J. Indian bot. Soc. ISSN - 0019 - 4468 Vol. 94 (1 & 2) 2015 : 111-117

EMERGING ROLE OF CO-INOCULATION OF *PSEUDOMONAS* WITH CYANOBACTERIA FOR INCREASING CROP PRODUCTIVITY

ANKITA KHEMKA AND MEENU SARAF*

Department of Microbiology, University school of sciences, Gujarat University, Ahmedabad 380009, India.

The assessment of performances of the selected bacterial strain- NT1 (*Pseudomonas* sp.) and two cyanobacterial strains CB1 and CB2 (*Desertifilum* sp. and *Leptolyngbya* sp.) and their combinations were done in a field experiment with *Pisum sativum* and *Cicer arietinum* crop. Current study is the novel approach towards utilizing *Desertifilum* sp. for plant growth promotion. The study comprises of seven treatments along with controls. The enhancement in percent germination was recorded in treatments involving combination of bacterial cyanobacterial strains T5 (CB2+NT1) and T7 (CB1+CB2+NT1) with 90 and 94 % in *P. sativum* while significant improvement in the fruit yield was obtained up to 63% in T7 (CB1+CB2+NT1) followed by 55% in T6 (CB1+CB2) with the same crop. Growth yield increased with T7 (CB1+CB2+NT1) over control by 230% in *P. sativum* while its rhizospheric soil analysis reveals enhancement in total nitrogen upto 130% compared to nitrogen content in control. The study with *C. arietinum* crop reveals significant increase in the fruit productivity by 48 % with T7 (CB1+CB2+NT1) with increase of 117% of total nitrogen in T7 (CB1+CB2+NT1). Comparative evaluation revealed the superior performance of strain CB2 and NT1 over CB1 in increasing the growth and grain yield of crop and improving soil health.

Keywords: *Cicer arietinum, Desertifilum sp., Leptolyngbya sp., Pisum sativum,* Plant Growth Promotion (PGP)

The usage of chemical fertilizers increased in agriculture over the globe with the food crises to enhance productivity. Also there is the emerging consciousness towards environment preservation, mainly land and water pollution, a paradigm shift occurs from chemical to organic farming in agricultural practices. The organic farming exploits microorganisms, algae, fungi, worms, etc. to enhance plant growth by a wide variety of mechanisms such as colonizing roots, or increase mixing of nutrients with the soil. Cyanobacteria are nitrogen fixing, colonizing and photosynthetic prokaryotes enhancing the plant growth (Karthikeyan *et al* 2008). Various studies reported the ability of cyanobacteria to improve soil composition through fixing atmospheric nitrogen(Osman *et al.*, 2010)and increasing the carbon and nitrogen status of soil (Maqubela *et al* 2009). Cyanobacteria are wide spread in diverse habitat in different ecosystem.Inspite of their abundancy and characteristic features, cyanobacteria are less explored towards agronomic practices with different crops and lands. Being eco-friendly, cyanobacteria can be used in seed inoculation, nutrient enrichment, reforestation, and rehabilitation of ecosystems. Being Plant growth promoting (PGP) organisms, cyanobacteria can affect plant growth by different mechanisms *viz.* siderophore production (Yadav *et al*. 2011) and production of phytohormone such as indole-3-acetic acid (IAA) (Sergeeva *et al.* 2002). Hegazi *et al.* (2010) had performed the studies for the improvement of plant growth and seed production using *Nostoc* sp., *Anabaena* sp., *Phormidium* and *Spirulina* sp. while *Subramaniyan et al*. (2012) used *Oscillatoria* and Westiellopsis sp. in his experiments.The present study is an unique approach utilizing the novel strains of cyanobacteria, *Desertifilum tharense* MSAK01 and *Leptolyngbya* sp. The research focuses towards the enhancement of *Pisum sativum* and *Cicer arietinum* crop through bio inoculation using cyanobacteria and a well established PGPR *Pseudomonas* NT1. The effect of these inoculations on the crop productivity and nutritional benefits shall be estimated.

