

Potential and Growth factor of Pteridophyte : Azolla

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Abstract

Azolla had, high multiplication rate, excellent source of protein for mono-gastric animals, high biomass production and increasing demand as organic food. *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. The present paper gives an overview of some important ecological factors affecting *Azolla*'s growth over the past few decades. Moreover, for the most ecological variables discussed in this study, the authors refer to their recent publications for the habitat requirements of *Azolla* in Anzali wetland. Water availability is the key factor for its growth. Growth is further promoted by optimal light intensity (15-18 Klux), temperature (18°- 28°C) and relative humidity (55-83%). Wind and turbulent water can fragment and kill *Azolla*. The importance of both macro (e.g. phosphorus, nitrogen, potassium, calcium and magnesium) and micronutrients (e.g. molybdenum, cobalt and etc.) has also been confirmed from literature. Various types of insects (e.g. caterpillars), bacteria, fungi and viruses can affect *Azolla* growth. As a conclusion, understanding the habitat requirements of *Azolla* is very helpful for managing this aquatic fern, also for decision making in the context of wetland restoration and conservation management.

Key words : *Azolla*, Bioremediation, Biogas, Reclamation, Sesbania, Desalinate, Sporulation and Fluctuations.

Introduction

The name *Azolla* is derived from Greek word azo (to dry) and allyo (to kill) meaning that plant dies when it dries. The genus *Azolla* established by J. B. Lamarck as early as 1783¹ was placed in the family Salviniaceae of the order Salviniales. However taxonomists have placed it now in monotypic family *Azollaceae*². There are seven or eight extant and more than forty fossil species of *Azolla* known^{3,4}. The genus is further categorized into two sub-genera *Euazolla* and *Rhizosperma*¹. *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 sub genus *Rhizosperma* consists of two old world species namely *A. pinnata* and *A.*

nilotica possessing floats in the megaspore apparatus. *Azolla imbricata nakai* has been reported as an independent species instead of a sub-species of *Azolla pinnata*⁵. The three Neotropical host species *A. microphylla*, *A. caroliniana* and *A. mexicana* are similar in vegetative morphology and eco physiology⁶. *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 microphylla⁷, short branched stem called rhizome covered with small alternate overlapping leaves the sporophyte has dorsiventral organization⁸. 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 enfolding of the adaxial epidermis. The cavity largely filled with gases is lined with mucilage⁹ which contains the cyanobiont *Anabaena azollae*¹⁰ and a gram positive non-nitrogen fixing bacteria¹¹ identified as *Arthrobacter* species¹².

BENEFICIAL ASPECTS FOR PLANTS

The experiments have shown that *Azolla* can overcome nutrient limitation to plant growth by enhancing nutrient acquisition, According to the plant species and to the growing practices and conditions, *Azolla* provide different benefits to the plants and to the environment :-

- Increase yields and crop quality
- Reduce disease occurrence
- Enhance flowering and fruiting
- Increase plant establishment and survival at seedling or transplanting
- Produce more vigorous and healthy plants
- Improve drought tolerance, allowing watering reduction
- Optimize fertilizers use, especially nitrogen
- Increase tolerance to soil salinity

- Contribute to maintain soil quality and nutrient cycling

1. 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.

2. As Green Manure

Azolla can also been used as green manure in the cultivation of water bamboo, arrowhead, taro, Wheat and rice¹³⁻¹⁵. 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 weeks¹⁶.

3. As Beneficial Effects on Crops

Azolla is beneficial to wheat when applied in a rotating rice-wheat cropping system¹⁷. Mahapatra and Sharma¹⁸ found that application of *Azolla* with *Sesbania* had beneficial residual effect on subsequent wheat crops, raising grain yield by 56-69 % over control.

4. As Beneficial Effects on Physio-chemical Properties of Soil

Azolla is used to increase soil fertility. Singh and Singh¹⁹ found that *Azolla* application improves soil fertility by increasing total nitrogen, organic carbon and available phosphorus in soil these findings were supported by^{20,21}. Van Hove¹³ found that *Azolla* improves soil structure.

5. 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 soil²².

