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IMPACT OF *CALOTHRIX SP.* A PROMOTIVE BIOFERTILIZER ON GROWTH PERFORMANCE OF *BARLERIA PRIONITIS* IN POT CULTURE

SAJAD HUSSAIN BAKSHI[1],SHABIR AHMAD LONE[2], MALIK TAFAZUL RASHID[1], SHAHIJAHAN DAR[2],KHURSHEED AHMAD SHAH[1]

[1] Department of Bioscience, Barkatullah Vishwavidhyalaya Bhopal.
[2] Govt. Motilal Vigyan Mahavidyalaya, Barkatullah Vishwavidhyalaya Bhopal.

Corresponding author's email: - sajad_bakshi@yahoo.com

ABSTRACT

As the problem of chemical fertilizers can be a restraining factor for agriculture development and crop production increases, the alternative methods have to be sought out and the present techniques discussed in this work appears to be a very suitable way to combat the pressing need of the nitrogen fertilizers using these simple nitrogen fixing cyanobacteria, which are low costing, high efficiency biofertilizers of today. An attempt was made to test a promotive biofertilizer- *Calothrix Sp.* on growth performance of *Barleria prionitis* in pot culture and the results revealed that all the growth parameters viz; Root length, Shoot length, Number of leaves, Fresh weight of leaves, Chlorophyll a content and Chlorophyll b content of *Barleria prionitis* treated with *Calothrix Sp.* and urea as compared with the initial values were found maximum as compared to control.

Key Words :- *Calothrix,* Biofertilizer, Growth, *Barleria prionitis*, Culture.

1. INTRODUCTION

Cyanobacteria (also known as blue–green algae) are a group of extraordinarily diverse Gram-negative prokaryotes that originated 3.5 billion years ago. Their diversity ranges from unicellular to multicellular, coccoid to branched filaments, nearly colorless to intensely pigmented, autotrophic to heterotrophic, psychrophilic to thermophilic, acidophilic to alkylophilic, planktonic to barophilic, freshwater to marine including hyper saline (salt pans). They are found both free living and potential organisms which can be useful to mankind in various ways.

The oxygenic photosynthetic bacteria, the cyanobacteria are autotrophs that fix CO_2 through the reductive pentose phosphate cycle. They make an important contribution to the Earth's nitrogen cycle by incorporating nitrogen into the biosphere through assimilatory processes. Organisms of this group frequently have the capacity to assimilate as sources of nitrogen a number of different simple N-containing compounds including ammonium, nitrate, nitrite and urea.

Baleria prionitis also known as **vajradanti** has gained tremendous importance in India. It is used for apthae, intermittent fever, paralysis, rheumatism, liver diseases, jaundice, dropsy, whooping cough, urinary troubles, bleeding gums, ear ache and cracking and laceration of the feet in the rainy season. A root decoction is taken as mouth wash in E. Africa, to relieve toothache. Tests for the plant's antimalarial activity have proved negative on avian malaria. The plant is found to be rich in potassium and this is said to contribute to its diuretic action. Important organic principles appear to be absent (Asolkar *et al*; 1988).

2. MATERIALS AND METHODS

The biofertilizer, ***Calothrix sp***. used for ***Barleria prionitis*** to enhance its vegetative growth parameters was made available from the Algal Biotechnology Laboratory, Department of Bio-Science, Barkatullah University.

Cloning and Purification

Standard microbiological techniques were employed for the selection, isolation and cloning of cyanobacteria in the pure culture. Cell mass almost broken into the individuals cells by vigorously shaking the sterilized glass beads. A drop of dilute suspension was streaked on agar plates and incubated in air conditioned culture room and maintained at a light intensity of 2500-3000 lux and temperature of 27±2 ºC and 14:00 hour light dark rhythm. Well separated single cell were marked externally by glass marking pencil. After 10-15 days of incubation those marked cells which produced small colonies were picked up with sterilized micropipette and transformed into culture tubes containing liquid medium. The culture was incubated in a culture room without shaking. After 3-4 days growth was visible in some tubes, these presumed clonal cultures were washed 3-4 times with sterile double distilled water then inoculated into fresh liquid medium lacking combined nitrogen.

