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ROLE OF EXOGENOUS GLUCOSE ON GROWTH PHYSIOLOGY OF BENGARD EXPOSED CYNOBACATERIUM *NOSTOC CALCICOLA*

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ABSTRACT :

At higher concentrations of fungicide, the heterocyst frequency of the cyanobacteria increased in N_2 as well as NH_4^+ media except at Bengard (250µg ml^{-1}) concentrations. The normal frequency of heterocyst was seen in 5 - 6% of nitrogen in normal culture condition. This stimulation action in heterocyst formation was observed after a time span of two to six days. Heterocyst did not appear in the fungicide supplemented or Unsupplemented culture in NO_3^- or fungicide interacted with organism, primarily with its N_2 and NH_4^+ mediated growth conditions. It was found that glucose protect the growth inhibitor effects of the higher concentrations of Bengard (100 - 250µg ml^{-1}) in all inorganic nitrogen media.

KEY WORDS : *Bengard, Optical density, Supplement and Fungicide.*

INTRODUCTION:

Life is a marvelous gift of nature and a living structure is a doll of chemicals comprising 40 elements, predominantly top ranked by nitrogen (N) as the integral component of the building blocks (proteins) and hereditary units (Nucleic acids) inside an organic body. In biosphere, only a few microorganisms are provided with specific gene organization to produce the enzyme nitrogenase, responsible for catalyzing the nitrogen - fixing reactions. Nitrogenase, isolated from any N_2- fixing organisms has been universally found to be extremely oxygen-sensitive (Brill, *et al.*, 1980). Therefore, only certain bacteria and Cyanobacteria perform the function of N_2-fixation under anaerobic or micro-aerophilic condition However, in sharp contrast, the majority of filamentous (Trichomous) Cyanobacteria endowed with the special nitrogen fixing anaerobic compartments, *i.e.*, heterocyst, fix considerable amount of nitrogen in aerobic state, Rather the combination of an oxygen - evolving photosynthesis with the oxygen- sensitive, but fully functional, nitrogen fixing system owned by the Trichomous and Heterocystous. Cyanobacteria is unique and rare among the entire microbial community.

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Out of various biological process on the earth, the photosynthesis and nitrogen fixation are the most important, because, this involve the fixation of atmospheric carbon and nitrogen respectively. It is wonder, however, to note that all the green plants, algae and Cyanobacteria have the capacity to photosynthetically fix inorganic carbon out of only 350ppm of CO_2 present in the atmosphere, while the ability to fix nitrogen is restricted to only certain microorganism *i.e.*, some of the bacteria and Cyanobacteria, despite the vast presence of nearly 79% N_2 in this atmosphere (Kumar, *et al.*, 1991). Therefore, in the agricultural fields the naturally occurring cyan bacteria are automatically exposed to a variety of toxic agro-chemicals, including the herbicides. The herbicides, although not designed for this purpose, often strongly inhibit the normal physiological processes of the cyanobacteria by impairing regulation of the growth cycle and ultimately disrupting their growth and development. In spite of the importance of N_2- fixing cyanobacteria in maintaining N fertility of the soil, only few studies have been undertaken on the herbicide-cyanobacterial interactions. In all such studies, largely conducted through laboratory trails, the herbicides have been found invariably toxic (and mutagenic in a few cases) and growth inhibitory to the cyanobacteria (Hawxby, *et al.*, 1977; Kar and Singh, 1978; Lundkvist, 1970; Pandey, *et al.*, 1984; Prasad, *et al.*, 1986; 1991; Singh, 1974). The toxicity has been, however, noted to be relatively less under the field condition possibly due to degradation of the herbicides in interaction with the several biotic and a biotic factors in the fields (Greave, 1982; Singh, *et al.*, 1986).

All those herbicides, which interfere with the photosynthetic reactions in their target herbs and weeds, have invariably proved toxic to the cyanobacteria (Brusslan and Haselkorn, 1988) as they share many of the unique pathways of plants, including the oxygenic photosynthesis. But apart from their "plant-type" characteristics these organisms possess the property and simple prokaryotic genome organization and short generation time (Doolittle, *et al.*, 1974), which has stimulated an easy isolation of herbicide resistant populations of these photosynthetic N2-fixers during the last decade (Brusslan and Haselkorn, 1988; Golden and Haselkorn, 1985; Prasad, *et al.*, 1986; Prasad, *et al.*, 1991) not only with a view to using them as a viable and self-renewable bio-N fertilizer in herbicide-treated fields, but also for investigating into the physiological / biochemical/ molecular modes of action of these synthetic agro-chemicals in a suitable and less time consuming biological system (Brusslan and Haselkorn, 1988) These results may be extrapolated for the higher plants. However, N_2-fixing cyanobacteria isolated from the paddy-fields have not been used so far for such studies which could have been otherwise after having acquired herbicide resistance) of considerable practical use (in view of their adaptability to field condition) as a viable bio-N fertilizer in the herbicide treated fields.

