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ANABAENA AZOLLAE CULTURE WATER, A SOURCE FOR BIOREMEDIATION

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ABSTRACT:
The *Azolla-Anabaena* symbiosis is outstanding due to its high productivity combined with its ability to fix nitrogen at high rates. *Anabaena azollae* a blue green alga, isolated from a pteridophyte, *Azolla pinnata* by growing in stress conditions. *Anabaena* culture was grown in Benecks media and further transferred in sterile soil and tap water medium (w/v). Water from the cultures of *Anabaena* contains nitrogen and can promote the growth of test crop. Taken this into consideration, the bioassays were performed on growth of wheat by evaluating different growth parameters, like germination percentage, seedling height, weight and chlorophyll content. The effect of *Anabaena* culture on soil texture was observed by analyzing the physico-chemical properties of treated soil against the control. In the experiment of presoaking treatment of wheat seeds, 12 hrs presoaking was found most promoting which showed 100% germination. Wheat seedlings growth enhancement was observed in treated wheat seedling with *Anabaena* culture water and a significant improvement in nitrogen, potassium, phosphorus content was observed with increased pH and reduced EC. The *Anabaena* culture water was analyzed in comparison with normal tap water as control, to evaluate the difference in the nutrient quality available for treated plants.

Key words: - Anabaena azollae, Azolla pinnata, Bioremediation, Blue green alga

INTRODUCTION:

Cyanobacteria plays an important role to build-up soil fertility consequently increasing the yield of crops. Bio fertilizers being essential components of organic farming plays a vital role in maintaining long term soil fertility and sustainability, by fixing atmospheric nitrogen, mobilizing fixed macro and micro nutrients or converting insoluble phosphorus forms available to plants. Cyanobacteria are one of the major components of the nitrogen fixing biomass in paddy fields. The agricultural importance of cyanobacteria in rice cultivation is directly related with their ability to fix nitrogen and other positive effects on plants and soil. Bio-fertilizers are eco-friendly and have been proved to be effective and economical alternate of chemical fertilizers.

The symbiotic association of the eukaryotic water fern *Azolla* with its prokaryotic cyanobacterial (blue-green algal) endosymbiont, *Anabaena azollae* has received considerable attention because of its ability to fix Nitrogen, allowing it to serve as a nitrogen source in agriculture. The usefulness of this association as a nitrogen fertilizer has been widely documented (Talley et al., 1977; Watanabe *et al.*, 1977).

In the *Azolla-Anabaena* symbiosis, specific functions are performed by each of the partners. It is believed that the fern provides the endosymbiont, the carbon source, probably sucrose (Ray *et al.*1979). *Anabaena,* the endosymbiont contributes by fixing atmospheric N_2 to its own requirement of nitrogen and that of its host (Peters and

Mayne, 1974a, 1974b; Ray *et al.*, 1978; Peters et al., 1980a, 1980b).

In the *Azolla-Anabaena* association the endosymbiont alga is present within specialized leaf cavities of *Azolla*, throughout formation and maturation of the leaf cavities the endosymbiont is associated with specialized multicellular hairs, produced by the fern (Moore, 1969; Calvert and Peters, 1981). These hairs have elaborate cell wall ingrowths and numerous organelles which are characteristic of transfer cells (Duckett *et al.*, 1975).

OBJECTIVE :

The *Azolla-Anabaena azollae* association fixes atmospheric nitrogen and offers a great promise as a biofertilizerr and feed. Taken this into consideration the experiments were carried out.

MATERIAL & METHODS:

To study the potential of *Anabaena azollae* as a biofertilizers, following experiments were performed, by following all the standard microbiological techniques and the experiments were carried in replicates.

1. Collection of plant material

Anabaena azollae a blue green alga is a symbiont of pteridophytic fern *Azolla pinnata*, which is mostly, found growing in ponds and lakes, and can be easily harvested. For the present study the plant material was collected from the botanical garden of Hislop college, Nagpur. The plants were identified in the PG Dept. Of Botany, Hislop College, Nagpur.

2. Isolation of *Anabaena azollae* from *Azolla pinnata*

Anabaena azollae was isolated from a pteridophyte, *Azolla pinnata* by growing the fern in distilled water. After 1 week due to nutrient deficiency the alga gets released from its host. *Anabaena* culture was then grown in Benecks media and then subcultured and incubated for growth under continuous illumination (2000 lux) and a temperature of 25°C± 2°C. After 20 days of incubation, the developed cyanobacterial cells were cultured on Petri dishes) with solid Beneck's medium. Plates were incubated in an illuminated growth chamber for colony development. The developed cyanobacterial colony was taken with a sterilized inoculation needle and subcultured to new conical flasks containing liquid Beneck's medium to get unialgal culture of *Anabaena azollae.* After one month profuse growth was observed. This unialgal culture was used for the further experiment.

