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Fodder Yield and Quality of *Azolla pinnata* Cultivated under the Different Water Sources

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ABSTRACT

Background: Azolla is a nature's gift and regenerates within 24 hours and has tremendous uses like supplementary feed to livestock. It is one of the most nutritive animal feeds and also can be used by livestock farming communities. Azolla has several times higher levels of crude protein than other forage crops and is also rich in minerals. The aim of the current study is to study growth in various water sources and their quality as supplementary feed to livestock.

Methods: A study was conducted in a randomized block design (RBD) with five different treatments and replicated five times to minimize the error. The study was carried out to explore the yield and nutritive value of *Azolla pinnata* cultivated under different water sources like well water, river water, bore well water, sewage water and drainage water. The duration of the study was three years from Jan 2019 to March 2022. The silpauline plastic bed having dimensions 12 × 4 ft size was used. Every alternate day, the fresh Azolla was harvested and recorded the weight.

Result: The pooled data for three years (2019 to 2022) showed a higher yield of 0.31 kg/day of fresh *Azolla pinnata* in Mula Mutha river water (T_2) and 0.30 kg in well water (T_1) and sewage water (T_4) crude protein content was highest (28.07 %) in sewage water (T_4).

Key words: *Azolla pinnata*, Protein, Sources, Water, Yield.

INTRODUCTION

Azolla is an aquatic free-floating fern belonging to the family Salviniaceae. There are more than seven species grown naturally in various geographical conditions but few species are cultivated considering its nutritional potentiality and adaptability to various climatic conditions. Due to the scarcity of concentrate and green fodder and its high prices in animal feeding, the Azolla is one of the best nutritive-rich feed for animals. Azolla is an invasive plant that grows faster in freshwater lakes, rivers, wetlands and ditches in both temperate and tropical countries by a few precautionary steps with low production costs. It can modify biodiversity and aquatic ecosystems substantially (El Naggar and El-Mesery, 2022). Among these, the Azolla *pinnata* is largely cultivated by the farm due to its high nutritive value, a good source of protein with almost all essential amino acids required for animal growth and milk yield. Besides the above, it also contains macronutrients like calcium, magnesium, potassium and vitamins A and B_{12} in large quantities. All these facts suggested that Azolla can be used as an unconventional feed with a protein supplement to livestock including ruminants, poultry and fish (Hossiny *et al*., 2008) and due to ease of cultivation, high productivity and good nutritive value it is used as a beneficial fodder supplement (Prabina and Kumar, 2010).

Since, it is a floating fern and is found in different water sources including rivers, wells, drainages, lakes and ponds etc. But there is not much precision of the quality and quantity of Azolla grown in these water sources. The increased Azolla production mainly depends on the chemical and biological properties of water and irrespective of water quality the consistent availability of water year around is very much important to produce the required quantity of Azolla for regular use. Therefore, House wastewater is one source that is continuously available and unknowingly wasted in large quantities.

From a farmer's perspective, the use of this water during summer will be the one suitable option to cultivate and produce the Azolla consistently at the farmer's doorstep. Similarly, the well and bore well water is also the alternate options to produce the Azolla on regular bases. Keeping this as important to the farmer's perspective, the study was conducted to understand the potential yield and nutritional quality of Azolla grown in different water sources.

MATERIALS AND METHODS

Considering the benefits and potential of Azolla as a supplementary feed to small and large ruminants. The water from different sources was collected like the river, bore well, well and drainage water. A shade net house was constructed

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using 90% shade net and 12 × 4 ft. size pits were prepared and a silpauline sheet of 450 GSM was laid on the pits for storing the water. 1 kg of *Azolla pinnata* culture was spread on each bed (12 × 4 ft) and allowed to grow freely in the water.

Treatment details: Different sources of water *viz*.

T_1- Well water, T_2- River water, T_3- Bore well water, T_4- Sewage water and T_5- Drainage water were used in the study. The study was conducted using Randomized Block Design (RBD) with five treatments and five replicates for each treatment to minimize the error. After 15 days of inoculation of Azolla culture, the fresh Azolla of one-fourth surface area was harvested from each of the beds and recorded the weight. The same was continued for every alternate day for 3 months. This study was conducted from Jan to March for 3 years *i.e*. in 2019 to 2022. The pooled data were taken into account to conclude the performance on yield and nutritional quality of *Azolla pinnatta* cultivated in different water sources.

