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ENHANCING SUSTAINABLE AGRICULTURE THROUGH ADVANCE UTILIZATION OF THE *AZOLLA-ANABAENA* SYMBIOTIC SYSTEM

Sanjay Koushal[1], Okram Ricky Devi[2]*, Bibek Laishram[2], Satsih Dileep Bhutekar[3], Hridesh Harsha Sarma[2] and Abhijit Debnath[4]
[1]Sher-e-Kashmir University of Agricultural Sciences & Technology,
Main Campus Chatha Jammu-180009, India
[2]Department of Agronomy, Assam Agricultural University, Jorhat, Assam, India
[3]Sun Grow Albion LLC New York, USA
[4]Subject Matter Specialist, Krishi Vigyan Kendra Dhalai, Tripura, India -799278
*Corresponding author E-mail: rickydeviokram@gmail.com

ABSTRACT

The ability of the Azolla-Anabaena relationship to fix atmospheric nitrogen at quicker and less expensive rates and make it accessible to crop plants makes it significant from an agronomic standpoint. The system's ability to fix nitrogen is caused by the cyanobacterium *Anabaena azollae*, which lives in harmony with the leaves' dorsal lobe. The system is significant because it contributes to soil fertility maintenance and enrichment, providing long-term ecological sustainability. due to its numerous other use like animal feed, water purifier, green manure, etc. The phrase "green gold mine" describes the system. It is still a very underused association, though, with a lot of room for abuse. The Azolla-Anabaena system's numerous applications would make its promotion and use environmentally friendly and appropriate for sustainable agriculture. This brief article provides an overview of the development of the Azolla-Anabaena system's application in agriculture and related fields.

***Keywords* :** *Azolla-Anabaena*; Biofertilizer; Nitrogen; Multifaceted Uses; Sustainable Agriculture

Introduction

With a worldwide distribution, the aquatic pteridophyte Azolla is a great biofertilizer and green manure. The Azolla-anabaena system is an excellent agronomic option for rice growing in tropical climates because to its quicker rate of atmospheric nitrogen fixation. One crop of Azolla supplied 20–40 kg N ha^{-1} to the rice crop in around 20–25 days, indicating that the Azolla-Anabaena system has an estimated 1.1 kg N ha^{-1} day^{-1} nitrogen fixation capability (Wat anabe *et al.*, 1977). The heterocystous cyanobacterium Anabaena azollae, which is restricted to the dorsal leaf cavity of the fern, is responsible for the capacity to fix nitrogen (Moor e, 1969). The adaxial epidermis folds into this depression in the dorsal leaf lobe (Petters, 1976). Azolla is a versatile plant that has grown in significance recently due to its usage as green manure, biofertilizer, bovine fodder, and chicken feed (Singh & Subudhi, 1978). It was noted that the chicks' body weight increased when they were fed Azolla. Azolla is high in vital amino acids and protein. It also includes beta-carotene, vitamin B-12, and vitamin A.

It also contains a lot of calcium, zinc, copper, phosphorus, and magnesium minerals. According to Parashura Mulu *et al.* (2013), Azolla's protein content ranges from 25 to 35% by dry weight and can be easily digested by poultry. Azoll A can be used for a variety of things, like making food for people and animals, making medicine, making biogas, making hydrogen fuel, cleaning water, controlling weeds, and reducing the amount of ammonia that volatilizes. It is likewise normally alluded to as an incredible gold minne (Wagner, 1997). Azolla ants immediately convert enormous amounts of ambient CO_2 into biomass after sequestering it (Speelman *et al.*, 2009). They claimed that the plants perished, sank to the Arctic Ocean's bottom, and evolved into sediments that are currently deposited as a layer there. Therefore, these plants had a significant impact on Earth's temperature 50 million years ago (Bujak, 2007). The plant's capacity for rapid multiplication makes it an outstanding biofertilizer. The organism's demonstrated agronomic potential and the effective utilization of Azolla in the agricultural setting of Asian nations have been noted (Singh, 1977a; Singh, 1989). In addition, numerous papers on the crop's potential and agronomic significance have been published by Watanabe (1982).

