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BIOMASS INCREASE AND PRELIMINARY PHYTOCHEMICAL SCREENING OF *AZOLLA MICROPHYLLA* KAULF GROWN UNDER FOUR SOIL NUTRIENT MEDIUM

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ABSTRACT

The Pteridophytic aquatic macrophyte, *Azolla microphylla* Kaulf collected from Tamilnadu Agricultural University, Coimbatore was made to acclimatize for a week before the commencement of the study. The aquatic fern was then grown under four different nutrient mediums consisting of soil and diluted fresh Cow dung. The four nutrient medium were similar except for the soil type which differed in each medium. The soil type chosen for the four nutrient mediums were garden, clay, red and black soil. Fresh cow dung was diluted in fresh water before adding them to the medium. No other additional fertilizers were added to the medium. The study was carried out for a period of one month to identify the best soil nutrient medium for the growth of *Azolla microphylla* Kaulf. The preliminary phytochemical analysis was conducted to determine whether the difference in soil types affected the presence and absence of phytochemicals. The biomass increase was calculated every seventh day, which proved that the medium containing clay soil suited the best, followed by the garden, black and red soil respectively. There was no considerable difference in the presence or absence of phytochemicals in *Azolla microphylla* Kaulf grown under four soil nutrient medium.

Keywords: *Azolla microphylla* Kaulf, Soil Nutrient Medium, Biomass Production, Preliminary Phytochemical Screening

1. INTRODUCTION

Azolla,a freshwater fern is a small floating macrophyte belonging to the family Azollaceae [1]. The fern is globally distributed and can be mostly found on the surfaces of rice paddies, lakes, ponds, and marshes, and is capable of tolerating a wide range of environmental conditions [2]. The Azolla ferns requires only 25-50% full sunlight for its normal growth; slight shade is of benefit to Azolla growth in field condition [3].

Under ideal growth conditions, Azolla undergoes its asexual multiplication by the breakage of side branches [3] and presents a high productivity and high protein content (generally 20-30%, on a dw basis) [4]. The fern is able to double its biomass in 3-5 days [5].

It has a symbiotic relationship with the nitrogen-fixing Cyanobacteria Anabaena azollae. This relationship makes Azolla a good source of nitrogen fertilizer for rice and other crops grown in flooded lands [1].

Azolla is of great interest to tropical agronomy, in particular with regard to lowland rice cultivation [5]. Other than the use of Azolla as a green manure for the rice fields, it is also used as a feed ingredient in producing low cost protein feeds [6]. Azolla is also proved to be successful in the process of phytoremediation of dyes [7] and heavy metals [2]. Since it has varied uses the wonderful fern is referred as "green gold mine" [8]. The plant system has the inherent capacity to synthesize several biologically active constituents which in turn protect them against the attack of insects and other plant pathogens such as bacteria and fungi [8].

The present study involves the use of four different soil types (Red, Garden, Clay and Black soil) to identify the best soil medium suitable for the biomass production of *Azolla microphylla* Kaulf and to analyse if there is any variations in the preliminary phytochemicals present in the azolla spp. grown in four different soil mediums.

2. MATERIAL AND METHODS

2.1. Preparation for *Azolla microphylla* culture

Four rectangular trays of 8 L capacity were taken. Thin layer of fine soil (Garden soil, Clay soil, Red soil and Black soil in respective trays) were added. Four litres of water was filled in all the four trays. The water and soils were analyzed for the important parameters before adding.

Cow dung (500 mg) was mixed with 1 litre of water and added to every four trays with four different types of soil. Triplicates were maintained for every treatment.

2.2. Inoculation of *Azolla microphylla* seeds

Preparation once completed, the four trays were inoculated with fresh *Azolla* seeds or *Azolla* mother fern (150 g) procured from Tamilnadu Agricultural University (TNAU). Cow dung was added weekly once, to avoid the nutrient deficiency. Water evaporated was compensated by adding water.

The whole set up was placed in the laboratory were plants obtained the sunlight needed for photosynthesis. Direct sunlight was avoided.

The growth of *Azolla* was calculated by taking the *Azolla* from all the four trays, washed, drained in filter paper and weighed in the weighing balance every week for one month.

2.3. Calculation of *Azolla microphylla* biomass production

$$\text{Biomass production B (\%)} = \frac{[bf - bi]}{bf} \times 100$$

Where, bf = biomass at the end of the experiment, bi = biomass at the start of the experiment [9].