MATERIALS AND METHODS

Micro-organisms

The two cyanobacterial isolates CB1 and CB2 were isolated from the dairy waste water and identified using the taxonomic keys of Iyenger and Desikachary(1981). The well established bacterial strain *Pseudomonas* NT1 had been used. The sterile synthetic, BG-11 Medium (Stanier *et al.* 1971) was used for isolation, cultivation and preservation of micro algae.The

Received on February 05, 2015 Accepted on February 06, 2015

cyanobacterial inoculation volume (suspended in cyanobacteria growth media) in each system was 10% (v/v), with an initial concentration of approx. 2x106 cells/ml. All the cultures were incubated at 25 ± 2^0C under a photoperiod of 16:8 h light: dark cycle and illumination (37 µ $Es^{-1}m^{-2}$) with cool white fluorescent lamps. The cultures were shaken manually once a day (without sparging with air or CO_2) to avoid adherence to the side of the flask.

PGP traits

Cyanobacterial strains were evaluated for different growth promoting traits such as phosphate solubilization, nitrogen fixation,hydrogen cyanide (HCN), IAA production and siderophore production. Phosphate solubilization was determined qualitatively by streaking strains on BG 11 agar plates containing tricalcium phosphate (0.3%).Development of a clear zone around the growth of cyanobacteria was observed after incubation at 24±2°C for 15 days (Mazhar and Hasnain 2011). Nitrogen fixation potential was examined by growing them in nitrogen free media (BG11 without $NaNO_3$). HCN production was determined as described by Ahmad *et al.* (2008).The production of indole acetic acid (IAA) by the cyanobacterial isolate was assayed by using Salkowski method (Gordon and Weber 1951) and quantified spectrophotometrically by measuring the intensity of pink colour at 530 nm, using calibration curve of standard IAA stock solution (10-100 µg ml^{-1}) prepared in 50% ethanol (Patel *et al* 2012). Quantitative estimation of total soluble proteins was done spectrophotometrically using the protocol of Herbert *et al.* (1971) against bovine serum albumin as standard. A qualitative assay of siderophore production was conducted in Chrome Azurole's (CAS) agar media (Jha *et al* 2012). The development of a yellow orange halo around the colony was considered as a positive result.

Field Trial of Promising Plant Growth Promoting Bacterial and Cyanobacterial Strains

The bacterial strain (*Pseudomonas* sp. NT1) and cyanobacterial strains showing potent PGP characters were selected and inoculated in field experiment with *Cicer arietinum* and *Pisum sativum* in plots of 8 sq m. The experimental site was located in Ahmedabad, Gujarat, situated at a latitude of 23.07 N, longitude of 72.49 E and altitude of 48.77 m above the mean sea level (Arabian sea). The climate of Ahmedabad is semi-arid with the annual mean pan evaporation of 955 mm. Both the crops are sown in the last week of November and harvested in the first week of February (Rabi season).

A suspension of log phase (12-14 day old) cultures of the cyanobacterial strains was applied at the rate of 2.5 g chlorophyll ha^{-1}. Bacteria and Cyanobacteria were mixed with Organic Compost Fertilizer Manure Plant Fertilizer as a carrier prior to sowing of seeds and the amended compost was used for seed coating at the rate of 500 g ha^{-1}. Preliminary evaluation undertaken on seed germination using single and dual strain combinations revealed the promise of seven treatments (Table 1).

Table 1: Details of treatments containing combinations of fertilizers, bacterial and cyanobacterial strains

Sr. No.	Treatments	Combinations
1	Control	
2	T1	*Desertifilum tharense* (CB1)
3	T2	*Leptolyngbya* sp. (CB2)
4	T3	*Pseudomonas* sp. (NT1)
5	T4	*Desertifilum tharense* and *Pseudomonas* sp.
6	T5	*Leptolyngbya* sp. and *Pseudomonas* sp.
7	T6	*Desertifilum tharense* and *Leptolyngbya* sp.
8	T7	*Desertifilum tharense*, *Leptolyngbya* sp. and *Pseudomonas* sp.