Chemical evaluation of *Azolla pinnata*

The DM content of collected Azolla samples was analyzed by drying to a constant weight in a forced hot air oven at 105°C. The ash content in the samples was estimated as residue after the incineration of samples at 600°C for 3 hours. Crude protein (N × 6.25) was analyzed using the Gerhardt digestion and distillation unit (AOAC, 2005). The ether extract (EE) content in the sample was analyzed after extraction with petroleum ether using the procedure of AOAC (2005). The mineral profile of Azolla was analyzed by an inductively coupled plasma-atomic emission spectrophotometer.

Table 1: Average Azolla yield Per day (3 years pooled data) from 1 kg of *Azolla pinnata* culture spread on an area dimension of 12 × 4 ft.

Treatment details	Mean (kg)
T_1: Well water	0.30
T_2: River water	0.31
T_3: Bore well water	0.27
T_4: Sewage water	0.30
T_5: Drainage water	0.25
SE (m)+	0.01
CD at 5%	0.03
CV %	7.9

RESULTS AND DISCUSSION

Fresh Azolla yield per day was highest (0.31 kg/day) in river water (T_2) and it was at par with well (T_1) and sewage water (T_4) as shown in Table 1.

Nutritive value of Azolla grown under different water sources (Dry matter basis)

In detail, the results of the proximate composition of sun-dried Azolla are shown in Table 2. The average DM content was 7.17%, crude protein 26.46%, total ash 21.24%, ether extract 5.33% ADF 21.29%, NDF 46.75% lignin 12.03% and it can be used as supplementary feed to animals. A lower DM of 8.07% was recorded in well water compared to the 12% DM reported by Akhud *et al*. (2017).

The average crude protein (CP) value of 26.46% was higher than the value reported by Ahirwar and Leela (2012) and many researchers reported as 21.17% (Sujatha *et al*., 2013), 21.66%, (Kavya 2014) and 22.5% (Ashraf and Sharma 2015). Similarly, the average ash percentage of 22.17% was higher in sewage water.

Mineral profiling of *Azolla pinnata*

Calcium is one of the major minerals required for growing and milking animals. The higher milk-producing animals utilize large amounts of calcium from the body, therefore the Azolla will be one of the best substitutes because of its high calcium content. The average value of calcium, phosphorus and potassium in fresh Azolla were 2.21%, 0.62% and 0.61% respectively (Table 3). Anitha *et al*. (2016) reported calcium, phosphorus and potassium contents of 1.64%, 0.34% and 2.71% in *Azolla pinnata*. However, it's important to note that the specific nutrient composition of *Azolla pinnata* can vary depending on factors such as cultivation practices, water quality and geographic location. If the farmers or researchers want to know precise information about the nutrient content of *Azolla pinnata* in a specific region or for a specific purpose, it is advisable to consult local research studies or laboratory analyses.

Analysis of water collected from different sources for growing Azolla

The growth performance of *A. pinnata* may vary with the types of water used for cultivation. The water from different sources has different chemical and biological properties that have an impact on the growth of the Azolla. Water collected

Table 2: Proximate composition of Azolla (on dry matter basis) grown in different water sources (3 years pooled data).

Nutrient (%)	T_1 (Well water)	T_2 (River water)	T_3 (Bore well water)	T_4 (Sewage water)	T_5 (Drainage water)	Average
Dry matter	7.43	6.37	8.07	6.47	7.51	7.17
Crude protein	26.18	27.73	25.19	28.07	25.14	26.46
Total ash	19.89	21.64	21.18	22.17	21.29	21.24
Ether extract	4.83	6.36	3.82	6.17	5.49	5.33
Acid detergent fiber (ADF)	20.98	24.10	18.90	23.19	19.29	21.29
Neutral detergent fibre (NDF)	46.32	51.81	42.62	49.44	43.55	46.75
Lignin	12.31	12.97	11.28	12.36	11.22	12.03

Table 3: Mineral profile of Azolla growing in different water sources (Dry matter basis) (3 Years pooled data).

