

## ORIGINAL ARTICLE

# Impact of endophytic bacterial inoculation on photosynthetic pigment of Sugarcane Plant

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### ABSTRACT

Sugarcane is an economically important crop of India which is cultivated in large area for production. But the main reason for the loss in productivity is the occurrence of various diseases in many cultivars of sugarcane. Currently, Plant growth promoting bacteria (PGPB) at present represents one of the useful and eco-friendly strategy to reduce the diseases to some extent. Endophytic bacteria are beneficial bacteria that survive within the tissue of plant and help in promotion of plants as well as fighting against various biotic and abiotic stresses. In the present study, sugarcane sett when incorporated with various endophytic bacterial treatments showed increased in the photosynthetic pigment when compared to control sett (without bacterial treatment). However, combination of two bacterial treatment showed higher photosynthetic pigment than the single bacterial inoculation.

**Keywords:** Endophytic bacteria, Plant growth-promoting bacteria, Photosynthetic pigment, Sugarcane.

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## INTRODUCTION

India is an agrarian based country which primarily depends on agriculture. At present, sustainable approaches is the need of hour in the field of agriculture. Sugarcane is an economically important crop of India which are grown in an area of 3.93 million hectares with a production of 167 million tonnes (Varma et al., 2019). It is one of the major crop on which livelihood of farmers depends. Productivity of Sugarcane is mainly hindered by various biotic stresses of which red rot caused by the fungus, *Colletotrichum falcatum* Went, is a serious threat to sugarcane production. When compared to chemical fertilizers and pesticides biological mediated agents played an important role in the enhancement of crop as well fighting against various diseases. Endophytic bacteria ameliorate the quality of soil and plants simultaneously like an organic way with high sustainability [1-7]. The aim of this study were to assess the impact of endophytic bacteria on photosynthetic pigment in sugarcane plant.

## MATERIAL AND METHODS

**Planting Material:** 3- budded set of sugarcane plant of cultivar Co1148 were used as planting material and used for sowing in the pot.

### Endophytic Bacteria used in the Experiment:

Four different endophytic Plant Growth Promoting Bacteria (PGPB) culture namely: *Bacillus aryabhattai*, *B. paramycoides*, *Pseudomonas aeruginosa* and combination of *B. paramycoides* + *B. aryabhattai* were used in the potting experiment. The bacterial cells were freshly grown in Luria Bertani broth and incubated in a shaking incubator for 24 h. Cells were harvested by centrifugation at the rate of 12,000 rpm for 3 min, then suspended in 100 ml of 0.85% saline to give a cell density of 10<sup>9</sup> cfu/ml. The prepared bacterial suspension was then applied by the method described by Viswanathan and Samiyappan [19], Muñoz-Rojas and Mellado [15], Hassan et al. [6, 7] with slight modification.

**Pot Experiment:**

Washed and sterile sets were planted in each pot in the sick soil (soil infected with fungal pathogen *C. falcatum*) as per the methodology of Viswanathan and Samiyappan [19], Hassan *et al.* [7]) and Hassan *et al.* [8]. Each treatment has three replications and the bacterial inoculum was applied twice in the soil, i.e. at 4 months and 5 months after sowing the sets into the soil near the root at the rate of 10ml per pot.

**Estimation of Chlorophyll a, chlorophyll b and total chlorophyll of the plants:**

Physiological parameters, such as chlorophyll a, chlorophyll b, total chlorophyll content in leaves were recorded using standard procedures [2].

One gram of leaf tissue was collected from each treatment and chlorophyll was extracted with 80% acetone followed by centrifugation at 5000 rpm for 5 min and the supernatant collected into a volumetric flask. The remaining residue was again grinded with 80 % acetone, centrifuged and supernatant was transferred into the volumetric flask and repeated until the residue became colourless. After, the volume was made upto 100 ml with 80% acetone and the absorbance of the solution was read at 645,663, and 652 nm against the solvent (80% acetone) blank., the calculation was done accordingly:

For Chlorophyll a:

$$\text{mg chlorophyll a/g tissue} = 12.7 (A_{663}) - 2.69 (A_{645}) * V/1000 * W$$

For Chlorophyll b:

$$\text{mg chlorophyll b/g tissue} = 22.9 (A_{645}) - 4.68 (A_{663}) * V/1000 * W$$

And for Total Chlorophyll:

$$\text{mg total chlorophyll/g tissue} = 20.2(A_{645}) + 8.02 (A_{663}) * V/1000 * W$$

Where, A= Absorbance at specific wavelengths

V= Final volume of Chlorophyll Extract in 80% acetone.

W=Fresh weight of the tissues extracted.

**Statistical analysis:**

The data were analyzed by ANOVA using Statistical Product and Service Solution (SPSS) version 20.0 software Developed by SPSS Inc., now IBM SPSS. All results were expressed at  $P < 0.05$  to compare the means among the treatment means.