Percent germination was measured during the initial stages of germination after 18 DAS (Jha and Saraf 2011). The equation to calculate germination percentage is:

$$\text{Germination \%} = \frac{\text{seeds germinated}}{\text{Total seeds}} \times 100$$

Table 2: Plant growth promotion traits of selected cyanobacterial strains

Strains	IAA production (µg IAA ml^{-1})	Siderophore Production (mM)	Nitrogen Fixation	Phosphate solubilization	HCN production
CB1	35	5.2	++	-	-
CB2	21	3.7	++	-	-

Table 3: Details of treatments containing combinations of fertilizers, bacterial and cyanobacterial strains

Treatment No.	Details	Shoot Biomass (gm) Chick Pea	Root Biomass (gm)	Shoot Biomass (gm) Garden Pea	Root Biomass (gm)
Control	-	50.2	3.04	27	7.6
T1	CB1	53	4.68	31	8.2
T2	CB2	57	5.12	28	7.9
T3	NT1	61	5.8	33	8.5
T4	CB1 + NT1	59	5.7	36	8.9
T5	CB2 + NT1	68	6.2	34	9.1
T6	CB1 + CB2	62	6.5	37	9.4
T7	CB1 + CB2 + NT1	87	7.2	38	10

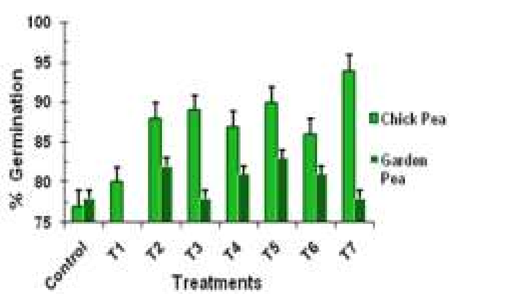

Fig. 1: Germination as influenced by inoculation of bacterial/ cyanobacterial strains

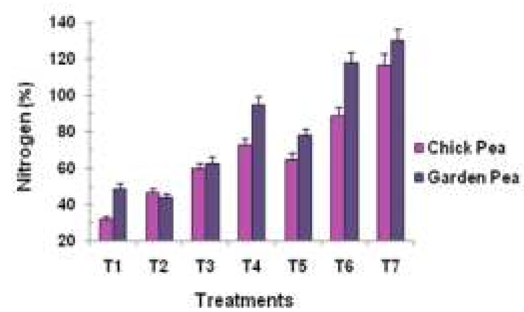

Fig. 2 (A): Nitrogen increment in soil inoculated with cyanobacteria & bacteria

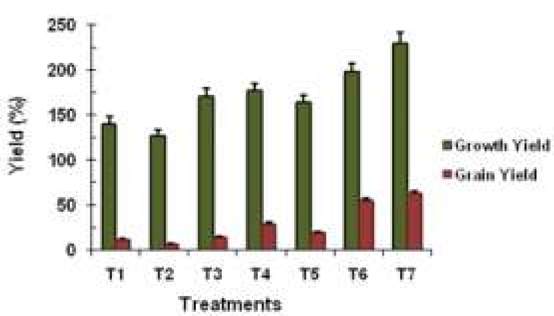

Fig. 2 (B): Fruit yield and growth yield of Chick Pea and Garden pea crop

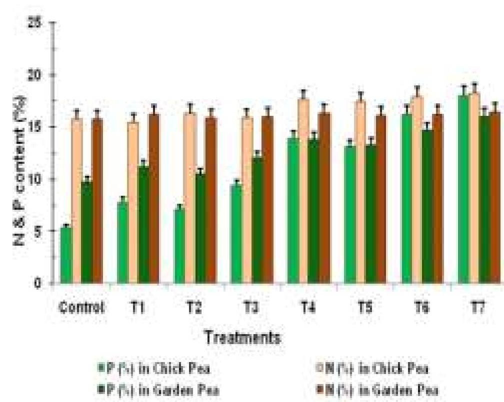

Fig. 2 (C): Nitrogen and Phosphorus measurement of grain after the treatment with bacteria and cyanobacteria mixture

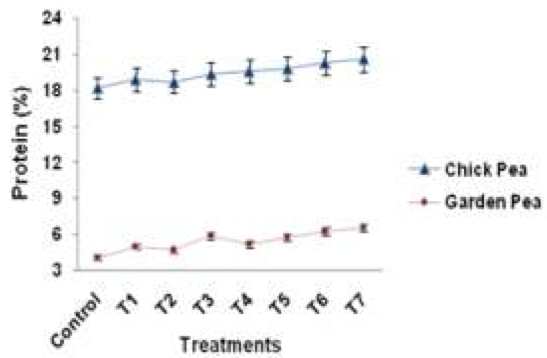

Fig. 3: Enhancement in protein content of the grains obtained during co-inoculation study of Bacteria-Cyanobacteria

The germination rate provides measure of the time course of seed germination. Rhizospheric soil samples were collected at mid and harvest stage and analyzed for nutrient analysis (Prasanna *et al.* 2011).