2.1 Measurement of cell by micrometry

The isolated *Anabaena* culture was examined under the light microscope (100 x) to study morphology characters like shape and color, presence or absence of heterocystes, site of heterocyst in filaments as well as shape, width and length of vegetative cells, and heterocystes was measured by micrometry. (The average size of the cells was measured by taking average size of five cells).

3. Effects of *Anabaena azollae* on growth of Test crop

The effect of algal culture water on wheat crop was evaluated by different growth parameters like, seed germination, seedling root shoot ratio, fresh and dry weight and chlorophyll estimation. Seed germination was checked by total no. of seeds germinated against total no of seeds sown. 100 seeds were surface sterilized by $HgCl_2$ then washed in autoclaved double distilled water in sterile condition. Then the seeds were transferred in petriplates containing Whatmann filter paper moistened with pure *Anabaena azollae* culture water, and kept for germination for two days. After the

emergence of radical germination percent was calculated by the given formula:

Germination Percent $=\frac{No.of\ seeds\ germinated}{Total\ no.of\ seeds\ sown} X100$

3.2.1 Effect on growth of wheat seedlings

The height of radicale and plumule, fresh weight and dry weight was measured from each test for1st week , 2nd and then 3rd week consecutively from the day of plantation.

3.2.2Chlorophyll estimation of test crop

Anabaena treated wheat plantets were grown in lab conditions. After 1 week, treated and control plants were uprooted for chlorophyll estimation.

Fresh samples were washed thoroughly first in tap water followed by distilled water in the laboratory, and analyzed for the estimation of chlorophylls (Ch-a and Ch-b) content. 1.5 gm. of fresh plant sample was taken, and homogenized in mortar and pestle with 10 ml of 80% acetone solvent. Homogenized sample mixture was centrifuge for 3000 rpm for 10 min in cooling centrifuge. The supernatant were separated and 0.5ml of it is mixed with 4.5ml of the 80% acetone solvent. The solution mixture was analyzed for Chlorophyll-a, Chlorophyll-b content in spectrophotometer. Now the quantification of Chlorophyll-a, Chlorophyll-b, is made by equations to determine concentrations (µg/ml) of chlorophyll a (Chl-a), chlorophyll b (Chl-b) by acetone extractant solvents in spectrophotometer.

4. *Anabaena azollae* and its effects on soil structure

The soil in which the *Anabaena azollae* treated wheat seeds were grown, was analysed in order to study the changes in soil structure and its physicochemical properties. 250 gram soil was air dried for analysis of physico-chemical properties, untreated soil was kept as control. The pH of the soil was determined using pH meter with glass column combination electrode in distilled water and 0.01M $CaCl_2$ solution at a ratio of 1:2 soil solutions. The organic matter was determined using Walkley and Black method (Jackson, 1967). Total nitrogen was determined by Kjeldahl method (Jackson 1973). Exchangeable K, Ca and Mg were extracted using ammonium acetate, K was determined on flame photometer and Ca and Mg by EDTA titration.

RESULT & DISCUSSION:

Anabaena azollae isolated from *Azolla pinnata* is characterized by filamentous brown to olive color in culture (plates 4 and 5) the shape of its vegetative cells is cylindrical and their diameters ranged from 8 to 9 µm width and from 5 to 6 µm length. Heterocysts are present and their sites are found to be internal and terminal in the filament, shape is spherical with diameters of 9 to 9.5 µm width and 9 to 9.5 µm length. Akintes are present and their shapes are cylindrical, their diameters ranged from 9 to 10 µm width and 6 to 8 µm length. Taxonomically, the *Azolla* cyanobiont is placed in phylum- Cyanophyta, Order-Nostocales, Family- Nostocaceae. It was first described as Nostoc and later renamed *Anabaena azollae* .

This treatment seems to supply the nitrogen demands of wheat plants. In, general terms, such behavior of wheat height was caused by the influence of nitrogen content in the medium, which could be supplied through different ways and means, as reported earlier (Singh 1989; Baker 1999; Bhuvaneshwari 2012; Bhuvaneshwari and Kumar 2013). Treatments combining both ways of using Anabaena *azollae* tended to produce more biomass than the remaining treatments with the same nitrogen dose.

Therefore, combining incorporation and association of this cynobacteria not only serve to provide plants with significant amount of nitrogen, but also enables a better use of nitrogen added by mineral fertilization and thereby, a higher dry mass production. Similar results were recorded when *A. pinnata with symbiotic association with Anabaena azollae* was used by Manna and Singh (1990); Baker (2000); Bhuvaneshwari and Singh (2012).

This increased concentration of various nutrients in soil might be reason for enhanced growth of seedling treated with anabaena treated water (Samarajeewa.1999) also attained similar results.