More than 128 million hectares of rice are grown in flooded conditions, making it suitable for the cultivation of Azolla. Rainfall patterns, flooding depths, and drainage patterns, as well as rice's adaptation to these agro-ecological factors, define rice ecosystems (IRRI, 1994, Gillar, 2002). Previous studies done at various places around the nation have showed an increase in yield resulting from the use of Azolla. Comparable outcomes have been reported when Azolla and chemical nitrogen fertilizers are used (Singh *et al*., 1992).

When using Azolla in compared to other biofertilizers, rice plants exhibit the highest grain production. Due to Azolla's ability to establish a thick mat in rice fields, weeds are suppressed and the volatilization of ammonia is reduced (Singh, 2000). In India, Azolla is widely used. The species that are most commonly found include *A. pinnata, A. fuiliculoides, A. rubra, A. microphylla, A. mexicana, and A. caroliniana*, in that order (Hills and Gopal, 1967). Reports exist on its effective use in crops other than rice. Azolla has been effectively used in trials to increase wheat output as a biofertilizer (Marwaha *et al*., 1992). Mahapatra and Sharma (1989) noted that Azolla had a positive impact on the next wheat harvest, increasing grain output. The maximum wheat yield was achieved by applying 20 tonnes of Azolla and 60 kg of nitrogen (Sharma *et al*., 1999). In phytoremediation projects, the plant is also utilized to clean up damaged and polluted streams. According to Arora *et al*. (2003), phytoremediation is quickly becoming recognized as a very effective way to protect aquatic ecosystems from pollution.

Nevertheless, despite the plant's benefits, its full potential is still untapped, and systematic efforts are needed to make the system more widely known. As a result, the current research evaluates the advancements made in the effective usage and exploitation of the Azolla-Anabaena system as well as its numerous applications for sustainable farming methods.

Distribution, Habit and Habitat and Morphology

Azolla may be found in tropical and temperate climates. It flourishes in paddy fields, ditches, and freshwater ponds. The delicate, tiny, triangular or polygonal Azolla plants are shaped as seen in Figure 1. It is aquatic and free-floating, but it may also grow on damp soils as long as the moisture stays there. The sporophytic plant features branches with tightly packed, overlapping leaves and a horizontal rhizome with a diameter of 0.5 to 7 cm. A leaf is made up of a tiny ventral lobe and a thick dorsal lobe. Only the dorsal lobe is home to the symbiotic Blue Green Alga (Peters and Mayne, 1974). The dorsal lobe's surface is covered in an epidermis, which includes vertical rows of single-celled stomata and trichomes made up of one or more cells. There are several stomata and trichomes on the ventral lobe, which aids in buoyancy because of its convex surface that touches water (Eames, 1936).

Growth and Multiplication

Azolla grows lushly in ditches, freshwater ponds, and paddy fields and may be found in both temperate and tropical climates. For optimal development, the soil must have a pH of 7.2 and a temperature of 32°C. Studies carried out in other parts of India, however, have demonstrated that the species chosen for use as biofertilizer varies depending on the locality.

At the Indian Agricultural Research Institute's Centre for Conservation and Utilization of Blue Green Algae in New Delhi, researchers compared the capacities of various Azolla species for nitrogen fixation and biomass production (Arora and Singh, 2003). *A. microphylla* was chosen for mass multiplication since it was shown to perform better based on the trials. Because it can tolerate both high and low temperatures, this strain is kept alive all year round.