Biometric Parameters

The plant parameters-root, shoot biomass and total biomass were noted on 45 DAS, and grain yield as an indicator of growth yield were recorded at the time of harvest (gathering of mature crops) after 85 DAS (Prasanna *et al.* 2011).

Analysis of NPK concentration in Chick pea and Garden pea

One gram (dry weight) of each legume was weighed and powdered in a pestle-mortar followed by suspending fine crushed sample in 5 ml of nitric acid: perchloric acid by 2:1 and digested using plant digestor (Gupta 2006)thus obtained digested samples were used for analysis. Potassium concentration was analyzed with Flame photometer and compared with standard ranging from 0 to 100 ppm of KCl. P concentration was measured by the method of Jackson (1958). The total nitrogen of grain samples and rhizospheric soil were estimated by Kjeldahl's method and the percent nitrogen content in the samples was recorded using the N-autoanalyzer(Kjeldahl 1883).

Statistical analysis

The triplicate sets of data for the various parameters evaluated were subjected to Chick . ANOVA (analysis of variance) ($p<0.05$) using Microsoft office Excel 2007. The experimental design (completely randomized block design)was performed using the software Statistical package for Social Sciences (SPSS Version 11.0) and PSPPire Software (Version 3)statistical package to quantify and evaluate the source of variation. The treatment means were compared at a significance level of 0.05.

RESULTS

PGP traits

The cultures were identified as Novel strains, *Desertifilum tharense* and *Leptolyngbya* sp. respectively. Both cyanobacterial strains showed positive results for nitrogen fixation (Table 2) with growth in nitrogen free media. Nitrogen is very essential nutrients for plant growth and inoculation with nitrogen fixing cyanobacteria has been shown to improve plant growth by increasing the availability of nitrogen content. Both cyanobacterial strains showed negative results for phosphate solubilization and hydrogen cyanide production. The cyanobacterial isolates (CB1 and CB2) exhibited property of heterotrophic growth with the production of siderophore and indolic compound. (Table 2).

Field experiment

Soil nutrients and plant parameters were measured at harvest stage while percent (%) germination was measured at germinating stage of Chick Pea and Garden Pea. The percent germination was highest in T7 (CB1+CB2+NT1) by 94%, followed by T5 (CB2+NT1) with 90% in Chick Pea. While in Garden Pea, the percent germination was highest in T5 (CB2+NT1) with 83% compared to control with 78% (Fig.1).

The biometric observation reveals the highest root and shoot biomass with the treatment T7 (CB1+CB2+NT1), followed by T6 (CB1+CB2) and T5 (CB2 + NT1) in both Pea and Garden Pea plant. The percent fruit yield calculated on the basis of fruit weight obtained per plant (g/plant) to determine crop yield reveals the highest percent fruit yield in T7

(CB1+CB2+NT1) in both the crops (Table 3).

The rhizospheric soil analysis for nitrogen content reveals the major and significant increment in total nitrogen in T7 (CB1+CB2+NT1) compared with the rhizospheric soil nitrogen of control plant. The NPK analysis of Chick Pea and Garden pea reveals the significant enhancement in all the treatment involving the use of combinations of cyanobacteria and bacterial strains. During the analysis in present experiment, the potassium content of Chick Pea and Garden Pea ranges from 200 – 250 kg/hec. There has been significant increment in nitrogen and phosphorus content of grains of Chick Pea and Garden Pea. The highest increment in T7 (CB1+CB2+NT1) with Garden Pea is 130 % followed by T6 (CB1+CB2) at 118 % (Figure 2A). Thus growth of Garden Pea was increased up to 230% compared to the plant biomass of control plant that is without treatment as shown in Figure 2 B.