2.4. Authentication of the plant

The pteridophytic aquatic macrophyte was authenticated as *Azolla microphylla* Kaulf by the Botanical Survey of India, Southern Regional Centre, TNAU Campus, Coimbatore.

2.5. PreliminaryPhytochemical screening

The fresh Azolla fronds was collected from the trays with different soil nutrient mediums and washed with tap water several times. The Azolla fronds were again washed in distilled water and air dried for a week and then finely powdered. The finely powdered material (50g) was extracted with methanol (250 ml) for 8-10 hrs at a temperature not exceeding the boiling point of the solvent in a soxhlet apparatus. The extract was evaporated at 40°C in a rotary evaporator and then the extract was refrigerated until further use. The phytochemical analysis was done using standard methods [15].

3. RESULTS AND DISCUSSION

3.1. Water quality parameters

The tap water was used for the biomass cultivation of *Azolla microphylla* Kaulf. The important physicochemical parameters were recorded before the beginning of the study. Temperature ranged between 24°C-26°C. The pH of the water ranged from 6.8-7.2. The salinity of the water ranged between 0.07-0.10 ppm.

3.2. Soil quality parameters

The soils (Garden, Clay, Red and Black) selected for the preparation of the soil nutrient mediums were analyzed for the soil quality parameters (pH, soil moisture, Nitrate, Phosphorus and Potassium). The results are depicted in the table 1.

Table 1: Soil quality parameters of the four soil types (Garden, Clay, Red and Black)

SOIL TYPE	pH	SOIL MOISTURE (%)	NITRATE (mg/g)	PHOSPHORUS (mg/g)	POTASSIUM (mg/g)
GARDEN SOIL	7.6±0.05	5.68±0.02	5. 68±0.01	4.49±0.01	3.9±0.01
CLAY SOIL	6.2±0.05	6.34±0.02	1.00±0.02	5.03±0.005	4.81±0.01
RED SOIL	4.7±0.05	2.83±0.47	0.82±0.005	0.80±0.005	5.15±0.01
BLACK SOIL	6.7±0.00	4.5±0.05	1.27±0.005	2.09±0.005	5.20±0.005

Values represented in mean ±SD, n=3

3.3. Biomass production of *Azolla microphylla*

The biomass increase of *Azolla microphylla* in the nutrient medium containing clay soil had a biomass percentage (B%) of 23.58 on 7th day,46.92 on 14th day,73.21 on 21st day and 85.03 on 28th day. The Black soil had a biomass percentage (B%) of18.03 on 7th day, 42.53 on 14th day,66.69 on 21st day and 78.26 on 28th day. The Garden soil had a biomass percentage (B%) of 16.66 on 7th day, 40.00 on 14th day,65.56 on 21st day and 76.93 on 28th day. The Clay soil had a biomass percentage (B%) of 23.58 on 7th day, 40.00 on 14th day,65.56 on 21st day and 76.93 on 28th day.

The biomass increase of *Azolla microphylla* Kaulf was comparatively high in the clay soil medium when compared to black, garden and red soil. This is because the Azolla is dependent on phosphate for its growth and nitrogen fixation [10] and the clay soil medium was found to have higher phosphate content when compared to the rest soil mediums for azolla growth. pH is an important parameter in the growth of Azolla. Slightly acidic to slightly alkaline pH is considered as optimum for the growth of Azolla [11] which matched with the results of the present study which had a increase in growth rate at pH 6.2±0.05, 6.7±0.00 and 7.6±0.05.