Taxonomic Status of Azolla

The Genus Azolla was established by Lamarck in the year 1783 and placed it in the family Salviniaceae under the order Salviniales. However,Azolla is placed in the monotypic family Azollaceae and there are seven extant Species ofAzolla (Hills and Gopal, 1967; Konar and Kapoor, 1972). Azolla is categorized into two Sub-Genus viz. EuAzolla and Rhizosperma (Svenson, 1944). The Sub-Genus EuAzolla is characterized by the presence of three floats of megasporocarps and consists of Species such as *A. caroliniana, A. filiculoides, A. mexicana, A. Rubra* and *A. microphylla.* In contrast, the Sub-Genus Rhizosperma consists of nine megaspore floats. *A. pinnata* and *A. nilotica* belong to this Sub-Genus. The trichomes are important in the identification of the organism at the Species level (Lumpkin and Plucknett, 1982; Nayak and Singh, 1988). The basic chromosome number in all Species of the section as well as the section Rhisosperma is n = 22 except in *A. nilotica* where n = 26. The cytological investigations show that the somatic chromosome numbers for Species of the Section Rhizosperma are Rhizosperma are *A. pinnata* R.Br. (India) 2n = 44, *A. pinnata* R.Br. (Africa) 2n = 44, *A. pinnata* R.Br. (Vietnam, Green) 2n = 66 and *A. nilotica* Decne. 2n = 52 (Tan *et al*., 1986). The somatic chromosome numbers for Species of the Section Euazolla are *A. mexicana* Presl. 2n = 48, *Azolla filiculoides* Lam. 2n = 40 with the basic chromosome numbers n = 24 and n = 20, respectively (Nayak and Singh, 1989). The taxonomic assignment of Azolla is difficult because many Accessions do not form sporocarps under culture conditions. The normal mode of reproduction is vegetative but sexual reproduction has also been noticed although this has been observed in limited time periods of the year. This has resulted in problems related to precise identification of the Species. Therefore molecular tools have been employed for precise identification of the species. RFLP and isozyme patterns to identify the Sections of Azolla are employed (Zimmerman *et al*., 1991a; Zimmerman *et al*., 1991b; Coppenolle *et al*., 1993). Taxonomy of the Family Azollaceae is highly controversial (Reid *et al*., 2006). The uniqueness of A. nilotica has been confirmed from the data obtained from the loci of plastid genome (Metzgar *et al*., 2007). Integration of different types of data based on morphology, vegetative characters and molecular biology to provide a firm footing for the taxonomy of Azolla has been suggested (Perreira *et al*., 2011). Species specific SCAR primers (sequence characterized amplified region) for the precise identification of different species of Azolla is developed recently (Abraham *et al*., 2013).

Sporulation and its Importance in *Azolla*

Due of Azolla's mostly vegetative mode of reproduction, biomass maintenance is required all year round. The fern is heterosporous, producing mega and micro sporocarps, and the germination of these depends on a number of variables.

Table 1: Sporocarp germination in *Azolla* as influenced by various conditions

Treatment	No. of sporocarps incubated	No. of sporocarps germinated	Germination (%)
Control (distilled water)	188	31	24.4
IRRI medium (solid)	155	44	37.1
IRRI medium (liquid)	181	37	26.7
Soil solution	184	46	28.9
Phosphorous (30 ppm)	126	100	63
Kinetin (100 ppm)	197	132	55.2
Gibberellic acid (100 ppm)	192	106	48

Singh *et al*., (1990)

Table 2: Effect of Azolla and various organic treatments on rice (Pusa Basmati 1) grain yield during kharif (2013-18)

Treatment*	Rice grain yield (t/ha)						
1	2013	2014	2015	2016	2017	2018	Mean
Azolla (A)	2.87	2.54	2.43	2.36	2.29	2.06	2.43
BGA (B)	2.70	2.46	2.35	2.29	2.19	1.90	2.32
FYM (F)	2.69	2.24	2.26	2.12	2.17	2.04	2.25
Vermicompost (V)	2.90	2.66	2.53	2.60	2.39	2.18	2.54
A+B	3.35	3.25	3.03	3.16	3.02	2.68	3.08
A+F	3.70	3.42	3.38	3.29	3.18	2.57	3.26
A+V	4.08	3.85	3.67	3.57	3.43	2.64	3.54
B+F	3.33	3.26	3.43	3.48	3.37	2.60	3.25
B+V	3.91	3.50	3.47	3.52	3.48	2.82	3.45
F+V	3.75	3.58	3.64	3.56	3.61	3.02	3.53
A+B+F	4.05	3.66	3.79	3.68	3.82	3.34	3.72
A+F+V	4.08	3.70	3.81	3.89	3.93	3.45	3.81
B+F+V	4.10	3.82	3.88	3.93	3.91	3.64	3.88
A+B+F+V	4.19	4.35	4.38	4.16	4.48	3.68	4.20
N80P40K30	4.93	4.68	4.21	4.34	4.61	3.46	4.37
N0 P0K0 (Control)	2.02	1.84	1.76	1.78	1.89	1.68	1.82
C.D (at @5 %P)	0.95	0.48	0.31	0.41	0.32	0.26	0.46

*Rate of application/ha: Azolla 1.0 t (fresh); BGA 2 kg (dry); FYM 5.0 t; Vermicompost 5.0 t.

