

Unlocking the Potential of *Azolla*: A Green Gold for Sustainable Cultivation and Diverse Applications

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Abstract

Azolla, a fast-growing aquatic fern, has emerged as a versatile and sustainable solution with numerous applications in agriculture, environmental conservation, and human nutrition. Known for its symbiotic relationship with nitrogen-fixing cyanobacteria. Serves as a natural biofertilizer, enhancing soil fertility and reducing the need for synthetic fertilizers. Its cultivation is cost-effective, requiring minimal resources, and it plays a significant role in improving crop yields, particularly in rice paddies.

Azolla's high protein content and nutritional value make it a valuable feed supplement for livestock, poultry, and aquaculture, while also holding promise as a potential food source for humans. This article explores the cultivation methods and applications of *Azolla*, underscoring its potential to address global challenges related to food security, sustainability, and environmental health.

Introduction

Azolla is already placed now by taxonomists in monotypic family Azollaceae (Konar & Kapoor, 1972) while it also goes by Mosquito fern, Water fern, Duckweed fern Azollais. Azollis are a dichotomously branched free floating aquatic ferns; they can be naturally found in moist soil, ditches and marshy ponds. Genus is further classified into two sub genera, i.e. *Euazolla* and *Rhizosperma*, while the name Azollais is derived from Greek word Azo means 'to dry' and Allyo means 'to dry' which means plant dies when it dries (Svenson, 1944). *Euazolla* is known by the presence of three megaspore floats and consist of *A. caroliniana*, *A. filiculoides*, *A. Mexicana*, *A. microphylla* and *A. rubraspecies* and *Rhizosperma* consist of *A. pinnata* and *A. nilotica*.

It has syntonous fronds, minuscule roots (or tiny roots), short branched stem known as Rhizome and covered with alternate small leaves divided into dorsiventral organization, ventral lobe and it is colorless and only one celled thick and dorsal lobe has ellipsoidal cavity and it is the lining with mucilage containing cyanobiont *Anabaena azollae* is largely filled with gases (Peters & Calvert, 1983; Lumpkin & Pluckmett, 1980) a blue green algae can photosynthesize independently and the entire nitrogen is supplied by the algal symbiont and gram positive non nitrogen fixing in the form of Ammonium which is identified as *Arthrobacter* species (Peters et al., 1980). Because of its rapid reproduction and its high content of nitrogen (3-6% N by dry weight) (Watanabe et al., 1981), this legume is commonly used as green manure, though it is also used, as a legume added with this, it is ideal for the cultivation of rice in tropical conditions, by owing the fix action of atmospheric nitrogen and the ability to reproduce increasing rates. Thus, it grows very well in flooded rice fields, widely used as a most suitable bio fertiliser to increase nitrogen content in rice fields within a few weeks after incorporation (Bhuvaneshwari & Singh, 2015).

Materials and Methods

Requirements

Growth development of aquatic fern *Azolla* demands particular environmental conditions for successful cultivation because it depends on temperature levels and humidity along with sunlight exposure. Each one varies in its growth rate, size and tolerance to environmental factors (Watanabe & Berja, 1983). *Azolla* grows best in partial sunlight under shade but in excess of sunlight, *Azolla* becomes stressed and produces deoxy anthocyanins if in large quantities in itself reduces nutrition value (Cohen-Shoel et al., 2002) and denies the food to the plant of polyunsaturated fatty acids. In order to prevent deficiency the levels of phosphorus should be in optimal values which causes the plant to turn pink to red (Abdalbakee & Mohammed, 2019).

Cultivation of *Azolla* Culture

Also, Fertile soil is sprinkled, water is poured into the pith, fertilizers are added: This is crucial for *Azolla*'s growth; the sheet is then laid down, spread uniformly over the pith and covered with a silpaulin sheets and the wanted materials are removed (Mohan Kumar et al., 2020), finally, the sheet must be free of pointed stones, roots, thorns that can percolate into the sheet and cause leakage (Temmink et al., 2018). Vermicompost or cow dung can be used as manure after that to obtain mother plants for the propagation as they reproduce through asexual methods that produce rapidly within a short span of duration (Qiu & Yu, 2003).

One kg of cow dung and 100 gms of Super phosphate should be applied once in a week for better growth of the culture, weeds should be cleaned and ponds should be cleaned for every six months and new culture should be prepared with fresh *Azolla* and soil. The growth will be completed in about 2- 3 weeks. After full growth, it can be harvested daily and must be washed very well so that the smell of dung is removed.



Figure 1 Cultivation of *Azolla*



Figure 2 *Azolla* sp.