6. As Bioremediation

It was found that *A. pinnata* and *Lamna minor* removed the heavy metals iron and copper from polluted water²³. The pollutants at low concentration could be treated by passing it through ponds and can be reused for Agriculture purpose. *Azolla* exhibits a remarkable ability to concentrate metals Cu, Cd, Cr, Ni, Pb and nutrients directly from polluted waters.

7. 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. It prevents breeding and growth of *Anopheles* and *Culex* mosquito.

8. In Weed Control

It was also seen that *Azolla* controls the weed growth. Our findings were supported by²⁴ 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.

9. 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 ammonia¹³.

10. As 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 nitrogenase in the symbionts, instead of fixing nitrogen evolves hydrogen, using water as the source²⁵. With cow dung azolla residues (1:0.4 ratio) give a gas production 1.4 times that of a cow dung alone.

11. As Human food

A few researchers have experimented with the preparation of *Azolla* in soups or “*Azolla* meatballs as food for man. However such recipes are as yet unpublished¹³. It is used as effectively as traditional cough medicine. It has high protein values so used as salad in Western countries.

12. As a Component of Space Diet

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.

13. As Nutritional Supplement for Livestock

Azolla is used as food supplement for variety of animals including pigs, rabbits, chickens, ducks and fish¹³. Seultrope²⁶ reported that *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.

Main abiotic and biotic factors affecting *Azolla*'s growth

Azolla is one of the fastest growing aquatic macrophytes, with a doubling time of only 2-5 days. We are briefly describe some of the most important structural habitat and physical-chemical factors to meet the habitat requirements of *Azolla* under laboratory and field conditions.

Structural habitat factors Water

This fern cannot survive without water. In other words, water is a vital and important factor for the survival of *Azolla*. This small aquatic fern should float on the water surface to stay alive. When enough water is available, it can form a layer with a height of around 2-3 cm that needs less space compared to other aquatic plants²⁷. Though, it is able to grow on a wet mud surface or wetted peat litter, this fern prefers to grow in free-floating conditions²⁸.

Air and water temperature

Azolla species and their eco-physiological strains indicating that a very high (above 30°C) or very low temperature (below -4°C) could play an inhibitory role in the growth of *Azolla*. The optimum temperature range for *Azolla* growth has been shown to be between 18 and 28°C²⁹. In fact, higher temperatures (e.g. 35°C) can inhibit or even be harmful for its growth. However, different *Azolla* species, strains or varieties have different temperature sensitivities.

Light intensity

Photosynthetic activity, growth and nitrogen fixation of *Azolla* and its symbiont are all affected by light intensity³⁰. Sporulation is regulated by the interacting effects of light intensity, photoperiod, temperature and other factors such as pH, nitrogen and phosphate supply³⁰. When light intensity is high and amount of nutrients in water is low, *Azolla* turns red. During hot summer or cold winter, it also turns red or brownish-red when under shaded conditions, whereas in nutrient-rich conditions, it becomes green. Irradiance interacts with temperature in influencing growth of *Azolla* species³¹.

Humidity

Increase in the biomass of *Azolla* relies to some extent on air humidity. At relative humidity of less than 60%, *Azolla* becomes dry and fragile³². Sadeghi *et al.* (2012a)³³ demonstrated that when the air humidity in the Anzali wetland exceeds 80%, the prevalence of *Azolla* would be

low. Mean relative humidity for allowing *Azolla* growth was estimated at 55-83% (Lumpkin and Bartholomew, 1986)³⁴ and it was in the range of 65-75% based on the study³⁵.

Growing seasons and day length

The length of the growing season and day length are other climatic factors which regulate production of aquatic plants²⁸. Biomass production and growth of *Azolla* are also dependent on the specific growing season. The growing seasons in *Azolla*, however, are linked with other factors such as nutrients, pH, salinity and wind. A water body with rich phosphorus and neutral pH is better than one with poor phosphorus and acidic conditions. Overall, production in summer is higher than in other seasons³⁶.

Wind and Waves

Wind and waves are other factors affecting the production of aquatic plants, in particular for *Azolla* which is a free-floating fern. In shallow lakes, wind-induced waves can have either direct effects on plant growth (e.g. mechanical damage) or indirect effects such as increased siltation³⁷. Since *Azolla* is a small free floating plant, wind and waves are not favourable for its growth. Water agitation can break up the fronds. This fragmentation as such can have a negative effect on N₂ fixation capacity. Therefore, wind and turbulent water can fragment and kill *Azolla*.

