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Effect of Biofertilizers on Chlorophyll contents of Maize (*Zea mays* L.) Variety Eco-92

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Abstract: **An attempt has been made to study the effect of different biofertilizers such as Azotobacter and *Phosphate solubilizing bacteria, (PSB)* on chlorophyll content on maize (*Zea mays* L.) variety Eco-92. The experiments were carried out in a randomized complete block design with three replications. The biofertilizers used were Azotobacter (A), phosphate solubilizing bacteria (P) and combine treatment Azotobacter + phosphate solubilizing bacteria (A +P), without treatment was control. The comparative extraction of chlorophylls (Chlorophyll a, chlorophyll b and total chlorophyll) And carotenoids from Eco-92 by 80% acetone as extraction method (Arnon, 1949) was studied. The study relates to the amount of concentration of chlorophyll and carotenoids between the control and treated of maize crop. Investigation revealed that method of Arnon (1949) [1], is simpler method for extracting the pigment molecules along with other methods used for extraction and results showed higher content of chlorophyll-a, Chlorophyll-b, total chlorophyll and Carotenoids in the treated plants in comparison with the control plants. By the application of biofertilizers treatment levels were corresponding to (TA_1), (TP_1),($TA+P_1$) respectively to the treated fodders, little amount of differences were observed in the concentrations of pigments between treated and control plants selected for present study.**

Keywords: **Chlorophyll, carotenoids Azotobacter, *PSB*, Eco -92 etc.**

I. INTRODUCTION

Maize is an important staple food crop, occupies a prominent place among cereals and first rank in terms of productivity and third in total area and production after wheat and rice, while in India it strands fourth ranks next to rice, wheat and Jowar in terms of area and production. Total pigment molecules present in the leaf, are chlorophyll-a, chlorophyll-b and total chlorophyll, carotenoids which are essential for photosynthesis[10],[11]reported that the chlorophyll coloration is related to the amount of nutrients absorbed by the plant from soil, This crucial Pigment also plays role as an index of plant growth and production of organic matter. Biofertilizers contain micro-organism that increases or promotes the important nutrients crucial for overall production the soil [9]. Biofertilizers applied to the soil supply of plant nutrients for crop growth and serve as important instruments in yield development and physiological processes. Moreover, they play important roles in photosynthesis capturing light energy which is converted into chemical energy [3], [15]. Most plants possess chlorophyll a and chlorophyll b which are the main photosynthetic pigments. Chlorophylls and carotenoids are essential pigments of higher plant assimilatory tissues and responsible for variations of color from dark-green to yellow. Carotenoids provide bright coloration, serve as antioxidants, and can be a source for vitamin A activity [4]. N is a key element in chlorophyll, therefore is usually a high correlation between them [13]. Positive correlation of nitrogen and chlorophyll is previously reported by some researchers [7]. The distribution of chlorophyll is the key indicator of crop

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photosynthesis within maize leaves is quite homogenous at a specific growth stage indicator. Chlorophyll content of leaf tissue is a good index of photosynthetic activity [6] and timing of fertilizer application [8], [14].of crop. Chlorophyll content is an indicator for crop growth and development, therefore accurately determining and assessing of chlorophyll concentration is essential [2]. The quantification of chlorophyll and carotenoids provides important information about the effects of environments on plant growth. Chlorophyll concentration usually is a good indicator of plant nutrient stress, photosynthesis and growing periods, the content of chlorophyll in the plant leaves indicates the growth status of the crops, also it is the important condition for exchange of mass and energy from the outside world and therefore real-time monitoring of the content of chlorophyll is a key step to complete crop monitoring and yield estimation [5], [12].

II. MATERIALS AND METHODS

The chlorophyll and carotenoids contents were quantitatively estimated by Arnon's (1949) [1] method. The results thus obtained were compared with the control.

Sample Collection: The biofertilizers used were Azotobacter (A), phosphate solubilizing bacteria (P) and combine treatment Azotobacter + phosphate solubilizing bacteria (A+P), without treatment was control. For the experimentation viz. to find out the chlorophyll and carotenoids contents in the maize crop treated with biofertilizers (TA_1), (TP_1) and ($TA+P_1$), the leaf samples were collected from the field in fresh and clean polythene bags from the plot in the morning, while bringing the leaf samples to the laboratory, Precautions were taken so as to avoid the mechanical or other damage. All the samples were washed under tap water to remove dust particles and other unwanted particles from the surface of leaves and were then analyzed for the determination of Chlorophyll-a, Chlorophyll-b, total Chlorophyll and Carotenoids.

Extraction of chlorophyll (Arnon, 1949):

The Quantitative estimation of chlorophyll-a, chlorophyll-b and total chlorophyll was carried out by the method of Arnon (1949), while carotenoids were determined by following method. 1g fresh leaf material was taken and homogenized with 80% acetone and centrifuged at 5000 rpm for 5 min. Supernatant was adjusted to 100 ml in the volumetric flask. The absorbance (O.D.) of this extracted solution was measured at 480, 510, 645 and 663λ. From these readings concentrations of chlorophylls and carotenoids pigment were determined by using following formula/equation:

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Table 1: Chlorophylls and carotenoids pigment were determined by using following formula/equation:

Solvent	Formula /Equation
80% Acetone	Chlorophyll -a mg/g tissue $= \frac{12.7\,(AR663R) - 2.69\,(AR645R) \times V}{1000} \times W$
	Chlorophyll -b mg/g tissue $= \frac{22.9\,(AR645R) - 4.68\,(AR663R) \times V}{1000} \times W$
	Total chlorophyll mg/g tissue $= \frac{20.2\,(AR645R) + 8.02\,(AR663R) \times V}{1000} \times W$
	Carotenoid mg/g tissue $= \frac{7.6\,(AR480R) - 1.49\,(AR510R) \times V}{1000} \times W$

Where, A = Absorbance at specific wavelengths

V = Final volume of chlorophyll extract in 80% acetone

W = Fresh weight of tissue extracted.