Nevertheless, the environment affects sporocarp production, and there is variation in the timing of sporocarp synthesis by the plants. Azolla microphylla, for instance, sporulates all year round (Kar *et al*., 1999). It has been discovered that plant population density, particularly high density, influences sporocarp production efficiency (Watanabe, 1982). In Cuttack, sporocarp production was often aided by cold nights and relatively short days (Singh *et al*., 1987). A combination of nitrogen sources influences the increase of sporocarp germination. *Azolla caroliniana* sporocarp germination is sensitive to light, carbohydrates, amino acids, and abscissic acid (Singh *et al*., 1990). There have also been reports of Azolla megasporocarps and their germination in various paddy soils (Nayak *et al*., 2004).

Chemical composition of *Azolla*

Gibberellic acid treatment has been shown to enhance sporulation in *Azolla microphylla* and *Azolla pinnata* (Kar *et al*., 1999). There have also been reports of similarly high sporocarp yields in connection with the exogenous administration of certain auxins in conjunction with gibbrellic acid (Kar *et al*., 2002). But in addition to being expensive, these chemical-based techniques also pollute the environment. Fertilizer administration has been shown to affect sporocarp production (Singh *et al*., 1987), and it has been documented that phosphorus deficiency can induce sporulation (Kannaiyan *et al*., 1988).The alteration in the phosphorous administration schedule in Azolla results in an increase in the frequency of sporulation and the amount of sporocarps, all without compromising the biomass production (Kar *et al*., 2001). After three to five months of dormancy, the sporocarps begin to germinate; from August to October, the percentage of germination is greater (Singh *et al*., 1984b). A significant amount of new inoculums must be transported by farmers each time they need to apply them in the field, which frequently results in the culture perishing. This is also one of the factors contributing to the farmers' poor *Azolla* adoption. So, in order to increase the efficacy of the *Azolla* biofertilizer technology, significant efforts must be undertaken to effectively propagate the fern through sporocarps.

Production Technology for *Azolla* Biofertilizer and Modes of Application

Azolla is often propagated in nurseries that are situated in soil. Nonetheless, it is advised to keep the cultures based on germplasm medium. There are reports on the impact of the medium's nutritional status on Azolla production and nitrogen fixation (Kushari and Taheruzzaman, 1990). The growth, pigments, nitrogen fixation, and nutritional status of Azolla grown in soil and nutrient-based cultures were compared, and the results indicated that the soil-based cultures were just as excellent as the ones grown on nutrient medium (Dawar and Singh, 2002). Nursery plots, ponds, ditches, canals, concrete tanks, and ditches coated with polythene can all be used for azolla cultivation. Azolla growing requires a carefully prepared and properly flat area. Plots of 20 m by 2 m are typically constructed in fields with appropriate bunds and irrigation canals that have water at a minimum depth of 10 cm. Water (20 liters) is given to each plot, and Azolla (8–10 kg) is infected. Every four days, a single super phosphate (100 g) in two to three split doses is administered to each plot. In the plots (100 g plot^{-1}), furadon or carbofuran (3% active granules) can be administered either before or after one week of inoculation. After 15 days, 100–150 kg of fresh azolla may be picked from each plot. Azolla may be made using the same process in bigger plots. The input amounts must be adjusted according to the needs. Azolla can also be kept in a nursery in trays or any size of earthen or cement pot, depending on the inocula's availability and requirements. Nutrients and pesticides are not used if the production is done in a pond or canal. There have been reports of using animal manure and cattle slurry to produce and use Azolla as a biofertilizer for rice (Singh *et al*., 1993). Animal dung and calf slurry work well as phosphorous fertilizers, and adding Azolla to the soil has been shown to increase its C, N and accessible P content.

Methods of Application of *Azolla*

Azolla is most frequently used in the field as dual crops with rice or as green manure. Azolla that has been directly gathered from ponds or ditches is spread in the field as green manure. It may be used in the field as well as cultivated in nurseries, as previously mentioned. After application, a thick layer of Azolla will form and may be mixed into the soil in two to three weeks. After that, rice can also be planted elsewhere in the field. Split dosages of a single super phosphate (25–50 kg ha^{-1}) are used. The dosage of the same might be lowered when the soil P-status has been examined. Alternatively, single super phosphate might be substituted with cattle manure or slurry. In case of pest infestation or attack, pest control measures have to be undertaken. *Azolla* application by this mode contributes around 20-40 kg N ha^{-1}.