For isolation of bacteria cultures the alga was homogenized with sterile glass beads and treated with 25 µg streptomycin for 10 minutes. After this the cells were washed 2-3 times and plated on agar plates and incubated in culture room. The presence or absence of bacterial contamination was checked both microscopically and by inoculating the following test media:

1. ***Caesinate glucose agar:***

Caesamimino acids	-	2.5 gm/100ml.
Glucose	-	10.0 gm/100ml.
Agar agar	-	2.5 gm/100ml.

2. ***Dextrose peptone broth:***

Peptone	-	1.0 mg/100ml.
Dextrose	-	1.0 mg/100ml.
Agar agar	-	2.5 gm/100ml.

Those colonies, which were found to be pure and axenic, were selected for experimental work. They were incubated on agar slants as well in the liquid medium. Cultures were regularly and periodically checked for bacterial contamination. When any contamination was found, these cultures were discarded and fresh axenic inoculums were taken from the original slants.

Culture Media.

Calothrix sp. grows well in **BG-11(N^-)** medium. The pH of the medium was adjusted to 7.5 after autoclaving. To avoid any change in the pH, the medium was buffered with Tris Hydroxymethylamine/HCl buffers.

Culture Vessels.

Glass wares of "Corning or Borosil" made were used. Cultures were maintained in culture tubes (15x1.5) cm each contained 30ml or 100ml medium respectively. Tubes and flasks were plugged with non-absorbent cotton plugs and were covered with aluminum foil for autoclaving.

Sterilization.

Culture media and culture vessels were sterilized by heat in an autoclave at 15 Ib/inch^2 pressure and temperature of 121ºC for 15 minutes. Some chemicals which could not be sterilized by heat, were filtered through Millipore filters with a pore size of 0.45 µm. Solution of inorganic nitrogen sources were autoclaved separately and added to the cold sterile medium.

Incubation and Maintenance of Culture.

Cultures were incubated in an air conditioned room and illuminated by 3-watt fluorescent tubes from a distance of 50cm for 12 hours daily. These

cultures were sub cultured at regular intervals and experiments were conducted on exponentially growing cultures.

Pot Experiments
Pot experiment was conducted on "***Barleria prionitis***" for 5 months commencing from October to February. Triplicate pots were used for different treatments. About 5kg of finely powdered dried washed and sterilized soil was taken in each pot.

Site of Experiment.
Department of Bio-Sciences.

Treatments.
The "***Barleria prionitis***" was treated with fresh ***Calothrix sp***. culture, 1% urea solution and control in four splits. First treatment, 10ml of ***Calothrix sp***. Culture, 1% urea solution was given when plants were in 6-7cm in height. The second treatment was given after 15 days with 20ml of ***Calothrix sp***. culture, 1% urea, 3rd treatment was given after 30 days with same amount of ***Calothrix sp***. culture and 1% urea solution was given and finally fourth treatment was given after 45 days with 20 ml of ***Calothrix sp.*** 1% urea solution. After this treatment vegetative parameters were analyzed and compared with the initial values of treatment.

Chlorophyll Estimation.
Chlorophyll was extracted by taking 0.5 gram leaves of ***Barleria prionitis*** to which 5 ml methanol or 80% acetone was added. It was kept overnight and the optical density of the chlorophyll was measured next day with a systronic 106 spectrophotometer at 663 nm. The amount of chlorophyll a extracted was calculated according to the equation of Mackinney (Mackinney, 1941).

Chlorophyll a μg/ml = O.D x 12.63 x D.F.

(Where O.D is optical density and D.F dilution factor).

The chlorophyll b was calculated according to the equation of Mackinney (Mackinney, 1941).

Chlorophyll b μg/ml = optical density x 19.3x D.F.

(Where O.D is optical density and D.F dilution factor)

2. RESULTS

Data presented in **(Table-1)** revealed the initial growth parameters of *Barleria prionitis* in the pot experiments, when the seedlings were planted from nursery pots, two such seedling of approximately 6.5-7cm of height were planted in each pot and for each treatment there were triplicate pots.

