AZOLLA- AN ENVIRONMENT ECO-FRIENDLY PTERIDOPHYTIC SPECIES

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ABSTRACT
The application of Azolla as biofertilizer and all other important uses play a significant role in maintaining or improving the state of global environment. There is a definite need to exploit the potential of the Aquatic Pteridophyte in a more efficient manner in the future, through biotechnological interventions. Azolla is one of the aquatic Pteridophyte that may be used as animal food, as green manure, biofertilizer, for increasing soil fertility, bioremediation of waste water and reclamation of saline soils. Due to its high nutritional values and protein content Azolla is suitable for human consumption and as feed supplement for variety of animals like fish, ducks, cattle, poultry etc. to reduce feed cost. It also finds use in biogas and hydrogen production, as space food, in controlling weeds and mosquitoes.

KEYWORDS: Azolla, Pteridophyte, Biological nitrogen fixation.

INTRODUCTION
Nitrogen (N₂) is an element essential for the support of all forms of life. It is found in amino acids and proteins and many other organic compounds are derived from the nitrogen fixation process. Biological nitrogen fixation is an important part of the microbial processes Biological nitrogen fixation is carried out only by prokaryotes, which may be symbiotic or free living in nature. It is well documented that biological nitrogen fixation mediated by nitrogenise enzymes is a process important to the biological activity.

The name Azolla is derived from Greek word azo (to dry) and allo (to kill) meaning that plant dies when it dries. There are seven or eight extant and more than forty fossil species of Azolla known. The genus is further categorized into two sub-genera Euazolla and Rhizosperma. Euazolla is characterized by the presence of three megaspore floats and consists of five new world species namely A. caroliniana, A. filiculoides, A. mexicana, A. microphylla and A. rubra.

The small fast growing free floating fern has global distribution. Azolla is a dichotomously branched free floating aquatic fern naturally available on moist soils, ditches and marshy ponds. The shape of Indian species is typically triangular measuring about 1.5 to 3.0 cm in length, 1 to 2 cm in breadth. Fronds has tiny roots usually associated with rich microphylla, short branched stem called rhizome covered with small alternate overlapping leaves the sporophyte has dorsiventral organization and each leaf is divided into dorsal and ventral lobe., the ventral lobe is thin almost colourless and distal half is only one celled thick. The aerial dorsal leaf lobe has multilayered mesophyll adaxial and abaxial epidermal tissues, numerous stomata and single celled papillae. In the dorsal leaf lobe there is an ellipsoidal cavity which is formed by the enfoldling of the adaxial epidermis. The cavity largely filled with gases is lined with mucilage which contains the cyanoabiotic Anabaena azollae and a gram positive non-nitrogen fixing bacteria identified as Arthrobacter species.

Azolla is a free-floating water fern that floats on the water and fixes atmospheric Nitrogen in association with N fixing cyanobacterium, Anabaena azollae. This lives endophytically in the dorsal lobes of Azolla leaves that supplies N to the rice crop. Azolla-Anabaena symbiosis is the only plant cyanobacterial symbiosis that is exploited as biofertilizer in agricultural practice. Azolla is the most important symbiotic system of the aquatic environment, which comprises seven species. Azolla contains high Nitrogen content and have used as a green manure and as organic matter sources for wetland rice in Northern Vietnam and China. About 70% of Azolla Nitrogen content was mineralized to NH₄⁺ after 20 days of inoculation. Rice plant can easily uptake of NH₄⁺. Excess amount of Azolla is utilized as compost that is fed in dry land crops and vegetables for the improvement of crop yield. Noteworthy, Azolla having high protein content approximately 23-27% of dry weight bases.
followed by 3-6% nitrogen, 0.5-0.9% phosphorus, and 2-4.5% potassium on dry weight basis respectively, beside other major and micro nutrients present. *Azolla* is able to reclaim soils reducing the weed infestations in rice crops and it was also used in waste water treatment and heavy metal degradation process. Beside these uses it can be also used directly as feed for aquacultures which is termed “Azobiofer”. [8]

As green manure, *Azolla* is grown alone for two to three weeks in flooded fields. Afterwards, water is drained out and *Azolla* fern is incorporated in the field before transplanting of paddy. Otherwise, 4-5 q of fresh *Azolla* is applied in standing water one week after planting of paddy. Dry *Azolla* flakes can be used as poultry feed and green *Azolla* is also a good feed for fish. It can be used as a bio-fertilizer, a mosquito repellent, in the preparation of salads and above all as a bio-scavenger as it takes away all heavy metals.