Chemical Composition

Azolla has outstanding composition that may contain up to 25–35% protein ranges of its dry weight and it also contains EAAs, lysine, methionine, cysteine, threonine, tryptophan, arginine, isoleucine, tyrosine, minerals such as calcium, phosphorus, potassium, zinc, cobalt, boron, nickel, iron, lead (Kabir et al., 2020; Mosha et al., 2020). Provitamin A, *azolla* contains chlorophyll and carotenoids, and growth promoter intermediate (ChiChilichi et al., 2015) indicates better animal consumption, low acid and neutral detergent fibre (NDF), *azolla* is also considered the source of biopolymers and probiotics (Ahmed et al., 2022).

It was claimed that the nutritional content of the *A. pinnata* is very high and especially large amounts of the calcium, vitamins and minerals were reported in it, as a substitute for the Commercial Fish Feed (CFF) of the *Barbonymus gonionnotus*, the use of 25% CFF was explored and concluded that the species

could be replaced with 25% replacement of the CFF without reduction (Das et al., 2018). Azolla is one of the available sources of protein, minerals, vitamins that has been documented. Secondly, it is ideally perfect for the tropical region and for livestock feeding, having higher quantity of carbohydrates, proteins, crude fat and complete digestible nutrients and lower crude fibre content. It is also used as human supplement because of its higher amounts of proteins as well as carbohydrates.

It is known that nutrient has been researched to have 206 to 619 mg/kg in Azolla and Azolla contains 206 to 619 mg/kg in nutrient rich growing conditions. Furthermore, the capacity of Azolla to fix nitrogen makes it one of the best biofertilizer for rice (Marzouk et al., 2023); amino acid compositions are presented in Table 1 and chemical composition in Table 2. Therefore, these attributes make Azolla an excellent organism as it is used as nutritional feeding to enrich the food for humans as biofertiliser and phytoremediator in sustainable agriculture.

Table 1 Amino Acid Composition of Azolla Plant (Azolla Pinnata)

Amino Acid	Dry Matter (%)	Protein (g/100 g)	Chemical Score (%)
Lysine	0.98	4.58	130.9
Methionine	0.34	1.59	45.4
Cystine	0.18	0.84	24
Threonine	0.87	4.07	116.3
Tryptophan	0.39	1.82	52
Arginine	1.15	5.37	153.4
Isoleucine	0.93	4.35	124.3
Leucine	1.65	7.71	220.3
Phenylalanine	1.01	4.72	134.9
Tyrosine	0.68	3.18	90.9
Glycine	1.00	4.60	131.4
Serine	0.90	4.21	120.3
Valine	1.18	5.51	157.4

Source: Alalade and Iyayi (2006)

Table 2 Chemical Composition of Azolla Meal on Dry Matter (%)

Index	Dry Matter (%)
Crude Protein	22.79
Crude fiber	15.49
Dry matter	90.3
Total ash	19.46
Ether extract	3.59
Nitrogen free extract	38.67
Calcium	2.03
Phosphorous	0.48

Source: Khursheed et al. (2019)

Availability of Mineral Nutrients

Rice yield is limited by other yield limiting nutrients or macro-nutrients which include Potassium and phosphorous, which, being able to accumulate Potassium is a remarkable source in its tissues in

the low environment, and decomposes quickly to release nitrogen, phosphorous and potassium to the field when the water dries (Bhuvaneshwari & Singh, 2015). The solubilizes Zinc, Iron and Magnesium to promote the yield and release vitamins and plant growth regulators for rice crop (Bhusal & Thakur, 2021). It is found that soil Phosphorus available at Paddy soils where Azolla was added did not show any significant difference at the beginning. Similarly, a group researches the two subsequent year research under Azolla treated. It was found that Azolla and cow dung treated soil increased the Phosphorus (Dey et al., 2018) by 42.94%, while soil showed a 29.12% increase of Potassium.

Phytochemicals and Bioactivity of Azolla Spp.

Thus, as compared to other green crops having similar crude protein content and amino acid composition, but exhibiting high level of amino acid, lysine, Azolla has its biomass and protein producing aquatic plants with potential to be used as direct fish feed. Results showed *A. microphylla* leaves have alkaloids, tannins, saponins, flavonoids, phenols and leaf extracts have been tested for anti microbial and antioxidant activities against bacteria species (Sathammapriya et al., 2018). Amongst these *A. pinnata* possesses amino acids, vitamins, beta carotene, and minerals and it is often considered a source high quality protein (Elrasoul et al., 2020).

Harnessing Azolla: A Sustainable Solution for Livestock Feed and Beyond Azolla as Biofertilizer

Azolla is also an important biofertilizer among all simply because it is capable of fixing up to 30 – 60 % kg which can replace upto 25% of the nitrogen mineral fertilization (Kollah et al., 2015) with its application in agriculture due to its properties that facilitate crop production. The nitrogen fixation to be ingested by the fern (Abd Elrasoul et al., 2020) is shown to be a symbiotic association; foliar spraying with Azolla extract yields 57.37 % and 51.71 % grain yield, 37.66 % and 21.57 % biological yield, respectively, compared to the control (Altai et al., 2019).

