan, M. (2011). Antibacterial and free radical scavenging potential of some cyanobacterial strains and their growth characteristics.*Journal of Chemical and Pharmaceutical Research*. **3**: 472-478.

Uma, D. and Kannaiyan, S. (1995). Monitoring the ammonia excretion by *Anabaena azolla*eimmobilized in polyurethane foam and its effect on the growth of rice seedlings. Paper presented at the National Seminar on *Azolla*eand Algal Biofertilizers for rice, Tamil Nadu Agriculture University, Coimbatore: pp 5662.

Witham, F.H., Blaydes, D.R. and Devlin, R.M. (1971). Experiment in plant physiology. Van Nostrad Reinhold Company, New York, USA.