



A Study on Biochemical analysis in aquatic fern *Azolla microphylla*

KEYWORDS

Azolla microphylla, cyanobacteria, nitrogen fixation, biochemical Compositions

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ABSTRACT

Azolla is a free floating aquatic fern and ranks among the fastest growing crops on earth and due to its association with the nitrogen fixing Cyanobacteria *Anabaena azollae*. In the present study an attempt was made to study the various biochemical composition in *A. microphylla* plant sample. *A. microphylla* (1/2 kg) was taken in plastic container containing 2 Kg of fine red soil, 1 Kg of cow dung and 15 gm of super phosphate in 5 litres of water and the pH was adjusted to 5.0-5.7. The *Azolla* culture was allowed to grow for a period of 21 days in green house. Various biochemical Compositions such as carbon, nitrogen, sodium, potassium and calcium were analysed using standard procedures. The results revealed that *A. microphylla* plant sample contains rich of organic carbon (36 ± 0.6 %), organic nitrogen (9.42 ± 0.08), sodium ($279.11\text{ppm} \pm 0.215$), potassium ($135.38\text{ppm} \pm 0.040$) and calcium ($318.70\text{ppm} \pm 0.933$).

Introduction

Azolla is a genus of small aquatic ferns with a world-wide distribution in temperate and tropical regions. Studies on *Azolla* have generated tremendous interest in the scientific community because of the nitrogen-fixing ability of this fern. Its capability to fix molecular nitrogen from the atmosphere is due to symbiosis with a heterocystous nitrogen-fixing blue-green algae, *A. azollae*, found within the cavities of the upper or dorsal lobes of *Azolla* fronds (Watanabe, 1978). *Azollae* which is able to fixable sufficient nitrogen for both itself and its host plant. In exchange, *Azolla* provides the *Anabaena* with a protected environment and a fixed source of carbon (Lumpkin and plucknett, 1982). The quick multiplication rate and rapid decomposing capacity of *Azolla* has become paramount important factor to use as green manure cum biofertilizer in rice field (Meghraj *et al.*, 1988). *Azolla* as biofertilizer for rice crop was realized in vietnam, later, it India and Bangladesh (singh, 1985). Thangam and Mahalingam (2010) cultivated the *Azolla* species using whey water as low cost medium. Keeping in view the biofertilizer potential of *Azolla*, the study was undertaken to culture *Azolla microphylla* and analyze biochemical characteristics.

Materials and Methods

I. Mass culture of *Azolla microphylla*

The *A. microphylla* (heat tolerant water fern) was collected from Tamilnadu Agricultural University, Coimbatore, Tamilnadu, India and mass cultured in the large plastic container containing 2 Kg of fine red soil, 1 Kg of cow dung and 15 gm of super phosphatate mixed in 5 litres of water and pH was adjusted to 5.0 to 5.7. In the prepared bed *Azolla* culture ½ kg was inoculated and incubated for 21 days.

II. Biochemical analysis of *Azolla*:

a) Determination of Nitrogen using micro kjeldahl method (Tandon, 1993)

In the micro kjeldahl apparatus 0.200mg of dried *Azolla* sample 3 gm of catalyst mixture mixing potassium sulphate with copper sulphate in 5:1 ratio 10 ml of H_2SO_4 is added to this mixture. Digestion process is carried out for 3 hours

at 420°C. After digestion the sample was cooled at room temperature. 10ml of distilled water was added in each digested sample tubes subjected to distillation using micro kjeldahl apparatus. In distillation 40% NaOH solution and boric acid (dissolve 20 g boric acid in 500 ml distilled water) with methyl red mixture (50 mg methyl red in 50 ml ethanol) solution are used to find the amount of nitrogen liberated from the sample. The whole process ends with turning of boric acid mixture with methyl red to green in color then, the amount of nitrogen in the sample was calculated using the following formula :

$$\text{Nitrogen (\%)} = \frac{T-B}{S} \times 0.2N \times 14 \times 100$$

S

Where, T - titrated value

B - Blank value

S - Volume of sample

b) Determination of carbon: (Walkley and Black, 1934)

Oven dried *Azolla* 0.200 mg of *Azolla* samples were mixed with 10 ml of potassium dichromate and 20ml H_2SO_4 . Then gentle stirring applied for 30 min and dilute the samples with 200ml of distilled water, and 10ml H_3PO_4 1ml of diphenylamine indicator. This mixture was titrated against 0.4N FAS (Ferrous Ammonium Sulphate) till to get brilliant green.. Then, the amount of total carbon in the sample was calculated using the following formula :

$$\text{Organic carbon(\%)} A = \frac{3.951}{g} (1-T/S)$$

where, g – weight of the sample

S – FAS with blank

T – FAS with sample

c) Determination of Sodium, Potassium and Calcium (Jackson, 1973)

Preparation of standard solution:

Dissolve 1 gram powdered sample of *A.microphylla* in the acid mixture contains 1 ml each of conc.H₂SO₄, 1 ml of perchloric acid and 3 ml of nitric acid in a heating mantle at 75° C. Dilute the digested sample with 25 ml Distilled water and filterd through Watmann filter. The amount of minerals such as sodium, potassium and calcium in filterate were determined using flame photometer (Flame Photometer 129^{pc}) the amount of above said minerals were calculated using the following formula :

Mineral (mole/l) = Photometer value * Standard value.

Where,

Standard value for Sodium (Na) is 23 ppm/l

Standard value for Potassium (K) is 39 ppm/l

Standard value for Calcium (Ca) is 20 ppm/l

Results and Discussion :

India being agricultural based country it could easily produce millions of liters of spent wash and considerably reduce the use of chemical fertilizers (Roger and Reynaud, 1982) *Azolla* act as dual crop with wet land rice is gaining importance as bio fertilizer for rice crop is its quick decomposition in soil and efficient availability of its nitrogen to rice (Tennyson, 2006). The application of *Azolla* may be very much beneficial to conserve the environment for de-

veloping renewable, sustainable resources and to provide a natural source of nitrogen.(Singh, 1992). In the present work, an attempt was made to analysis various biochemical composition in *A. microphylla*. In the present preliminary studies, observed an increased level of Carbon, Nitrogen, Potassium, and Calcium in *A.microphylla* Cultures (Table 1). Similar analysis was carried out by Lumpkin (1985) and his results shows Potassium concentration was high in all leaf cavities with a small increase in the basal ones.

Table 1 . Biochemical Composition of *Azolla microphylla* dried culture

Sl. No	Name of the constituents	Contents (in percentage/ ppm)
1	Organic carbon	36 ± 0.6 %
2	Nitrogen	9.42 ± 0.8 %
3	Na	279.11 ± 0.215 ppm
4	K	135.38 ± 0.040 ppm
5	Ca	318.70 ± 0.933 ppm

Values are mean of three replicates ± standard error

Conclusion :

The results of this study clearly shows the presence of increased levels of various biochemical composition such as organic Carbon, Nitrogen, Potassium, Sodium and Calcium in *A.microphylla* culture. Hence it can be effectively used as biofertilizer to promote agriculture practices in rural India.

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