**Beneficial Aspects of Azolla**

1. It easily grows in wild and can grow under controlled condition also.
2. It can easily be produced in large quantity required as green manure in both the seasons – Kharif and Rabi.
3. It can fix atmospheric CO$_2$ and nitrogen to form carbohydrates and ammonia respectively and after decomposition it adds available nitrogen for crop uptake and organic carbon content to the soil.
4. The oxygen released due to oxygenic photosynthesis, helps the respiration of root system of the crops as well as other soil microorganisms.
5. It solubilises Zn, Fe and Mn and make them available to the rice.
6. *Azolla* suppresses tender weeds such as *Chara* and *Nitella* in a paddy field.
7. *Azolla* releases plant growth regulators and vitamins which enhance the growth of the rice plant.
8. *Azolla* can be a substitute for chemical nitrogenous fertilizers to a certain extent (20 kg/ha) and it increases the crop yield and quality.
9. It increases the utilisation efficiency of chemical fertilizers.
10. It reduces evaporation rate from the irrigated rice field.
11. Reduce disease occurrence
12. Enhance flowering and fruiting
13. Increase plant establishment and survival at seedling or transplanting
14. Improve drought tolerance, allowing watering reduction

**Advantages of Azolla**

1. **Azolla as Biofertilizer in Rice Cultivation**
   *Azolla* is used as a biofertilizer and produces around 300 tonnes of green bio- hectare per year under normal subtropical climate which is comparable to 800 kg of nitrogen (1800 kgs of urea). The important factor in using *Azolla* as a biofertilizer for rice crop is its quick decomposition in soil and efficient availability of its nitrogen to rice plant. 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.

   *Azolla* suitable as a biofertilizer in rice are as follows:-
   1. *Azolla* fixes nitrogen at substantial rates.
   2. *Azolla* has rapid growth
   3. Since *Azolla* floats at the water surface, it cannot complete with rice for light and space.
   4. In most climates, *Azolla* grows best under a partial shade of vegetation which a rice canopy, in its early and intermediate stages of growth can easily provide.
   5. When rice approaches maturity, due to low light intensities under the canopy and depletion of nutrients, *Azolla* begins to die and decompose, thus releasing nutrients into the medium.
   6. *Azolla* decomposes rapidly and therefore the nitrogen it has fixed and the phosphorus and other nutrients it may have observed from the water, perhaps in competition with the rice are rapidly released back in to the medium and made available for uptake by rice during grain development.
   7. *Azolla* has great ability than rice to accumulate potassium in its tissues in low potassium environment; thus, after decomposition, it makes this nutrient available to rice.
   8. A thick *Azolla* mat in a rice field has the side benefit of suppressing weeds.

2. **Azolla as Green Manure**
   *Azolla* can also been used as green manure in the cultivation of water bamboo, arrowhead, taro, Wheat and rice.[9] Incubation of *Azolla* as green manure in water logged soil resulted in rapid mineralization with a release of 60-80% of the nitrogen within two weeks.[10]

3. **Azolla: Beneficial Effects on Crops**
   *Azolla* is beneficial to wheat when applied in a rotating rice-wheat cropping system.[11] *Azolla* increases water holding capacity, organic carbon, ammonium nitrogen, nitrate-nitrogen and its available phosphorus, potassium, calcium and magnesium, while it decreased pH and bulk density, such incorporation significantly raised the yield of mung beans.