The various studies made so far now clearly suggest that (i) field treatment with N_2^- fixing cyanobacteria, even without weeding increased rice yield up to the level that attained on herbicide and biofertilizer treatment (Singh, *et al.,* 1988); (ii) herbicide treated fields are not suitable for the application of N_2^- fixing cyanobacteria as bio-N fertilizer (Singh, *et al.,* 1988); (iii) for algalization of agricultural fields with N_2 - fixing cyanobacteria successful it is essential to first develop the pesticide resistant inoculants for thriving in the chemicalized fields (Kumar, *et al.,* 1991).

MATERIALS AND METHODS:

Cynobacterium *Nostoc calcicola* was isolated and identified form the pure culture. Bengard (250 - 500 gm /hect /500 litre) concentration was dissolved in double distilled water. Stock solution was prepared in 1µl/ml concentration and further dilution was prepared for experiment. The results were plotted by providing different nitrogen sources that is N_2, NO_3 and ammonia. Suplimented and Unsuplimed with Glucose. Optical density was measured by the help of Systronic UV visual spectrophotometer under different condition of exposed organism.

RESULTS AND DISCUSSION:

Data was analyzed for heterocyst formation and growth behavior of Nostoc calcicola. It was grown on N_2, NO_3^-, NO_2^- and NH_4^+ containing media with different concentration of Bengard (5 to 50µg ml^{-1}). Unsupplemented and supplemented with 5mM glucose were incorporated in Table1 and 2. The Bengard fungicide at a threshold concentration (25

Table 1 : Growth of WT Organism, untreated or treated* with graded Concentration (0, 100, 150, 200, 250µg ml $^{-1}$) of the pesticide Bengard in N^2, NO_2^-, NO_3^- and NH_4^+ media , unsupplemented or supplemented with 5mM glucose.

Period (Days)	Concentration of the pesticide Benegard µg ml^{-1}	Growth (Optical density)							
		N_2		NO_3^-		NO_2^-		NH_4+	
		Nil	+Glucose	Nil	+Glucose	Nil	+Glucose	Nil	+Glucose
0	Initial OD	0.09±0.00	0.09±0.00	0.09±0.00	0.09±0.00	0.09±0.00	0.09±0.00	0.09±0.00	0.09±0.00
2	0	0.129±0.012	0.146±0.012	0.205±0.012	0.243±0.014	0.168±0.011	0.208±0.009	0.146±0.009	0.191±0.011
	100	0.087±0.009	0.098±0.011	0.098±0.011	0.125±0.009	0.096±0.010	0.112±0.011	0.087±0.012	0.096±0.009
	150	0.078±0.011	0.094±0.010	0.088±0.09	0.099±0.011	0.092±0.009	0.098±0.012	0.068±0.011	0.088±0.012
	200	0.057±0.014	0.065±0.009	0.072±0.012	0.084±0.014	0.068±0.011	0.084±0.009	0.062±0.009	0.078±0.014
	250	0.035±0.010	0.041±0.014	0.043±0.011	0.052±0.009	0.048±0.009	0.058±0.014	0.051±0.014	0.064±0.009
4	0	0.221±0.009	0.265±0.011	0.352±0.010	0.421±0.011	0.272±0.014	0.326±0.011	0.272±0.012	0.318±0.011
	100	0.118±0.012	0.149±0.014	0.213±0.09	0.268±0.012	0.13±0.011	0.184±0.012	0.127±0.011	0.163±0.012
	150	0.096±0.012	0.128±0.009	0.181±0.012	0.223±0.014	0.114±0.014	0.154±0.009	0.092±0.009	0.131±0.014
	200	0.055±0.013	0.078±0.012	0.098±0.011	0.146±0.009	0.071±0.009	0.122±0.014	0.082±0.012	0.102±0.009
	250	0.028±0.009	0.048±0.013	0.032±0.013	0.069±0.011	0.042±0.010	0.082±0.011	0.046±0.013	0.071±0.011
6	0	0.325±0.014	0.366±0.011	0.412±0.012	0.592±0.010	0.414±0.011	0.478±0.009	0.382±0.011	0.432±0.010
	100	0.198±0.011	0.252±0.010	0.314±0.011	0.386±0.010	0.21±0.010	0.267±0.012	0.192±0.012	0.241±0.009
	150	0.167±0.014	0.208±0.014	0.264±0.009	0.331±0.009	0.174±0.009	0.236±0.014	0.141±0.009	0.208±0.014