Dual cropping involves growing Azolla alongside rice, with each crop providing an average of 30 kg N

ha^{-1}. Following a period of 7–10 days following transplantation, new Azolla inoculums are sown at a rate of 0.50–1.0 tons per hectare. Split doses of one superphosphate are administered at a rate of 20 kg ha^{-1}. Azolla grows into a thick mat in 15 to 20 days. As a result, Azolla breaks down and releases the fixed nitrogen in 8 to 10 days. During the rice crop cycle, another crop of Azolla can be grown in a manner similar to this. The technique used to produce azolla is straightforward, reasonably priced, and very effective in terms of nitrogen and biomass accumulation. The rice growing season is also conducive for the growth of Azolla plants. The dual application does not have any negative influence on the rice crop.

Biological Nitrogen Fixation and *Azolla* in Relation to Soil Fertility

The organism is being used as biofertilizer because to its significantly greater rates of atmospheric nitrogen fixation. Enhancing the soil fertility index is one of the benefits of using Azolla in rice paddies. The symbiotic cyanobacterium Anabaena, which lives in the dorsal leaf chambers of the fronds, is what gives the fronds their capacity to fix nitrogen (Peters and Meeks, 1989). The symbiont is able to supply all of the association's nitrogen needs. According to Van Hove (1989), the Calvin cycle functions in both partners, and sucrose is the main byproduct of photosynthesis. There is a close relationship between nitrogen fixation and photosynthesis, which produces ATP and NADPH. According to Plumpkin and Plucknett (1980), Azolla has the ability to fix nitrogen in the field at a rate of approximately 1.1 kg N ha^{-1} day^{-1}. This nitrogen fixed is enough to meet the rice crop's entire nitrogen requirement in a matter of weeks. The organism is an excellent agronomic choice due to its high biomass output and quick, significantly higher rates of nitrogen fixation.

Because Azolla strains have the ability to fix nitrogen, they have been effectively used as an effective biofertilizer for rice paddies. The use of Azolla as a prospective biofertilizer has been successfully disseminated in the Eastern regions of India by Dr. P K Singh and colleagues from the Central Rice Research Institute, Cuttack. A. pinnata generates 347 tons of fresh weight ha^{-1} of biomass annually and fixes 75 mg N g^{-1} dry weight day^{-1}. There are 868 kg N in this biomass, which is the same as 1900 kg urea. Azolla strains vary greatly in their capacity to fix nitrogen and proliferate, according to research done at the Central Rice Research Institute in Cuttack (Singh, 1988). Nutrient availability, pace, and timing of inoculation are a few of the variables that affect Azolla's capacity to develop and fix nitrogen (Kannaiyan, 1993; Singh and Singh, 1995). It has been documented that Azolla inoculation improves the biological health of the soil in addition to maintaining rice production. To maintain crop yield, it's critical to maximize the utilization of organic, inorganic, and biological inputs in an integrated way while taking the soil's ecology and ecological circumstances into account. One measure of microbial activity and soil fertility is soil enzyme activity. Azolla breaks down quickly in the soil and gives crop plants nitrogen. In addition to adding nitrogen, it also provides noteworthy levels of potassium, sulfur, phosphorus, zinc, iron, and molybdenum, among other micronutrients. The application of Azolla has improved the biological health of the soil, leading to an increase in the microbiological condition of the soil and improved mineralization. One essential mechanism in lowland rice farming is the conversion of organic nitrogen to ammonia (Sahrawat, 1983). A few examples of the parameters that affect the rate of mineralization include C: N. Low C:N Azolla species took two days to mineralize, but high C:N Azolla species took five days (Wang *et al*., 1987). Regardless of how long it takes for mineralization to occur, the microbial population is actively developed by the degraded organic waste. The humic materials produced during Azolla's breakdown have an impact on soil fertility as well (Bhardwaj and Gaur, 1970). Azolla and Sesbania are employed as green manures for wetland rice farming (Ventura and Watanabe, 1993). The soil's organic nitrogen concentration considerably rose with ongoing treatment. There was a notable rise in the number of heterotrophic bacteria, as well as increased celluloytic and urea hydrolyzing activities. (Kannaiyan and Subramani, 1992; Kannaiyan and Kalidurai, 1995). Increased soil urease and phosphatase activity has also been observed due to incorporation of *Azolla* (Thanikachalam *et al*., 1984; Thangaraju and Kannaiyan, 1989).