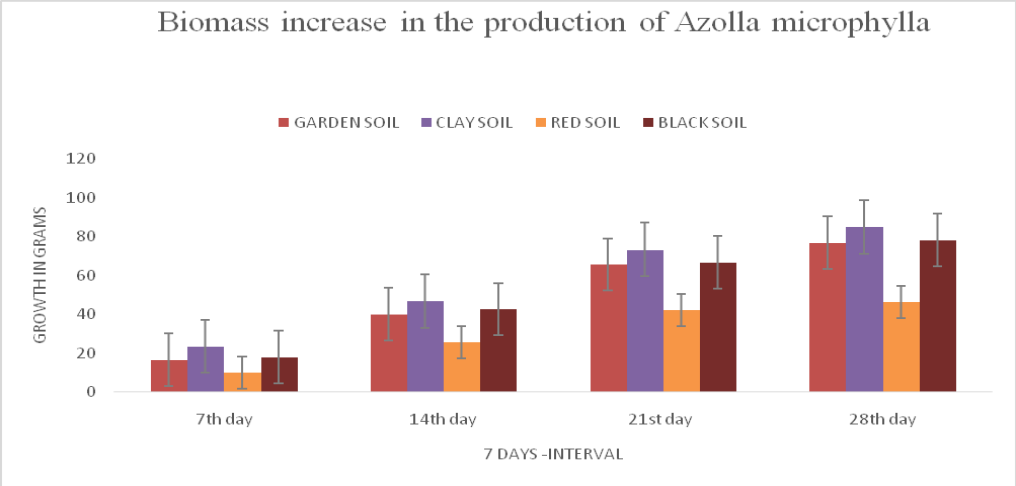

Fig. 1: Biomass increase in the production of Azolla microphylla

Response of Azolla to pH is greatly affected by other environmental factors like temperature [12]. Water temperature of nearly 25° C along with slightly acidic to slightly alkaline pH can contribute to a better growth environment [13]. The present study also had a water temperature range between 24°C-26°C which resulted in better growth rate.

3.4. Preliminary phytochemical screening of *Azolla microphylla* grown in four soil nutrient mediums.

The phytochemical screening of the methanolic extract of *Azolla microphylla* Kaulf grown in four soil nutrient mediums are shown in the table 2.

Alkaloids were weakly present in the methanolic extract of Azolla collected from all the four soil mediums. Tannins were fairly present in the methanolic extract of Azolla collected from clay, garden and black soil mediums, and weakly present in methanolic extract of azolla grown in red soil. Phenols were highly present in the azolla extract from clay and black soil mediums whereas it was fairly present in the azolla extract of garden and red soil mediums. Saponins were fairly present in the azolla extract grown in clay soil and weakly present in the azolla extract from garden, black and red soil. Flavanoids were weakly present in the methanolic azolla extract of clay and garden soil whereas absent in the methanolic azolla extract taken from black and red soil. Terpenoids were fairly present in the methanolic azolla extract from clay soil, absent in the azolla extract from garden soil and weakly present in the methanolic extract in black and red soil mediums. Steroids were highly present in the methanolic azolla extract taken from clay, garden, black and red soil mediums. Quinones were absent in the methanolic extract in all the four soil mediums. Amino acids were fairly present in the methanolic extract of azolla collected from clay soil and weakly present in the azolla extract from garden, black and red soil mediums.

Table 2: Phytochemicals present in the methanolic extracts of *Azolla microphylla* Kaulf grown in four different soil nutrient mediums

PHYTOCHEMICALS	CLAY	GARDEN	BLACK	RED
Alkaloids	+	+	+	+
Tannins	++	++	++	+
Phenols	+++	++	+++	++
Saponins	++	+	+	+
Flavonoids	+	+	-	-
Terpenoids	++	-	+	+
Steroids	+++	+++	+++	+++
Quinones	-	-	-	-
Amino acids	++	+	+	+

+++: Highly present, ++: Fairly present, +: Weakly present, - Nil

Alkaloids were weakly present in the extracts taken from Azolla from all the four soil nutrient mediums which were in correlation with the results of Sathammaipriya *et al.*, 2018 [14]. But, the results of Abraham *et al.*, 2012 [8] and Veerabahu *et al.*, 2015 [15] the methanolic extracts of Azolla microphylla did not show the presence of Alkaloids. Tannins, Phenols, Saponins and Steroids were present in the methanolic extract of the present study which was supported by the results of Sathammaipriya *et al.*, 2018 [14] and Abraham *et al.*, 2012 [8]. Quinones were absent in the extracts. The results coincided with the results of Veerabahu *et al.*, 2015 [15]. Terpenoids were fairly present in the extract of the present study which correlated with the results of Sathammaipriya *et al.*, 2018 [14] except in the extract from garden soil medium which did not show any presence of terpenoids. Flavanoids were weakly present or absent in the methanolic extract of the present study. Amino acids showed its presence in the extract of the present study which was in accordance with the results of Sathammaipriya *et al.*, 2018 [14].

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