Conclusion

The mankind is threatened by drastic global environmental changes triggered by his own activities, we need to investigate and develop alternative strategies for conducting our affairs. 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. Therefore a combination of approaches involving basic and applied research should be taken towards making *Azolla* more resistant to environmental fluctuation and also less labour-intensive, so that its actual utilization is diversified and enhanced in Agriculture, industry and environmental

management.

The

most

important

structural habitat variables in order to meet the habitat requirements of *Azolla* including water, light intensity, air and water temperature, relative humidity, wind velocity and waves. Moreover, the importance of physical-chemical variables for *Azolla* has been confirmed from the cited literature.

References:

1. H.K. Svenson, The new world species of *Azolla* Tam .Fern. J. 1944 pp. 34-69.
2. R.N. Konar, R.K. Kapoor, Anatomical studies on *Azolla pinnata* *Phytomorphol.* 22 (1972) 211-223.
3. K. Fowler, Megaspores and massulae of *Azolla prisca* from Oligocene of the isle of weight Palaeontology. 18 (1975) 483-486.
4. L. V. Hills, B. Gopal, *Azolla* its phylogenetic significance *Can. J. Bot.* 45 (1967) 1179-1191.
5. Y.X. Lin, Classification of *Azolla* and wide use of certain species *Acta. Phytotox. Sin.* 18 (1980) 450-456.
6. W.J. Zimmermann, I. Watanabe, T.A. Lumpkin. The *Anabaena-Azolla* symbiosis: diversity and relatedness of neotropical host taxa. *Plant. Soil.* 137 (1991) 167-170.
7. P.A. Roger, P.A. Renaud, Ecology of blue green algae in paddy fields in nitrogen and rice IRRI, Los Benos.1979 pp. 289-309.
8. G.A. Peters, H.E. Calvert. The *Azolla-Anabaena*, symbiosis, Cambridge Univ. Press.Cambridge, 1983 pp. 109-145.
9. T.A. Lumpkin, D.L. Pluckmett. *Azolla-* Botany, Physiology and use as a green manure, *Econ. Bot.* 34 (1980) 111 – 153.
10. G.A. Peters, W.R. Evans, D.R. Crist, B.C. Mayne, R.E. Poole, Characterization and comparison of five nitrogen-fixing *Azolla-Anabaena* association I. optimization of growth conditions for biomass increase and N- content in controlled environment, *Plant Cell Environ* 3 (1980) 261-269.
11. B. Hates, O. Frank, B.D. Angells, S. Feingold, Plasma tocopherol in man at various times after ingesting free or acetylated tocopherol, *Nutr. Rep. Int.*, 21 (1980) 531 – 536.
12. M. Grilli Caiola, C. Fornic, M. Castagnola, Bacteria in the *Azolla–Anabaena* association *Symbiosis*, 2 (1988)185–198.
13. C. Van Hove, *Azolla* and its multiple use with emphasis on Africa, Food and Agriculture Organization, Rome, 21 (1989) 112-116.
14. T.S. Marwaha, B.V. Singh, S.K Goyal, Effects of incorporation of *Azolla* on wheat (*Triticum aestivum* var. HD-2329), *Acta. Bot. Indica.*, 20 (1992) 218-220.
15. E.V.D. Teckle-Haimanot, Comparison of *Azolla mexicana* and N and P Fertilization on Paddy taro (*Colocasia esculenta*) yield, *Trop. Agric. (Trinidad)*,72 (1995) 70-72.
16. O. Ito, I. Watanabe, Availability to rice plants of nitrogen fixed by *Azolla*, *Soil Sci. Pl. Nutr.*, 34 (1985) 91-104.