The activity of soil enzymes like dehydrogenase, phosphatase, cellulose, and amylase is significantly increased when nitrogen-fixing green manures like sesbania and azolla are combined (Kumar & Kannaiyan, 1992). According to Gopalaswamy and Kannaiyan (2000c), there was a comparable increase in the microbial population, total bacterial, cellulolytic, phosphate solubilizing, and urea hydrolyzing bacteria. By replenishing the soil with nitrogen in amounts that are about equivalent to those that the rice plants remove from it, azolla aids in maintaining the availability of nitrogen in the soil (Cisse and Vlek, 2003). According to reports, organic farming utilizing Azolla as one of the components resulted in the maximum population of bacteria, fungus, and actinomycetes as well as high urease and dehydrogenase activities (Krishnakumar *et al*., 2005). The Indian Agricultural Research Institute in New Delhi is home to the Centre for Conservation and Utilization of Blue Green Algae, where field experiment research have been carried out. The best production of organic Basmati rice was seen in these investigations, along with improvements in the quality of the grain and soil. Azolla, bluegreen algae, farmyard manure, and vermicompost were among the organic amendments employed in this study (Singh *et al*., 2007).

Azolla has been shown to be useful in the production of rice in experiments carried out at Banaras Hindu University in Varanasi (Bhuvaneshwari, 2012). According to Bhuvaneshwari and Kumar (2013), the application of Azolla has been found to greatly enhance the physical and chemical qualities of the soil, notably nitrogen, organic matter, and other cations released into the soil, such as calcium, sodium, and magnesium.

Improvement in Crop Productivity Due to Application of *Azolla*

Rice productivity and the availability of nitrogenous fertilizers are correlated. Additionally, rice crops can receive nitrogen supplementation from biofertilizers such cyanobacteria and Azolla. Since both organisms have comparable development requirements, applying Azolla to rice plants is excellent. One notable feature of the organism's use as biofertilizer is its rapid multiplication rate. Singh (1989) has emphasized the successful utilization of this organism in the Asian environment. High yielding rice types were used in field tests at the Central Rice Research Institute in Cuttack, which revealed that applying 10 tons ha^{-1} of fresh Azolla is just as effective as applying 30 kg of N at the basal level (Singh, 1977; Singh, 1978). Increases in height, tiller, dry matter, panicle weight and number, grain and straw output, etc. were the outcomes of their research. Numerous tests carried out at various sites have proven an increase in yield as a result of the use of Azolla. Azolla yields 20–40 kg N ha^{-1} in a single crop (Singh, 1977b). Comparable outcomes have been seen when Azolla and chemical nitrogen fertilizers are used together. The maximum grain production in rice is noted when comparing Azolla with other biofertilizers (Singh *et al*., 1992). The nitrogen component of the organism is released for the rice plants to use when it has broken down. The plant broke down 8–10 days after being incorporated into the soil, and rice plants started to benefit 20–30 days later (Singh, 1977d). Azolla releases nitrogen slowly, and the first rice crop may use it at a rate that is almost 70% higher than that of ammonium sulphate. Furthermore, compared to cyanobacteria that fix nitrogen, the release of nitrogen occurs more quickly (Saha *et al*., 1982).

Fresh algal lamination releases nitrogen more quickly than dried algal lamination because nitrogen mineralizes more quickly at ambient temperature (Singh, 1979a; Singh, 1979b). The N release effectiveness of chemical N fertilizers, such as Azolla and BGA, and various organic manures, was compared in Cuttack. Azolla was discovered to release nitrogen at a much slower rate; in contrast, artificial fertilizers release 87% of its nitrogen within ten days.