DISCUSSION

Preliminary studies revealed the growth enhancement of crop due to cyanobacterial and bacterial co-inoculation. Research work that tested the PGP potential of the cyanobacterial isolates was systematically carried out. PGP potential of novel strain, *Desertifilum tharense* and *Leptolyngbya* sp was studied by inoculating *Cicer arietinum* (Chick pea) and *Pisum sativum* (Garden pea) crop. Both the cyanobacterial isolates were found to produce phytohormone (IAA) and ironchelating substances, that is, siderophores. The Higher levels of IAA and siderophore were produced by *Desertifilum tharense* in comparison to *Leptolyngbya*. Hashtroudi and others (2013) reported that *Anabaena vaginicola* and *Nostoc calcicola*, isolated from the paddy soil, showed significant production of IAA and growth-promoting effect on vegetables and herbaceous plants. Rana and others (2012) reported the production of indolic compounds and siderophore by *Anabaena* sp. and *Calothrix* sp.The growth of cyanobacteria in nitrogen deficient media indicates its stimulatory potential due to the atmospheric nitrogen fixation and making it available to the associated plants (Karthikeyan *et al*. 2007) and significant growth of both the strains had been noted in the given experiment.

The effect of the two cyanobacterial isolates along with bacterial strain in our study was then observed in field trials using chickpea and gardenpea. The effect of plant growth could be relieved in plants inoculated with selected PGP organisms compared to plants not treated with PGP organisms. Germination percentage was maximum in T7 (CB1 + CB2 + NT1) followed by T5 (CB2 + NT1). Similarly Rana and others (2012) reported enhanced germination of wheat inoculated with bacteria and cyanobacterial strains under field trials. The seeds treated with both cyanobacteria and bacterial strains showed maximum root and shoot biomass, resulting in growth promotion and higher yields. Karthikeyan and others (2007) reported increases in the plant height and dry weight of wheat crop when inoculated with *Nostoc sp,Hapalosiphon intricatus* and *Calothrix ghosei*. The observation of biometric study reveals the increase in crop productivity, Similar to the improvement observed with the co-inoculation of bacteria and cyanobacteria on rice crop with yield enhancement up to 19% concluded by Prasanna *et al.* (2011). The significant increase noted in root biomass, shoot biomass and crop productivity may be responsible for increased the fruit productivity with more than 48%.Muthukumaravel-Chinnusamy *et al.* (2006) and Dhar *et al.* (2007) concluded that application of biofertilizers (BGA) resulting in significant improvement in rice yield parameters. The enhancement influence of BGA on the biological activity and chemical properties of the soil positively affected plant characteristics which led to improve in common bean yield and quality of seeds. The significant increase is noted in NPK content of grains as compared to the results of Hegazi *et al*. (2010). Available N in soil significantly increased due to the cyanobacterial inoculation compared to the non inoculated treatments due to nitrogen fixing ability of heterocystous cyanobacteria .

Thus the present study revealed the percent nitrogen increment up to 117% in chick pea and 113% in Garden Pea, therefore the organisms in the current study, when co-inoculated, that is *Desertifilum tharense* and *Leptolyngbya* sp. together with bacterial culture, can be regarded as efficient biofertilizers.

CONCLUSION

The application of cyanobacteria with PGP traits is becoming widespread worldwide to reduce usage of chemical fertilizers, achieving sustainable agriculture and improving soil nutrition through remediation using plants, microbes and algae. The present study concludes the potential of cyanobacterial strain coinoculated with *Pseudomonas* in plant growth and fruit productivity. Among the isolated cyanobacterial strains, both the strains equally prove to be efficient PGP strains. Cyanobacterial isolate CB2 alone increased plant biomass by 171% in chickpea and 14% in garden pea compared to untreated plants. Treatments with cyanobacterial isolate CB1, CB2 and *Pseudomonas* NT1 yields maximum increase in the plant biomass and fruit productivity by 230% and 48%, respectively. Thus the organisms utilized in the current study can be regarded as efficient PGP organisms and biofertilizer when co-inoculated with bacterial strain.

Thanks are due to the Department of Microbiology and Biotechnology, Gujarat University to provide required facility for conducting research work.

REFERENCES

Ahmad F, Ahmad I and Khan M S 2008 Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiol Res* **163** 173-181.

Muthukumaravel-Chinnusamy, Kaushik B D and Prasanna R 2006 Growth, nutritional, and yield parameters of wetland rice as influenced by microbial consortia under controlled conditions. *J Plant Nutr* **29(5)** 857-871.

Dhar D W, Prasanna R and Singh B V 2007 Comparative performance of three carrier based blue green algal biofertilizers for sustainable rice cultivation. *J Sust Agri* **30(2)** 41-50.

Gordon S A and Weber R P 1951 Colorimetric estimation of indole acetic acid. *Plant Physiol* **26** 192–195.

