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Bio-fertilizer to Enhance the Chlorophyll Content

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ABSTRACT

Air pollution is the contamination of the indoor or outdoor environment by any chemical, physical or biological agent that modifies the natural characteristics of the atmosphere. The exhaust of industries, automobiles, household combustion and various devices cause air pollution. Oxides of nitrogen, sulphur, particulate emissions are a few of the examples as pollutants from industries. These air pollutants drastically affect photosynthetic pigments and reduce chlorophyll content of the plants growing in polluted sites. The reduction in chlorophyll is due to conversion of chlorophyll into pheophytin by loss of magnesium ions. In the present study, formulation of bio-fertilizer has been made using nitrogen fixing bacteria and phosphate solubilising bacteria and its effect has been studied under various parameters. Bio-fertilizers overcome the effects of air pollution on plants. It can enhance the chlorophyll content of Fenugreek and spinach. Effectivebio-fertilizer has great potential to replace chemical fertilizers.

Key words : *Air pollution, Chlorophyll content, Biofertilizer, Sustainable development*

Introduction

Chlorophyll is a green pigment consisting of a tetrapyrrole ring with a central magnesium ion. It has a long hydrophobic phytol chain in its structure. It is found in some varieties in plants and algae (Aminot, 2000). Two types of chlorophyll, chlorophyll-a and chlorophyll-b are present in green algae and terrestrial plants. The difference between these two chlorophyll is a methyl moiety in chlorophyll-a replaced by a formyl group in chlorophyll-b. The ratio of chlorophyll-a to chlorophyll-b in higher plants is approximately 3:1. Chlorophyll absorbs light mainly in the red (650 – 700 nm) and the blue - violet (400 – 500 nm) regions of the visible spectrum. Green light (~550 nm) is not absorbed but reflected giving chlorophyll its characteristic color. Chlorophyll a possesses a green-blue color, and chlorophyll b possesses a green-yellow color (Arnon, 1849).

Industrialization, economic growth and associated increase in energy demands for mankind have deteriorated the air quality in developing countries. Oxides of nitrogen and sulphur and fly-ash constitute the major proportions for the gaseous and particulate emissions from industries and automobiles. The exposure of these pollutants to the leaves causes a reduction in the concentration of their photosynthetic pigments such as chlorophyll, carotenoids (Giri *et al.*). Air pollution has long been known to have an adverse effect on plants. chlorophyll and carotenoids, which affects the plant productivity, germination of seeds, length of pedicles, and number of flowers inflorescence. Air pollution has long been known to have an adverse effect on plants. At first , it was only Sulphur dioxide that was considered a dangerous pollutant. Now, with the use of various pesticides and chemical fertilizers, the range of harmful pollutants has multiplied tremendously.

Nitrogen and magnesium alongwith iron are two of the important elements used during chlorophyll

([1]Assistant Professor, [2] Assistant Professor & Head, [3]Student) synthesis. This can be provided to plants with the help of Nitrogen Fixing Bacteria(NFB) and Phosphate Solubilizing Bacteria (PSB). Growth promoting substances are produced in huge quantities by the action of these rhizosphere microorganisms that directly or indirectly influence the overall morphology and physiology of the crops. Recent advances in the field of sustainable development relies on the use and diversity of PGPR (Plant Growth Promoting Rhizobacteria), their colonizing capability and the mechanism of action that may be used to facilitate their application as a dependable element in the management of sustainable agricultural system (Bhattacharyya and Jha, 2012 and Di Benedetto *et al.,* 2017).

The aim of this study is to formulate a biofertilizer using the rhizobacteria isolated from different sites of Palghar district that may help in the enhancement of the chlorophyll content of plants that are growing in polluted sites and whose chlorophyll synthesis is affected due to polluted soil.

Materials and Methods

3.1 The chlorophyll and carotenoids contents from the heavily air polluted area were quantitatively estimated by Arnon's (1849) method. The results thus obtained were compared with the control.

3.2 Formulation of biofertilizer by using nitrogen fixing bacteria and phosphate solubilizing bacteria (PSB).

3.3 Plants grown under formulated biofertilizer and further chlorophyll content were estimated and studied

Sample Collection: The leaf samples were collected 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 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.

Chlorophyll Extraction (Arnon's, 1949)

Take 1 gram of finely cut fresh leaves with 10-20 ml of 80% acetone. It was then centrifuged at 5000-10000 rpm for 5min. The supernatant was transferred and the procedure was repeated till the residue became colorless. The absorbance of the solution was red at 645 nm and 663 nm of visible spectrophotometer against the solvent (acetone) blank.