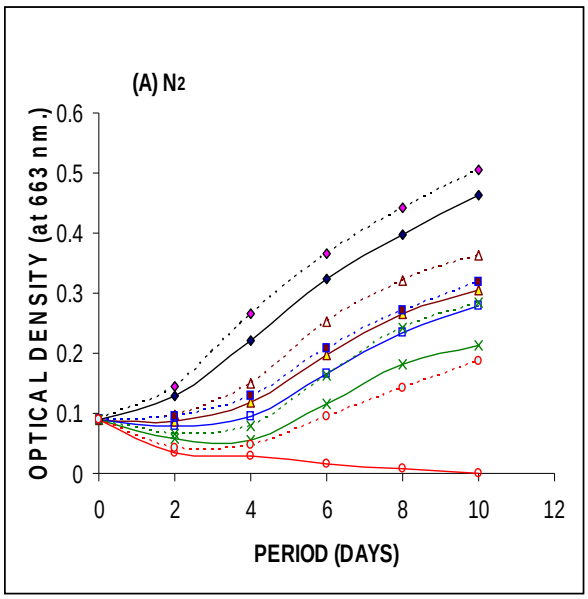
	200	0.115±0.012	0.164±0.009	0.138±0.010	0.258±0.011	0.137±0.014	0.194±0.011	0.108±0.014	0.158±0.011
	250	0.016±0.014	0.095±0.014	0.024±0.012	0.089±0.012	0.028±0.011	0.118±0.009	0.026±0.011	0.121±0.009
8	0	0.398±0.009	0.442±0.012	0.552±0.011	0.653±0.009	0.472±0.014	0.548±±0.012	0.458±0.012	0.526±0.012
	100	0.265±0.010	0.322±0.014	0.372±0.09	0.443±0.013	0.283±0.009	0.36±0.011	0.264±0.014	0.325±0.014
	150	0.234±0.011	0.271±0.009	0.318±0.010	0.388±0.011	0.239±0.011	0.316±0.010	0.214±0.009	0.293±0.009
	200	0.181±0.012	0.241±0.010	0.174±0.012	0.338±0.012	0.195±0.010	0.263±0.009	0.157±0.010	0.243±0.011
	250	0.008±0.014	0.143±0.011	0.011±0.09	0.114±0.009	0.016±±0.013	0.168±0.011	0.014±0.011	0.198±0.012
10	0	0.463±0.009	0.505±0.010	0.576±0.011	0.705±0.014	0.498±0.009	0.563±0.012	0.514±0.012	0.571±0.013
	100	0.306±0.012	0.364±0.012	0.405±0.014	0.486±0.012	0.326±0.014	0.385±0.009	0.301±0.009	0.371±0.009
	150	0.278±0.014	0.318±0.009	0.362±0.012	0.428±0.009	0.296±0.011	0.346±0.010	0.276±0.014	0.324±0.012
	200	0.213±0.014	0.285±0.010	0.19±0.009	0.382±0.010	0.248±0.012	0.318±0.011	0.208±0.012	0.287±0.010
	*250	0.00±0.011	0.168±0.011	0.000±0.011	0.172±0.011	0.005±0.014	0.208±0.012	0.006±0.011	0.254±0.011

Treatment time was 15 minutes.

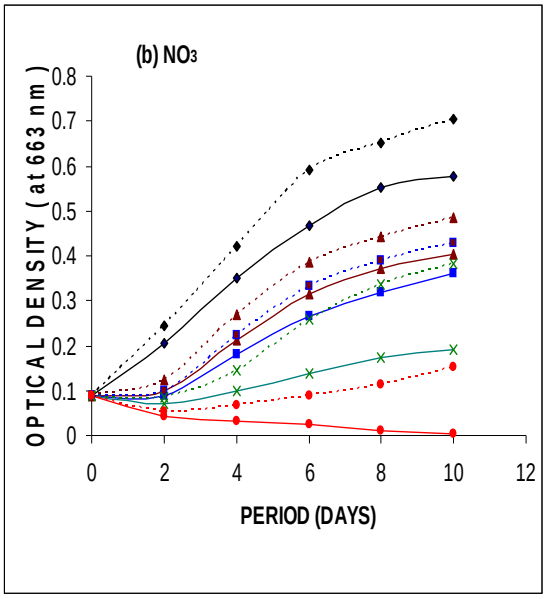
Table 2 : Heterocyst frequency of *Nostoc calcicola* in N_2 and 1mM NH_4^+ media supplemented or unsupplemented with 5mM glucose with graded concentration of the pesticide bengard.