CONCLUSION:

The use of *Anabaena azollae* as a green manure benefits wheat crop, It also enhances the structure of the soil.

REFERENCES:

Bhuvaneshwari K. Beneficial effects of bluegreen algae and *Azolla*in rice culture. Environ Conserv J. 2012;13(1 & 2):1–5.

Bhuvaneshwari K, Kumar Ajay. Agronomic potential of the association *AzollaAnabaena*.Sci Res

Report. 2013;3(1):78–82.

Bhuvaneshwari K, Singh PK. Organic rice production using organic manures and bio inoculants in alkaline soil. J Recent Adv Agric. 2012;1(4):128–134.

Calvert,H.E. and Peters,G.A. (1981) New Phytol., 89, 327-335.

Moore,A.W. (1969) Bot. Rev., 35, 17-34.

Peters,G.A., Ray,T.B., Mayne,B.C. and Toia,R.E.,Jr. (1980a) in Newton,W.E. and Orme-Johnson,W.H. (eds.), Nitrogen Fixation, Vol. 2, University Park Press, Baltimore, pp. 293-309.

Peters,G.A., Toia,R.E,Jr., Evans,W.R., Crist,D.K., Mayne,B.C. and Poole,R.E.(1980b) Plant, Cell Env., 3, 261-269.

Ray,T.B., Peters,G.A., Mayne,B.C. and Toia,R.E.,Jr. (1978) Plant Physiol.,62, 463467.

Talley,S.N., Talley,B.J. and Rains,D.W. (1977) in Hollaender,A. (ed.), Genetic

Engineering for Nitrogen Fixation, Plenum PubI.Corp., New York, pp.

259-281.

Watanabe, N, Ota, Y, Minoda, Y, Yamada, K. 1977. Isolation and iden-tification of alkaline lipase producing microorganisms, cultural conditions and some properties of crude enzymes. *Agricultural and Biological Chemistry* 41:1353–1358

Jackson, M.L., 1967. Soil Chemical Analysis. *Prentice-Hall of India Pvt. Ltd.*, New Delhi, 498p

Jackson, M. L. Soil chemical Analysis.Prentice Hall of Englewood cliffs, New Jersey, USA, 1973.

Table No .01 -Morphological characteristics of the cyanobacteria strain *Anabaena azollae*

Type of Cell	Length (µm)	Width (µm)	Shape
Vegetative Cell	8±0.2	5.4±0.2	Spherical
Akinetes	8.5±0.3	7.8 ±0.6	Cylindrical
Heterocyst	9±0.2	8.4±0.1	Cylindrical

Table No. 02: The rate of germination of test plant seeds in percentage (%)

Type of test plants	Seed germination (%)		
	4hrs	8hrs	12hrs
Control	85%	87%	87%
Treated	92%	96%	100%

Table No.3 Effect of *Anabaena* on wheat seedling growth

Growth parameters	Control			Treated		
	1st week	2nd week	3rd week	1st week	2nd week	3rd week
Shoot (cm)	4.3±0.1	6.4±0.1	11±0.3	5.1±0.1	8.9±0.1	14.5±0.1
Root(cm)	4.2±0.08	7.9±0.02	8.7±0.2	5.2±0.06	8±0.05	13.5±0.4
Fresh weight (gm.)	0.8 ±0.02	1.9±0.01	3.5±0.1	1.5±0.02	3.5±0.04	4.3±0.03
Dry weight (gm.)	0.1±0.01	0.5±0.02	0.9±0.01	0.2±0.09	1.5±0.01	1.9±0.05

Table No.4: Chlorophyll estimation of wheat seedlings

Chlorophyll Content Mg/gm	Control			Treated		
	1st week	2nd week	3rd week	1st week	2nd week	3rd week
Chlorophyll A	0.00108	0.00274	0.2553	0.0017	0.09441	0.2718
Chlorophyll B	0.0018	0.00776	0.4708	0.0031	0.00163	0.4910
TotalChlorophyll	0.00245	0.001048	0.4648	0.0039	0.00156	0.5022

Chemical properties			
S.No	**Property**	**Control**	**Treated**
1	Organic carbon	2.16	2.41
2	Nitrogen	1512	1687
3	Phosphorous	38.00	45.00
4	Pottassium	1640	1761
5	Calcium	40.08	46.44
6	Mangnese	22.18	30.18
7	Sodium	0.63	0.70
8	Calcium carbonate	3.62	3.62
Physical properties			
1	pH	8.00	8.00
2	EC	0.23	0.37
3	Soil percentage	27.00	31.00
4	Silt percentage	28.00	28.00
5	Clay percentage	45.00	45.00
6	Moisture	8.65	9.00
7	Moisture holding capacity	32.98	42.72
8	Partical density	2.60	2.50
9	App. Density	1.04	1.02
10	Porocity	28.40	22.24