The observations on the growth performance of *Barlaria prionitis* in pot culture with respect to *Calothrix sp.* are depicted in (**Table-2 to7)** and are summarized as under:

Root length of Barlaria prionitis.
The comparative length of root of *Barleria prionitis* before and after different treatments revealed **(Table-2)** that maximum increase in root length is observed with *Calothrix sp.* (11.47cm) followed the urea treated pot (8.43cm). The increase in root length of *Barleria prionitis* with *Calothrix sp.* was nearly 3.3 fold increase over initial values, with urea there was 2.45 fold increase compared to initial values.

Shoot length of Barlaria prionitis.
(**Table-3)** shows the comparative length of shoot of *Barleria prionitis* before and after different treatments. Here again the best results were obtained with pots treated with *Calothrix sp*. (19.8 cm) followed by the urea treated pots (12.71 cm). The increase in shoot length was nearly 2.71 fold increase over initial values and was followed by pots treated with urea, which showed 1.75 fold increase over initial values of the treatment.

Number of leaves of Barlaria prionitis.
When the number of the leaves per plants was counted after the treatments compared with the initial values, maximum numbers of leaves were recorded in *Calothrix sp.* treated pots (26.66) followed by urea treated pots (19.33) (**Table-4**).The increase in the number of leaves was (3.71) fold with *Calothrix sp.* after receiving the different treatments compared to initial values followed by the Urea treated (2.76).

Weight of leaves of Barlaria prionitis.

Results depicted in **(Table-5)** revealed the fresh weight of the leaves after receiving the treatments. Maximum increase in weight of leaf was observed with *Calothrix sp.* (25.5 mg), which was followed the urea treated pots (19.51 mg) and finally that of control (17.36). Calothrix sp. gave (2.4) fold increase weight of leaf after receiving the different treatments compared to initial values. The next best result was obtained with Urea (1.8)

Chlorophyll a and b content of the Barleria prionitis.

(**Table-6**) shows the chlorophyll a content of the *Barleria prionitis* after treatments with cyanobacteria and urea. It was observed that maximum enhancement was observed with *Calothrix sp.* (19.56 µg/ml) followed by urea (16.91 µg/ml). 2.06 fold increase in chlorophyll a content was observed in plants treated with Calothrix sp. followed by (1.78) fold increase in Urea treated pots and (1.57) fold increase in control. Similarly when the chlorophyll b content of plant was measured after different treatments, maximum enhancement was observed with *Calothrix sp.* (19.36 µg/ml) followed by urea treated pots (16.69 µg/ml) and finally that of the control (14.14 µg/ml). *Calothrix sp.* showed (1.95) fold increase in chlorophyll b when compared to its initial values followed by Urea (1.6) fold increase in Urea treated plants.

4. DISCUSSION

From the results obtained it is evident that *Calothrix spp*. is an important bio-fertilizer for *Barleria prionitis*. This is because of its nitrogen fixing ability and its extra cellular secretion of it in medium. Release of nutrients through microbial decomposition after the death of the algae appears to be principal means by which nitrogen is made available to the crops. Besides increasing nitrogen fertility, blue green algae have been said to benefit the plants by producing growth promoting substances. These observations are in line with (Watanabe, 1984 and Fogg, 1949) who demonstrated that certain microorganisms liberate appreciable amount of fixed nitrogen into the medium as polypeptides or free amino acids and were suggested as promising components of biofertilizers because of nitrogen fixing ability. (Singh and Trehan, 1993; Maliga *et al.,*) are of opinion that besides nitrogen fixation they contain several extra cellular products like growth promoters, amino acids, vitamins useful enzymes and nutrients like carbohydrates. Cyanobacteria produce a variety of bioactive compounds including growth phyto-regulators that could be used *in-vitro* production of other plants (Melting and Pyne, 1986). The probable nature of the substances have been found to be similar that of gibberellins, auxin and in some cases like vitamins B-12, which improves the macromolecular composition of the plants (Banerjee *et al*; 1986.).

The developmental effect on vegetative characters such as stem height, root length, numbers of leaves, chlorophyll content are because of the increased level of protein synthesis. The enhancement in chlorophyll content is also attributed to the *Calothrix spp.* which makes available nitrogen, as it is important for the chlorophyll biosynthesis. The great increase in chlorophyll content is due to nitrogen contribution by the algae. The porphyrin structure, the formation of which is dependent on nitrogen is present in the structure of chlorophyll and cytochrome enzyme. With increase in chlorophyll content there is corresponding increase in the photosynthetic activity of the plant hence results in overall increase in growth.