It is also added with Nitrogen, Phosphorus, Iron, Magnesium. Application of *A. microphylla* fertilizers led to an increase in *Spirulina platensis* population density and chlorophyll content while *A. microphylla* fertilizers can be made as liquid fertilizer that increases the population concentration of the above mentioned microalgae at some concentrations. An optimal AZO based fertilizer of 5mL/L was determined at 321,500 cells/mL and 5mL/L Azolla based fertilizer concentration. It is worth noting, particularly for its potential for rapid growth, which mainly hinges upon its N fixation ability (through symbiotic cyanobacteria – *Nostoc* attached to leaf cavities [*Azolla*] of Azolla that provides a range of ecological services).

Recently several researches in Egypt stated the different applications of *A. caroliniana* and *A. pinnata* as a green manure for cowpea plants (Ismail, 2017). However, the plant height, number of branches per plant, fresh and dry shoot weights, and number of inflorescences of chamomile plants growing in sandy soil were increased greatly by mineral nitrogen added as fertilizers.

Azolla in Bioremediation of Environment

On a biotechnological basis recently the process of polluted water decontamination has achieved good yields at low cost, on the base of it several plant species such as *Eichhornia* sp., *Lemna* sp., *Azolla* sp. had been used widely (Senthil Kumar et al., 2020; Wiangkham & Prapagdee, 2018). In its own way, mosquito fern also serves as a phytoremediation for clearing up the environment of pollution. Proper biomass management is required once phytoremediation process is complete because the produced biomass may be contaminated with toxic elements, which may then pose an environmental issue (Naghipour et al., 2018; Saxena et al., 2020).

Azolla as Mosquito repellent

That is because mosquitoes laying their eggs on the surface of the water could be prevented from making these eggs hatch due to the heavy Azolla mat on water. They are studied almost eliminated in such water bodies as are completely covered with Azolla (Verma et al., 2022).

In addition to this cultivation of wetland Azolla in and around the poultry farm, this also serves to reduce the odor, house fly and mosquito load (Mahanthesh et al., 2018). LC50 of the *A. pinnata* extract is from 1000 to 1500 ppm and all of the 'Adulticidal assays' can be performed against *Aedes* Mosquitoes. More effective and longlasting method of controlling *Aedensaeypiti* and *Aedesalbopictus* mosquitoes will be provided with alternative applications of bio insecticides (Ravi et al., 2020).

Azolla in Livestock feeds

At present, the dairy farmers depend on commercial feeds, but these are not economical for dairy milk production. However, few studies are available on the feeding of Azolla supplementation on milk production performance and milk constituents of cattle to provide a possible green fodder feed ingredient (Senthil Kumar et al., 2020). It multiplies rapidly and every few days it doubles and contains 25-30% of protein which makes many animals digestible with low content of lignin and rich in amino acids, vitamins, growth promoter intermediates and minerals such as calcium, phosphorus, potassium (Hasan & Chakrabarti, 2009). On its dry weight it contains 25-35% protein, 10-15% minerals and 7-10% amino acids and bioactive and bio polymers substances.

In the year 2020-21, the business exported 255686.92 MT of poultry goods in the global market at a cost of Rs. 651.2143505. However, they have high growth rate, high fertility and low (feed, management, etc.) and tolerances for various climatic and environmental changes (Munnarwar et al., 2022). Conclusion from the study was that there was no adverse effect on carcass quality, slaughter features or production cost of Azolla meal for body weight gain, feed conversion ratio and nutrient retention up to 7.5 percent of the diet (Mishra et al., 2016).

Azolla as Human Supplement

The protein content of azolla is almost the same as in soybean. Amongst the vegetables, Azolla is very rich in minerals (10 to 15% of the dry weight), essential amino acids (7 to 10% dry weight), vitamins and carotenoids. Azolla is also a very good source of protein (20 to 30% of the dry weight). Azolla protein is of fairly good quality (Roy et al., 2016).

Conclusion

Azolla, often referred to as the "green gold mine," is a remarkable aquatic fern with a wide array of applications that benefit both the environment and human industries. Azolla as a natural biofertilizer, revolutionizing sustainable agricultural practices. Beyond agriculture, Azolla's potential extends to livestock and poultry feed, aquaculture, bioremediation, and even as a promising source of biofuel, making it an invaluable asset in various sectors.

With its high protein content and nutrient density, it also shows great promise as a potential food source for both humans and animals, contributing to the fight against food insecurity. Moreover, its role in carbon sequestration and water purification emphasizes its importance in addressing environmental challenges, particularly in combating climate change and promoting ecosystem health.

As research into Azolla's applications continues, it is clear that this versatile fern offers a sustainable, cost-effective solution to numerous global challenges, from improving agricultural productivity to enhancing environmental conservation. Azolla's wide-reaching potential makes it an essential tool for building a greener, more sustainable future.

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