4. **Azolla: Beneficial Effects on Physio-chemical Properties of Soil**
   *Azolla* is used to increase soil fertility. *Azolla* application improves soil fertility by increasing total nitrogen, organic carbon and available phosphorus in soil these findings were supported by.[12]

5. **Azolla in Reclamation of Saline Soils**
   Although, *Azolla* is relatively sensitive to salt, cultivation in saline environment for a period of two consecutive years decreased salt content from 0.35-0.15 and desalinate rate (71.4%) was 1.8 times faster than through water leaching and 2.1 times faster than *Sesbania* and...
also reduced the electrical conductivity, pH of acidic soil and increased calcium content of soil.[13]

6. Azolla in Bioremediation
It was found that A. pinnata and Lammna minor removed the heavy metals iron and copper from polluted water. The pollutants at low concentration could be treated by passing it through ponds and can be reused for Agriculture purpose. It has been found that tolerance and phytoaccumulation of chromium by three Azolla species and biofiltration of toxic elements by Azolla biomass. Azolla exhibits a remarkable ability to concentrate metals cu, Cd, Cr, Ni, Pb and nutrients directly from pollutants or sewage water.[14]

7. Azolla as Mosquito Repellent
Azolla can also be used in the control of mosquitoes, for a thick Azolla mat on the water surface can prevent breeding and adult emergence. In a survey of pools, ponds, wells, rice fields and drains found that breeding by Anopliies spp. was almost completely suppressed in water bodies that were completely covered with Azolla. A. pinnata greatly reduced both oviposition and adult emergence of Culex quinquefasciatus say and Anopheles culicifacies giles, but not larval survival. Egg hatchability was partially reduced.[15,16]

8. Azolla in Weed Control
It was also seen that Azolla controls the weed growth. Our findings were supported by and found that an Azolla cover significantly reduced the total amount of weeds; particularly the predominant weed Monochoria vaginalis, through grasses and hedges could not always be controlled.[17]

9. Azolla in Production of Biogas
Anaerobic fermentation of Azolla (or a mixture of Azolla and rice straw) results in the production of methane gas which can be utilized as fuel and remaining effluent can be used as a fertilizer because it contains all the nutrients originally incorporated in plant tissues except for a small percentage of nitrogen lost as ammonia.[8]

10. Azolla and Bioenergy
A non-polluting, high energy fuel when Azolla-Anabaena is grown in a nitrogen-free atmosphere and or a water medium containing nitrate, the nitrogenise in the symbionts, instead of fixing nitrogen evolves hydrogen, using water as the source.[18]

11. Suppressing the Growth of Aquatic Weeds
Weed growth is suppressed when azolla forms a thick, virtually light-proof mat. There are probably two mechanisms for this suppression, the most effective being the light-starvation of young weed seedlings by the blockage of sunlight. The other is the physical resistance to weed seedling emergence created by a heavy, interlocking azolla mat. In some weed-infested rice fields, the benefit from azolla weed suppression may even surpass its benefis as a nitrogen source. Rice seedlings are not affected by azolla’s weed suppression effect because, when transplanted, they stand above the azolla mat.

12. Azolla as Human food
A few researchers have experimented with the preparation of Azolla in soups or “Azolla meatballs as food for man. In Tanzania. Azolla has been reported to be used effectively as traditional cough medicine.[19] Azolla is also used as a salad in western countries due to high quantity of protein.

13. Azolla as a Component of Space Diet
Recent research by Katyama[20] in collaboration with Space Agriculture Task Force suggested Azolla as a component of the space diet during habitation on Mars and found that Azolla was found to meet human nutritional requirements on Mars.

14. Azolla as Nutritional Supplement for Livestock
Azolla is used as food supplement for variety of animals including pigs, rabbits, chickens, ducks and fish.[8] Azolla is harvested in large quantities and utilised as fodder for cattle and pigs. It was also found that broilers feed with Azolla resulted in growth and body weight values similar to those resulting from the use of maize-soya bean meal. Digested Azolla slurry remaining after biogas production was suitable as fish pond fertilizer, in the study lactating cows[21] found that Azolla could be used as feed ingredient with milk yields and fat percentage being maintained at the same levels as with conventional feeds.

Present status of Azolla
Azolla is already being grown commercially in China and Vietnam, where its usefulness has been known for years. Once restricted in use because of propagation problems, the fern is now being used in larger crop areas, Chinese use of Azolla goes back hundreds of years, at least to the Ming dynasty. Its use in Vietnam dates to the 11th century. These two are the only countries with a long history of Azolla cultivation. The practice probably began with recognition that the spontaneous growth of wild Azolla in rice fields had a beneficial effect on the crop. Organized use of the fern could not occur, however, until reliable methods were developed to overwinter and over summer the fern. Since Azolla can only be grown from vegetative material, it must be protected during seasons that are too severe for its survival.
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