Thus, using Azolla in addition to chemical fertilizers presents a great option for rice farming (Singh *et al*., 1981). Azolla alone and chemical nitrogen fertilizer (150 kg N ha^{-1}) have been shown to increase grain output (Singh *et al*., 1992). Field tests conducted at the Central Rice Research Institute in Cuttack showed that the application of Azolla considerably increased crop production and crop N absorption when compared to treatments that did not include Azolla (Manna and Singh, 1989). There have also been reports of *Azolla pinnata* being used as a biofertilizer in Kerala's acidic soils (Sevichan and Madhusoodanan, 1998). Additionally, it is noted that Azolla's creation of a thick mat in rice fields suppresses weeds and reduces ammonia volatilization (Singh *et al*., 1981). By adding phosphorous to P-enriched inocula, Azolla dual cropping with rice increased biomass and N production when compared to un-enriched *Azolla caroliniana* (Singh and Singh, 1995). In an effort to enhance the nitrogen and phosphorous balance in contaminated settings, the system was potentially exploited as phosphorous biofertilizer (Singh *et al*., 2010).

Azolla as an Input in Organic Agriculture

There has been a recent increase in interest in organic farming, and Azolla is utilized as a significant and possible ingredient in organic rice growing. Prior research has indicated a positive correlation between rice yield and the BGA and Azolla (Singh and Mandal, 1997; Singh and Mandal, 2000). Azolla has shown to be an effective addition to organic farming practices for agricultural systems centered around rice. From 2003 to 2009, field tests were carried out at the Indian Agricultural Research Institute in New Delhi to determine the most appropriate organic amendments for the Basmati rice, wheat, and green gram cropping system's sustainable production (Singh *et al*., 2011). Various combinations of treatments including organic additions like Azolla (1.0 ton ha^{-1}) and Blue Green

Algae (2.0 kg ha^{-1}), Vermicompost and farm yard manure treated alone or in combination at a rate of 5.0 tons ha^{-1} were studied. The application of organic amendments, such as Azolla, either alone or in combination, was found to significantly increase rice grain production above the absolute control (Table 2). When four amendments (Azolla, BGA, vermicompost, and FYM) are applied simultaneously, the best yield of Basmati rice (cv. Pusa Basmati 1) may be achieved every year. Higher productivity of vegetables including cauliflower, broccoli, cabbage, and carrot produced after organic rice under organic nutrition were noted in addition to improving and maintaining the productivity of the organic rice-wheat system (Singh *et al.*, 2012). During these years, there was no significant occurrence of any disease or insect pest in the rice crop farmed organically. The application of organic amendments, as opposed to total control and recommended fertilizer application, was found to improve microbial populations (Actinomycetes, Bacteria, Fungi, and BGA) over time. This improvement was observed to be accompanied by a notable increase in dehydrogenase enzyme activity. Because of fertilization, application of organic matter, and other treatments, microbial communities' composition has changed (Irisarri *et al.*, 2001; Jha *et al.*, 2004; Singh and Dhar, 2011; Singh *et al.*, 2012). The important ion levels of iron, zinc, manganese, and copper in rice grains were significantly higher in the treatments where two or more organic amendments were provided overall over the control. Bhattacharya and Chakraborty discovered similar outcomes (2005). Azolla inoculation and organic nutrient management demonstrated a significant build-up in the organic carbon content of the soil. In comparison to inorganic nitrogen management (INM) and chemical fertilization, the values of soil physical parameters such as accessible water content (AWC) and water retention capacity (WRC) are greater under organic management. Both micro- and macro-porosity increased in tandem with the rise in AWC. In comparison to INM and chemical fertilizer treatments, lower bulk density (BD) was noted in the organic treatment (Singh *et al.*, 2012). The integrated nutrient management (INM) of rice, which included the use of Azolla in addition to chemical fertilizer, also yielded the highest net return. Organic management, which included four inoculants, including Azolla, came in second. These findings unequivocally demonstrate how Azolla might be effectively used with other bio-inoculants in organic rice and vegetable-based farming.