Nitrogen source	Concentration of fungicide in µg ml^{-1}	Supplemented glucose (+ or -)	Heterocyst frequency						
			0 Hr.	24 hr.	48 Hr.	72 Hr.	96 Hr.	120 Hr.	144 hr.
N_2	Nil	-Gl	0.00	4.25±0.28	5.25±0.06	6.20±0.31	6.00±0.34	6.10±0.24	6.15±0.38
		+Gl	0.00	5.650±0.16	7.80±0.380	7.40±0.36	6.950±0.35	6.750±0.42	6.580±0.42
	100	-Gl	0.00	0.00	0.00	9.10±0.40	10.35±0.38	12.34±0.40	13.15±0.31
		+Gl	0.00	0.00	0.00	12.80±0.5	13.48±0.0.37	13.63±0.28	13.82±0.40
	150	-Gl	0.00	0.00	0.00	6.40±0.32	9.82±0.48	11.68±0.412	11.726±0.35
		+Gl	0.00	0.00	0.00	12.15±0.6	10.15±0.28	12.28±0.365	12.675±0.44
	200	-Gl	0.00	0.00	0.00	5.200±0.2	6.68±0.42	7.82±0.275	8.35±0.46
		+Gl	0.00	0.00	0.00	8.40±0.42	8.78±0.34	9.315±0.425	9.42±0.238
	250	-Gl	0.00	0.00	0.00	0.00±	0.00	±0.00	5.26±0.12
		+Gl	0.00	0.00	0.00	8.80±0.48	10.30±0.28	11.40±0.36	12.20±0.44
NH_4^+	Nil	-Gl	0.00	0.00	0.00	0.00	0.00	0.00±	0.00
		+Gl	0.00	0.00	0.00	0.00	0.00	0.00±	0.00
	100	-Gl	0.00	0.00	0.00	0.00	0.00	3.68±0.27	4.12±0.38
		+Gl	0.00	0.00	0.00	0.00	0.00	4.84±0.29	5.156±0.32
	150	-Gl	0.00	0.00	0.00	0.00	0.00	5.725±0.282	5.752±0.321
		+Gl	0.00	0.00	0.00	0.00	6.18±0.336	5.830±0.281	±8.2150.416
	200	-Gl	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		+Gl	0.00	0.00	0.00	0.00	0.00	4.41±0.25	8.12±0.159
	250	-Gl	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		+Gl	0.00	0.00	0.00	0.00	0.00	0.00	0.00

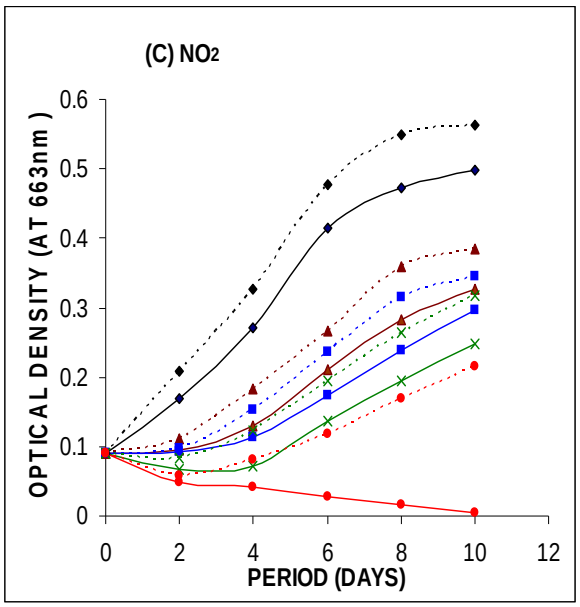
to 30µg ml^{-1}) was found to stimulate the growth of the Cyanobacteria is NO_3^- and NO_2^- medium in comparison to that of the control cultures. Such growth and stimulatory effect of Bengard, was not observed in either N_2 or NH_4^+ mediator. No any significant change was noticed in the heterocyst frequency (Table - 2) of *Nostoc calcicola* growing with 5 to 50µg ml^{-1} Bengard, fungicide stimulated the formation of heterocyst in NH_4^+ medium is sharp contrast to the control cultures.