**RESULT****Chlorophyll content:**

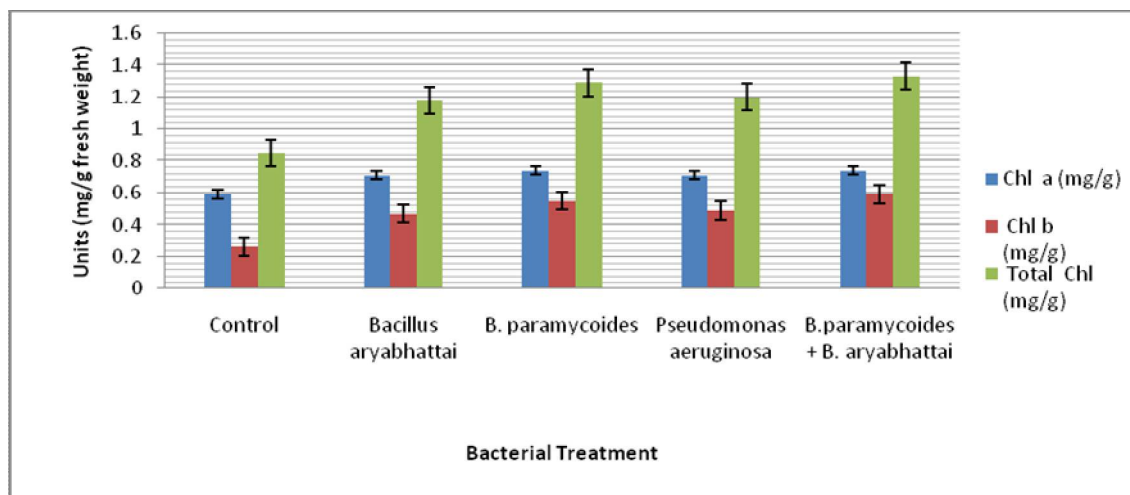
The endophytic bacteria had profound effect on the photosynthetic pigment contents. The results are depicted in Table. 1 and Fig. 1 for chlorophyll a, b, and total chlorophyll.

The effect of bacterial inoculation on the photosynthetic pigments was significantly higher in all the treatments when compared with the control (without bacterial inoculation).The chlorophyll 'a' pigment was significantly higher in all the treatment with equivalent value in *B. paramycoides* and *B. paramycoides* + *B. aryabhattai* treatment (0.74 mg/g) and equivalent value was also obtained for other treatment of *B. aryabhattai* and *Pseudomonas aeruginosa* with 0.71 mg/g fresh weight. Similarly, Chlorophyll 'b' pigment was found to be highly significant in *B. paramycoides* + *B. aryabhattai* (0.59 mg/g fresh weight) followed by *B. paramycoides* (0.55 mg/g fresh weight). The total chlorophyll pigment was found to be highest in *B. paramycoides* + *B. aryabhattai* (1.33 mg/g fresh weight) followed by *B. paramycoides* (1.29 mg/g fresh weight). In all the cases, higher pigment is present in the combination bacterial treatment.

**Table 1.** Effect of inoculation with bacterial endophytes on photosynthetic pigments of sugarcane.

Treatments			
	Chl a (mg/g)	Chl b (mg/g)	Total Chl (mg/g)
Control	0.59 a	0.26a	0.85a
<i>Bacillus aryabhattai</i>	0.71b	0.47 b	1.18b
<i>B. paramycoides</i>	0.74 b	0.55 c	1.29c
<i>Pseudomonas aeruginosa</i>	0.71b	0.49 b	1.20b
<i>B.paramycoides</i> + <i>B. aryabhattai</i>	0.74b	0.59c	1.33c

Data in a column designated by the same letter(s) are not significantly different according to DMRT rule.



**Fig 1** Chlorophyll content in leaves of sugarcane plant treated with different bacterial treatment in. Control represent treatment without any endophytic bacterial inoculation. All values are the mean of three replicates. Vertical Bars represent the standard error of the means.

## DISCUSSION

Chlorophyll is a significant part of plant pigment that help in the photosynthesis; without which plant fails to perform photosynthesis. Chlorophyll presume to be a vital part in the ATP generation and an elemental plant constituents [13]. Chlorophyll analysis is one of the important physiological parameters which is used as an indicator of plant protection capacity. Chlorophyll 'a', 'b', and total chlorophyll content are signal of photosynthetic and metabolic activity [5, 21]. In the present study, the efficiency of cane plant got improved with endophytic bacterial treatments; thus, enhanced the performance of photosynthetic apparatus of the plant. Present study investigated that all the photosynthetic apparatus i.e. Chlorophyll a, chlorophyll b and total chlorophyll contents were found significantly higher in all the cane plants inoculated with bacterial treatments compared with the control at all the time intervals studied. However, higher level of pigment were found in the combination of two bacterial culture. It is also suggested by Lenin and Jayanthi [14] that the consortium treatment of *Azotobacter*, *Azospirillum*, *Pseudomonas* and *Bacillus* enhanced the chlorophyll content of plant *Catharanthus roseus*. In a study, it is suggested that the combined application of *Bacillus lentus*, *Pseudomonas* sp., and *Azospirillum brasilense* enhanced the chlorophyll content of *Ocimum basilicum* [9]. Higher chlorophyll content were also reported in sugarcane plant treated with bacteria as reported by Muthukumarasamy *et al.* [16], Sevilla [17], and Chauhan *et al.* [4]. The growth responsible trait namely, chlorophyll content, and the total biomass were increased due to Plant Growth Promoting Rhizobacteria (PGPR) inoculation [11]. Bashan *et al.* [3] also suggested that the increased chlorophyll content in plant leaves as a result of bacterial isolate co-inoculation could be due to the increased accumulation of plant nutrition and photosynthesis.

Kang *et al.*, [10] also reported that chlorophyll contents got improved in the PGPR-treated plants under salinity and drought stress in *Cucumis sativus*. The PGPR (*Azospirillum*, *Azotobacter* and *Pseudomonas*) application increased Chlorophyll 'a', Chlorophyll 'b' and total chlorophyll [1]. The current study is also supported by the study of Karlidag *et al.* [12] where PGPR inoculations significantly increased the chlorophyll content of strawberry plants.

## CONCLUSION

In the present study, significant variations were noticed in various parameters under endophytic bacterial treatments in sugarcane. The chlorophyll pigment were enhanced significantly. This can be further explored in a sustainable way for using it as a prospective tool to increase the crop yield.

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