Minerals	T_1 (Well water)	T_2 (River water)	T_3 (Bore well water)	T_4 (Sewage water)	T_5 Drainage water	Average
Calcium (%)	2.13	2.34	2.24	2.43	1.90	2.21
Phosphorus (%)	0.59	0.73	0.65	0.48	0.67	0.62
Potassium (%)	0.60	0.62	0.57	0.54	0.72	0.61

Table 4: Analysis of different water sources used for growing Azolla (3 years pooled data).

Test	Unit	T_1 (Well water)	T_2 (River water)	T_3 (Bore well water)	T_4 (Sewage water)	T_5 Drainage water	Average
pH		7.40	7.11	7.20	7.42	7.51	7.33
Total dissolved solids	mg/l	817.33	421.40	508.47	840.00	1468.25	811.09
Total hardness as $CaCO_3$	mg/l	278.88	300.20	260.67	239.67	367.00	289.28
Alkalinity as $CaCO_3$	mg/l	261.00	141.85	145.37	127.67	164.48	168.07
Chlorides	mg/l	220.78	145.97	96.97	98.57	223.40	157.14
Electrical conductivity	s/cm	653.67	543.00	243.67	640.17	980.40	612.18
Potassium	mg/l	0.61	4.08	2.15	6.50	10.01	4.67
Nitrogen	mg/l	7.90	4.48	6.19	20.81	37.91	15.46
Phosphorus	mg/l	0.06	1.36	0.16	3.69	4.19	1.89

Table 5: Profiling of amino acid in Azolla (DM basis).

Parameter	Test value (%) (DM)*
Methionine	0.194
Cystine	0.100
Methionine + Cystine	0.294
Lysine	0.551
Threonine	0.595
Arginine	0.675
Isoleucine	0.578
Leucine	1.016
Valine	0.709
Histidine	0.217
Phenylalanine	0.618
Glycine	0.655
Serine	0.59
Proline	0.539
Alanine	0.758
Aspartic acid	1.21
Glutamic acid	1.586
NH3	0.339
Total including NH_3	10.93
Total without NH_3	10.591

*DM= Dry matter.

from different sources was tested in the laboratory and it was found that the pH level (7.11 to 7.51) of all the water samples were within normal range and hence there is no adverse effect on Azolla growth. The total dissolved solids (TDS) collected sample ranges from 421 to 1468.25 (mg/l) (Table 4). The TDS of water of mula mutha river was 421.40 mg/l and it was lower than the samples of other water sources used for Azolla cultivation. As per the report, if the tested value of TDS was found above the 2000 mg/l then it was unsuitable for irrigation purposes or growth of Azolla. (Ayers and Westcot, 1994). Hence, the lower TDS in river water (T_2) may have supported a high fresh Azolla yield (0.31 kg/day). Besides, the lower total hardness (mg/l), alkalinity (mg/l) and chlorides and higher phosphorus (mg/l) in mula mutha river water may have the added advantages for better growth of the Azolla.

Profiling of amino acids in Azolla

There is a very limited work was done in profiling of Amino acids in Azolla. Considering this fact the composite samples of Azolla from all the water sources were analyzed to know the Amino acid content in the Azolla. It is a richest source of Protein and the protein quality is a function of Amio acids present in it. It contains almost all essential amino acids as shown in (Table 5).

CONCLUSION

The study indicated that the Azolla *pinnata* grown (0.31 kg per day) in mula mutha river water is more beneficial as compared to other water sources like sewage, well, bore well and drainage water. But growing Azolla only in river water may not be suitable for all the farmers who are no access to it. Even they can grow the Azolla in wells and sewage water sources as they are at par with the Azolla grown in mula mutha river water. The higher crude protein (CP) of 28.07% was recorded in sewage water as well and this may also be beneficial to the farmers rearing the livestock in the peri-urban area. However, it needs to study further to understand the toxic elements present in the different water sources before cultivating the Azolla.

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Conflict of interest

There is no conflict of interest on the behalf of all authors of the manuscript

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