Gupta P K 2006 Soil, plant, water and fertilizer analysis. *Agrobios India* **6** 264-266.

Hashtroudi M S, Ghassempour A, Riahi H, Shariatmadari Z and Khanjir M 2013 Endogenous auxins in plant growth-promoting cyanobacteria—*Anabaena vaginicola* and *Nostoc calcicola*. *J Appl Phycol* **25** 379-386.

Hegazi A Z, Soha S M, Mostafa Hamdino and M I Ahmed 2010 Influence of different cyanobacterial application methods on growth and seed production of common bean under various levels of mineral nitrogen fertilization. *Nature and Science* **8** (11).

Herbert D, Phipps P J and Strange P E 1971 Chemical analysis of microbial cells. *Methods Microbiol* **VB** 249-344.

Iyengar M O P and Desikachary T V 1981 *Volvocales*. Indian Council of Agricultural Research, New Delhi.

Jackson M L 1958 *Soil Chemical Analysis*. Prentice Hall London.

Jha C K and Saraf Meenu 2011 Effect of plant growth promoting rhizobacteria on Seed germination behaviour and seedling vigor of *Jatropha curcas* plant. *Int J Biotechnol Biosci* **1(1)** 101-113.

Jha C K, Patel B and Saraf M 2012 Stimulation of the growth of *Jatropha curcas* by the plant growth promoting bacterium *Enterobacter cancerogenus* MSA2. *World J Microbiol Biotechnol* **28** 891-899.

Karthikeyan N 2006 Characterization of cyanobacteria from the rhizosphere of wheat. M.Sc dissertation Division of Microbiology Post Graduate School Indian Agricultural Research Institute New Delhi.

Karthikeyan N, Prasanna R, Nain L and Kaushik B D 2007 Evaluating the potential of plant growth promoting cyanobacteria as inoculants for wheat. *Euro J Soil Biol* **43** 23-30.

Kjeldahl J 1883 A new method for the determination of nitrogen in organic matter. *Zeitschreft fur analytische chemie* **22** 366.

Maqubela M P, Mnkeni P N S, Issa M O, Pardo M T and D'Acqui L P 2009 Nostoc cyanobacterial inoculation in South African agricultural soils enhances soil structure, fertility and maize growth. *Plant Soil* **315** 79-92.

Mazhar S and Hasnain S 2011 Screening of native plant growth promoting cyanobacteria and their impact on *Triticum aestivum* var. Uqab 2000 growth. *African J Agri Res* **6(17)** 3988-3993.

Osman M E H, El-Sheekh M M, El-Naggar A H and Gheda S F 2010 Effect of two species of cyanobacteria as biofertilizers on some metabolic activities, growth, and yield of pea plant. *Biol Fertil Soils* **46** 861-875.

Prasanna R, Joshi M, Rana A, Shivay Y S and Nain L 2011 Influence of co-inoculation of bacteria-cyanobacteria on crop yield and C–N sequestration in soil under rice crop. *World J Microbial Biotechnol* DOI 10.1007/s11274 011 0926 9.

Patel D, Jha C K, Tank N and Saraf M 2012 Growth Enhancement of Chickpea in Saline Soils Using Plant Growth-Promoting Rhizobacteria. *J Plant Growth Regul* **31** 53–62.

Rana A, Joshi M, Prasanna R, Shivay Y S and Nain L 2012 Biofortification of wheat through inoculation of plant growth promoting rhizobacteria and cyanobacteria. *European J Soil Biol* **50** 118-126.

Sergeeva E, Liaimer A and Bergman B 2002 Evidence for production of the phytohormone indole-3-acetic acid by cyanobacteria. *Planta* **215** 229-238.

Stanier R Y, Kunisawa R, Mandel M and Cohen-Bazire G 1971 Purification and properties of unicellular blue-green algae (order Chroococcales). *Bacteriol Rev* **35** 171-205.

Subramaniyan V, Krishna Moorthy S and Malliga P 2012 Analysis of biochemical and yield parameters of *Zea mays* (Corn) cultivated in the field supplemented with coir pith based cyanobacterial biofertilizers. *J Algal Biomass Utln* **3(3)** 54– 57.

Yadav S, Sinha R P, Tyagi M B and Kumar A 2011 Cyanobacterial secondary metabolites. *Int J Pharma Biosci* **2(2)** 145-167.