Chlorophyll Estimation

Total chlorophyll= 20.2(A645) + 8.02(A663)
Chlorophyll-a = 12.7(A663) - 2.69(A645)
Chlorophyll-b = 22.9(A645) - 4.68(A663)

Formulation

The preserved mother culture activated for the next step of formulation, i.e log phase.

The Rhizobacterial strain and Azotobacter strain and Phosphate Solubilizing Bacteria were multiplied in desired medium by inoculating and incubating at 28 ± 2°C in a shaking incubator (180 rpm) for 24 h for them to attain log phase growth with a cell load of about 1.8 × 10^9 CFU mL"1. The culture volume can be increased according to requirements by using large scale fermentation.

Plants growth using bio-fertilizer

Take 400 kg of soil sample and add 4-8 ml of formulation and distribute it in 2 separate pots. Sow the Fenugreek and spinach seeds. Monitor on a daily basis in terms of seed germination, stem length, no. of leaf, overall growth etc. Repeat the same procedure for control for both the seeds without formulation. After the plant's growth, extract and estimate the chlorophyll contents(follow protocol 3.1.1 - 3.1.1.a) to check chlorophyll contents. Compare it with control.

Results

Table 1. The chlorophyll contents (Fenugreek and spinach) from polluted site and non polluted site - Before treatment with formulation

Sites	Chlorophyll contents		
	Chl-a (μg/ml) chlorophyll (μg/ml)	Chl-b (μg/ml)	Total
S_1	8.120	12.822	20.938
S_2	8.156	14.424	22.187
S_3	8.313	14.944	23.279
S_4	8.023	12.854	20.860

S1 sample from polluted site (spinach)
S2 sample from non-polluted site (spinach)
S3 sample from non-polluted site (Fenugreek)
S4 sample from polluted site (Fenugreek)

Table 2. The chlorophyll content(Fenugreek and spinach) after using formulated bio-fertilizer.

Sites	Chlorophyll contents		
	Chl-a (μg/ml)	Chl-b (μg/ml)	Total chlorophyll (μg/ml)
Spinach			
Rhizobium spp.	7.797	12.025	19.815
Azotobacter spp.	7.963	12.577	20.512
PSB	7.826	11.269	19.018
Symbiotic	8.080	13.322	21.395
Control	7.627	10.992	18.612
Fenugreek			
Rhizobium spp.	8.772	16.967	25.569
Azotobacter spp.	8.396	16.032	24.418
PSB	8.022	12.926	20.919
Symbiotic	8.420	15.826	24.236
Control	7.533	13.656	21.181

Discussion

The samples for polluted sites collected from Pam, Boisar, Palghar (District) Maharashtra and samples for non polluted sites collected from Parnali, Boisar, Palghar (District) Maharashtra. The Chlorophyll content was estimated from Fenugreek and spinach for samples from both sites. The total chlorophyll content in spinach for polluted site was found to be less as compared to spinach from non polluted site with <2μg/ml per gm of sample. The total chlorophyll content in Fenugreek for polluted site was found to be less as compared to Fenugreek from non polluted sites with < 3 μg/ml per gm of sample. (Table 1).

The Fenugreek and spinach grown in normal soil and sterile soil treated with *Rhizobium spp*. formulation, *Azotobacter spp*. formulation, PSB formulation and combinations of all (for symbiotic activity), controls were also kept without any formulation. Growth of Fenugreek and spinach observed in terms of length of stem was higher. The stems length of Fenugreek and spinach for combination's observed more showing symbiotic activity. The total chlorophyll content in spinach as well as fenugreek for combinations were found to be more as compared to control in both - normal and sterile soil (Table 2).

Conclusion

The decrease in the total chlorophyll content in spinach and fenugreek plants in natural conditions reveal the air pollution effects on plants. For the reduction in chlorophyll content of spinach and fenugreek, air pollution may be one of the reasons. There may be some other reasons for chlorophyll reduction that need to be investigated. If chlorophyll content reduction is because of air pollution that may cause harmful effects on plants that directly affects biodiversity.

In the present paper, the chlorophyll content was estimated in spinach and fenugreek, which was grown in soil treated with four different bio-fertilizer formulations in normal soil and sterile soil separately. The growth of spinach and fenugreek in terms of stem length and chlorophyll content was observed to be higher when used in combination. This indicates positive activity of *Rhizobium spp.*, *Azotobacter spp.*, *PSB* towards complex nutrients breakdown and increased bio-availability for plants.

The chlorophyll content estimated by Arnon's (1949) method in spinach and fenugreek grown in soil treated with formulation. The chlorophyll content was found to be comparatively higher when *Rhizobium spp*. formulation, *Azotobacter spp*. formulation, PSB formulation used in combination. The enhancement in chlorophyll content is because of symbiotic activity of bacteria used in bio-fertilizer formulation.

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