Table-1: ***Initial growth parameters of Barleria prionitis before giving different treatments.***

S.No.	Parameters	Initial readings*
1.	Root length.	3.43 ± 0.096 (cm)
2.	Shoot length.	7.23 ± 0.233 (cm)
3.	Number of leaves.	7.00 ± 0.57
4.	Fresh weight of leaves.	10.6 ± 0.152 (mg)
5.	Chlorophyll a content.	9.46 ± 0.028 (µg/ml)
6.	Chlorophyll b content.	9.95 ± 0.020 (µg/ml)

(Mean of two readings± S.E)

Table-2: ***Root length of Barlaria prionitis before and after different treatments given to pots in which plants were grown***

Treatments	0 days	15 days	30 days	45 days
Control	3.43±0.096	4.43 ± 0.145	5.26±0.352	6.70 ± 0.057
Urea	3.43±0.096	5.51± 0.152	7.46 ± 0.176	8.43 ± 0.008
Calothrix sp.	3.43±0.096	7.56± 0.138	8.48 ± 0.098	11.47 ± 0.098

Mean root length in cm ± SE

Table - 3: ***Shoot length of Barlaria prionitis after different treatments compared with initial values***

Treatments	0 days	15 days	30 days	45 days
Control	7.23 ± 0.0233	8.78 ± 0.121	9.74 ±0.150	10.93 ± 0.137
Urea	7.23 ± 0.0233	9.88 ± 0.075	10.79 ± 0.175	12.71 ± 0.153
Calothrix sp.	7.23 ± 0.0233	11.55 ± 0.124	14.23 ± 0.153	19.80 ± 0.175

Mean shoot length in cm ± SE

Table - 4 : ***Number of leaves of Barlaria prionitis after different treatments compared with initial values***

Treatments	0 days	15 days	30 days	45 days
Control	7 ± 0.57	11.00 ± 0.578	14.33 ±0.882	17.33 ± 0.769
Urea	7 ± 0.57	13.50 ± 0.289	17.00 ± 0.578	19.33 ± 0.410
Calothrix sp.	7 ± 0.57	14.44 ± 0.233	18.83 ± 0.441	26.66 ± 0.882

Mean of five readings ± SE

Table - 5: ***Weight of leaves of Barlaria prionitis after different treatments compared with initial value***

Treatments	0 days	15 days	30 days	45 days
Control	10.60 ± 0.152	12.46 ± 0.260	15.73±0.120	17.36 ± 0.008
Urea	10.60 ± 0.152	14.06 ± 0.070	16.00 ± 0.091	19.51 ± 0.101
Calothrix sp.	10.60 ± 0.152	15.91 ± 0.037	18.92 ± 0.014	25.50 ± 0.456

Mean weight in mg ± SE

Table - 6: ***Chlorophyll a content of Barlaria prionitis after different treatments compared to initial values***

Treatments	0 days	15 days	30 days	45 days
Control	9.46 ± 0.082	11.46 ± 0.041	13.13±0.120	14.87 ± 0.046
Urea	9.46 ± 0.082	12.41 ± 0.192	14.92 ± 0.091	16.91 ± 0.046
Calothrix sp.	9.46 ± 0.082	13.97 ± 0.011	16.06 ± 0.014	19.56 ± 0.060

Table-7: ***Chlorophyll b content of Barlaria prionitis after different treatments compared to initial values***

Treatments	0 days	15 days	30 days	45 days
Control	9.95 ± 0.20	11.66 ± 0.010	12.94±0.023	14.10 ± 0.028
Urea	9.95 ± 0.20	12.46 ± 0.062	15.23 ± 0.120	16.69 ± 0.157
Calothrix sp.	9.95 ± 0.20	13.61 ± 0.072	17.10 ± 0.028	19.36 ± 0.570

Mean of five readings ± SE

5. CONCLUSION

The above mentioned growth parameters viz: root length, shoot length, number of leaves, weight of leaves, chlorophyll **a** and chlorophyll **b** content of *Barlaria prionitis* were found maximum when treated with *Calothrix sp.* –a promotive biofertilizer.

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