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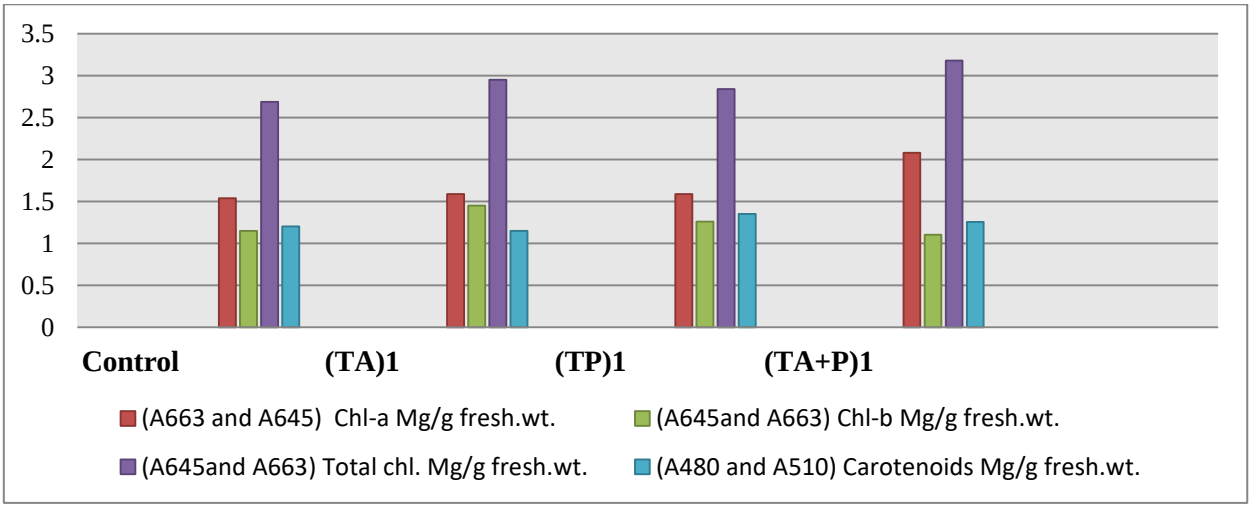
Table 2: The Spectrophotometric determination of absorbance for Chlorophylls and Carotenoids

Eco.92	(A_{663} and A_{645}) Chl-a Mg/g fresh.wt.	($A_{645}$and A_{663}) Chl-b Mg/g fresh.wt.	($A_{645}$and A_{663}) Total chl. Mg/g fresh.wt.	(A_{480} and A_{510}) Carotenoids Mg/g fresh.wt.
Control	1.536	1.149	2.685	1.202
$(TA)_1$ ***Azotobacter***	1.585	1.449	2.950	1.149
$(TP)_1$ ***Phosphate solubilizing bacteria***	1.585	1.256	2.840	1.349
$(TA+P)_1$ ***Azotobacter + Phosphate solubilizing bacteria***	2.079	1.100	3.178	1.255

A=Absorbance, Ch-a=Chlorophyll-a, Ch-b=Chlorophyll-b, Total chl. =Total Chlorophyll.

III. RESULT AND DISCUSSION

Leaf pigment content provides valuable information about the physiological status of crops. The content of foliar pigment varies depending on leaf pigments (chlorophyll and carotenoids) and its relation due to the internal factors and environmental conditions. The chlorophyll and carotenoids contents were quantitatively estimated by Arnon‟s (1949) [1] method. The results thus obtained were compared with the control. In this study control and treated plant leaves were used to estimate the chlorophyll content. A total 10 healthy plants of each variety (Eco-92 and African tall) were selected for this study. The extractions of Chlorophyll and carotenoids pigments molecules by 805 acetone method from the treated and control maize variety Eco-92 were measured by spectrophotometer. Chlorophyll estimation was done in the fresh green leaf samples extracted with the acetone solvent the absorbance Reading of chlorophyll extracts were measured in two different wavelengths 645nm and 663 respectively. Based on the absorbance value calculations were made using Arnon's (1949) equation and the amount of chlorophyll a. chlorophyll b, total chlorophyll and carotenoids were estimate and tabulated. (Table: 2) For cultivars (Eco-92) concentration of total chlorophyll (chlorophyll a+b), carotenoids and chlorophyll a/b were ratio significantly different as compared to control. Result showed that, the effect of biofertilizer *Azotobacter* (A), *phosphate solubilizing bacteria* (P) and interaction between them *Azotobacter + phosphate solubilizing bacteria* (A+P) on chlorophyll-a, Chlorophyll-b, total chlorophyll and Carotenoid content of variety Eco-92 in combine treatment of biofertilizer ($TA+P_1$) ,were highest in 2.079, 1.100, 3.178, 1.255 mg/g fresh wt respectively as compared to the chlorophyll-a, Chlorophyll-b, total chlorophyll and Carotenoid content of control plant 1.536,1.149,2.685,1.202 mg/g fresh wt. respectively.



Graph no.1: Effect of biofertilizer on chlorophyll and carotenoids content (mg/g fresh wt.) in Maize variety Eco-92.

IV. CONCLUSION

It is concluded that, the treatment of biofertilizer chlorophyll-a, Chlorophyll-b, total chlorophyll and Carotenoid content increase the more effectively than the control. The use of biofertilizer influenced the Maize variety Eco-92 positively. The application of biofertilizers as a source in agricultural production, and its proper use is an environmental friendly way of strengthening plant growth and improvement for farmers.

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