17. S.S. Kolhe, B.N. Mitra, *Azolla* as an organic source of nitrogen in rice-wheat cropping system, Trop. Agric. (Trinidad), 67 (1990) 267-269.
18. B.S. Mahapatra, G.L. Sharma, Integrated management of *Sesbania*, *Azolla* and urea nitrogen in lowland rice under a rice-wheat cropping system, J. Agric. Sci. (Cambridge), 113 (1989) 203-206.
19. A.L. Singh, P.K. Singh, Intercropping of *Azolla* bio fertilizer with rice at different crop geometry, Trop. Agric (Trinidad) 67 (1990) 350-354.
20. K.B. Satapathy, Effect of different plant spacing pattern on the growth of *Azolla* and rice, Indian J. Pl. Physiol.,36 (1993) 98-102.
21. S. Kannaiyan, S.J. Arun, S.M.P. Kumari, D.O. Hall, Immobilized cyanobacteria *A. azollae*- a symbiont of *azolla* as a biofertilizer for rice crops, J. Appl. Phycol.,7 (1997)1-9.
22. P. Anjuli, R. Prasanna, P.K. Singh, Biological significance of and its utilization in agriculture, Proc. Indian Natl. Sci. Acad., 70 (2004) 299-333.
23. S.K. Jain, P. Vasudevan, N.K. Jha, Removal of some heavy metals from polluted water by aquatic plants: studies on duckweed and water valvet. Biol. Wastes., 28 (1989) 115-126.
24. T. Krock, J. Alkamper, I. Watanabe, *Azolla* contribution to weed control in rice cultivation. Pl. Res. Develop., 34 (1991) 117-129.
25. J.W. Newton, Photoproduction of molecular hydrogen by a plant-algal symbiotic system, Science., 191 (1976) 559-561.
26. C.D. Sealthrope, The biology of aquatic vascular plants, Edward Arnold (Pub) Ltd. London., 1967, pp. 610-615.
27. X. Liu, Min, C., Xia-shi, L. and Chungchu, L. (2008) Research on some functions of *Azolla* in CELSS system. Acta Astronautica. 63, 1061-1066.
28. M.S. Serag, El-Hakeem, A., Badway, M. and Mousa, M.A, (2000) On the ecology of *A. filiculoides* Lam. in Damietta District, Egypt. Limnologica. 30, 73-81.
29. D.T. Tuan and Thuyet, T.Q. (1979) Use of *Azolla* in rice production in Vietnam. In: Nitrogen and Rice. International Rice Research Institute, Los Banos, Philippines, pp 395-405.
30. A. Pabby, Prasanna, R. and Singh, P.K. (2003) *Azolla*-*Anabaena* symbiosis- from traditional agriculture to biotechnology, Indian Journal of Biotechnology. 2, 26-37.
31. R. Janes, (1998) Growth and survival of *A. filiculoides* in Britain.1. Vegetative reproduction. New Phytologist. 138, 367-376.
32. M. Biswas, Parveen, S., Shimosawa, H. and Nakagoshi, N. (2005) Effects of *Azolla* species on weed emergence in a rice paddy ecosystem. Weed Biology management. 5, 176-183.
33. R. Sadeghi, Zarkami, R., Sabetraftar, K. and Van Damme, P. (2012a) Use of support vector machines (SVMs) to predict distribution of an invasive water fern *Azolla filiculoides* (Lam.) in Anzali wetland, southern Caspian Sea, Iran. Ecological Modelling. 244, 117-126.
34. T.A. Lumpkin and Bartholomew, D.R. (1986) Predictive models for the growth response of eight *Azolla* accessions to climatic variables. Crop Science. 26, 107-111.
35. M. Biswas, Parveen, S., Shimosawa, H. and Nakagoshi, N. (2005) Effects of *Azolla* species on weed emergence in a rice paddy ecosystem. Weed Biology management. 5, 176-183.

36. E.N. Speelman, Van Kempen, M.L., Barke, J., Brinkhuis, H., Reichart, G.J., Smolders, A.J.P., Roelofs, J.G.M., Sangiorgi, F., De Leeuw, J.W., Lotter, A.F. and Sinninghe Damste, J.S. (2009) The Eocene Arctic Azolla bloom: environmental conditions, productivity and carbon drawdown. *Geobiology*. 7, 155–170.
37. J.E. Vermaat, Santamaría, L. and Roos, P.J. (2000) Water flow across and sediment trapping in submerged beds of contrasting growth form. *Arch Hydrobiology*. 148, 549-562.