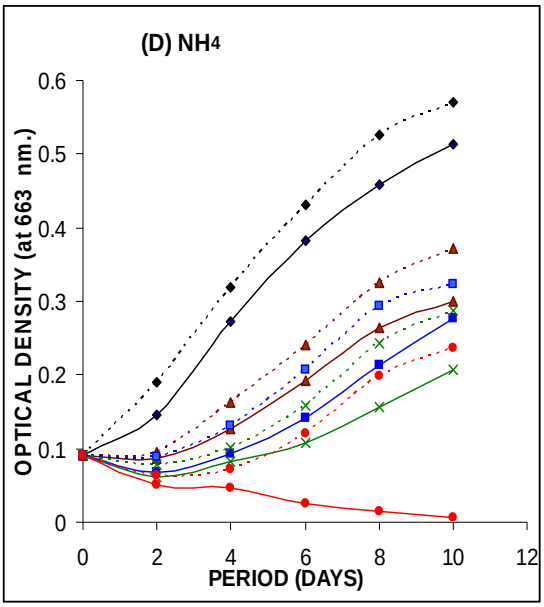
Hist. 1 **Graph of different concentrations of Glucose**



Hist. 2 **Graph of different concentrations of Glucose**



Hist. 3 Graph of different concentrations of Glucose



Hist. 4 **Graph of different concentrations of Glucose**

Table -3: The growth (increase in optical density at 663 nm) and heterocyst frequency (number of heterocyst /100 vegetative cell) of 10 days old cultures of *Nostoc calcicola* in N_2, 5m NO^-_3 mM NO^-_2 1mM NH_4^+ ± 5mM Glucose medium, supplemented with various concentration of Bengard*

Concentration of benegard µg /ml.	Growth								Heterocyst frequency			
	N_2		N_2		NH_4^+		NH_4^+		N_2		NH_4^+	
	-gl	-gl	+gl	-gl	+gl	+gl	-gl	+gl	-gl	+gl	-gl	+gl
Nil	0.48 ± 0.002	6.680 ± 0.168	9.42 ± 0.176	0.00	0.00	0.524 ± 0.036	0.548 ± 0.074	0.683 ± 0.008	6.680 ± 0.168	9.42 ± 0.176	0.00	0.00
5.0	0.332 ± 0.012	6.25 ± 0.50	8.34 ± 0.32	1.32 ± 0.011	2.28 ± 0.03	0.468 ± 0.028	0.308 ± 0.026	0.482 ± 0.012	6.25 ± 0.50	8.34 ± 0.32	1.32 ± 0.011	2.28 ± 0.03
10.0	0.338 ± 0.022	6.66 ± 0.42	8.92 ± 0.02	3.22 ± 0.03	4.32 ± 0.51	0.490 ± 0.065	0.298 ± 0.038	0.472 ± 0.016	6.680 ± 0.42	8.92 ± 0.02	3.22 ± 0.03	4.32 ± 0.51
20.0	0.352 ± 0.036	7.12 ± 0.42	9.014 ± 0.44	5.58 ± 0.16	6.66 ± 0.12	0.504 ± 0.046	0.305 ± 0.074	0.484 ± 0.072	7.12 ± 0.42	9.014 ± 0.44	5.58 ± 0.16	6.66 ± 0.12
30.0	0.385 ± 0.015	6.82 ± 0.060	8.78 ± 0.18	3.22 ± 0.42	4.99 ± 0.24	0.674 ± 0.056	0.344 ± 0.024	0.518 ± 0.062	6.82 ± 0.060	8.78 ± 0.18	3.22 ± 0.42	4.99 ± 0.24
40.0	0.346 ± 0.012	6.21 ± 5.422	8.90 ± 0.02	3.40 ± 0.21	4.48 ± 0.50	0.546 ± 0.028	0.312 ± 0.026	0.424 ± 0.020	6.21 ± 5.422	8.90 ± 0.02	3.40 ± 0.21	4.48 ± 0.50
50.0	0.330 ± 0.021	5.422 ± 0.055	8.626 ± 0.250	3.11 ± 0.20	4.22 ± 0.32	0.460 ± 0.035	0.302 ± 0.052	0.405 ± 0.031	5.422 ± 0.055	8.626 ± 0.250	3.11 ± 0.20	4.22 ± 0.32

*Number of heterocyst was found in nitrate or nitrite medium an hence data on the heterocyst frequency has been provided for N_2 and NH_4^+ media only.

CONCLUSION:

Therefore, suggesting that possibly at higher concentrations the fungicide created a condition. Supplementation of exogenous carbon *i.e.*, Glucose uniformly stimulated the growth as well as heterocyst formation capacity of the Cyanobacteria growing with various lower concentration of Bengard. Similar to the control cultures, no any heterocyst was traced in culture growing in NO_3^- and NO_2^- medium with graded concentration of fungicide supplemented or un-supplemented with glucose.

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