Use of *Azolla* as Livestock Feed

Azolla's significance as a sustainable feed source for poultry and cattle has recently been examined (Gouri *et al.*, 2012). They claim that Azolla is a perfect feed for fish, pigs, sheep, goats, cattle, and rabbits (Fig. 3). Azolla's high nutritional content makes it suitable for use as fish and cow fodder in addition to poultry feed. It contains amino acids, proteins, vitamins, calcium, phosphorus, iron, copper, magnesium, and beta-carotene (Table 3). Azolla may be kept alive all year long with the right planning and production techniques, which will guarantee the organism's availability for use as fodder throughout the year. Since birds dislike dried azolla, fresh biomass makes a good poultry feed that has no negative consequences (Singh and Subudhi, 1978a). Fresh Azolla biomass may be added to commercial feed to replace around 20–25% of it (Subudhi and Singh, 1978b). It is proposed that using azolla as animal feed will benefit livestock production, which is a crucial aspect of agriculture (Banerjee and Matai, 1990).

Research on *Azolla pinnata* shows that the leaf protein's amino acid content compares nicely to both the FAO reference pattern and chick needs (Dewanji, 1993). According to Bishwanath and Vishwanath (1997), adding Azolla powder to the fish meal and feeding trials increases the weight of the carp *Osteobrama belangeri* and improves feed conversion efficiency and protein efficiency ratio. *Tilapia mossambica* fish were given an Azolla protein supplement, and researchers saw increases in eating, absorption, and growth rate (Sithara and Kamalaveni, 2008). They discovered that combining the Azolla biomass with other agricultural wastes, such rice and wheat bran, would enhance the protein's digestion and quality. Azolla meal may be a viable and novel source of protein, according to feeding studies done on buffalo calves (Indira *et al.*, 2012).

Even though the majority of these research support using fresh biomass, some demonstrate that *Labeo rohita* significantly increased when heat-treated Azolla meal was partially substituted for fish meal (Maity and Patra, 2008). An investigation was conducted to find out how broiler chicken production performance was affected by adding ground and sun-dried Azolla (*Azolla pinnata*) to the diet (Balaji *et al.*, 2009). It is discovered that birds given 4.5% Azolla had a much greater percentage of geittal yield compared to control and other treatments. The trials' results indicate that adding dry Azolla to the feed up to a 4.5% level has no negative effects on broiler chicken productivity. Due to their ease of cultivation, productivity, and nutritional content, aquatic plant species, particularly Azolla, are noted to have a considerable potential as an animal source of protein (Prabha and Kumar, 2010). Consequently, given the significance of organic aquaculture today, the application of Azolla is discovered to be among the least expensive methods of raising fish yield in organic fish farming.

Azolla has been shown to boost fish output in organic aquaculture, and it is highly recommended that this technique can be a useful means of raising farm revenue and living standards for low-income farmers in the Meghalaya region (Majhi *et al*., 2006). According to Rai *et al*. (2012), poultry fed on Azolla exhibit a notable increase in their body weights, which has led to a rise in the net return. The overwhelming majority of these research point to the enormous potential for both lowering the cost of feeding and using Azolla biomass in feed. Nonetheless, consideration must be given to the protein quality as well as the nutritional and amino acid content of the various species of these plants. This will help in the selection of promising species with superior protein quality, amino acid and nutrient composition that can be used as efficient dietary supplement.

Bioremediation Potential of *Azolla*

Azolla's use in the bioremediation process is another intriguing and practical use for the material that has gained favor recently. Massive amounts of contaminants have been released into water resources as a result of population growth and fast industrialization. Because of their detrimental effects on the health of plants and animals, the discharged pollutants are hazardous in nature and should be taken very seriously. Consequently, phytoremediation presents a great way to protect aquatic environments from pollution. The contaminated and polluted waterways can be cleaned up using azolla (Table 3). According to Antunes *et al*. (2001), the fern *Azolla filiculoides* may effectively remove gold from waste water solutions.

Similarly Elmachliy *et al*. (2010) demonstrated removal of Silver and Lead ions from waste waters using *Azolla filiculoides*. Successful cultivation of *A. microphylla* biomass in secondary treated Municipal waste water of Delhi is attempted by Arora and Saxena (2005).

Additionally, azolla biomass is used in the phytoremediation of heavy metals that are poisonous. *Azolla microphylla, A. pinnata* and *A. filiculoides* are three distinct species that have been researched recently for their potential as phytoremediation sites. The results show that *A. microphylla* has acquired more metal than the other species (Arora *et al*., 2006). Azolla's biomass, both dead and living, has also been utilized to biosorb a number of heavy metals (Umali *et al*., 2006; Mashkani and Ghazvini, 2009). The removal of heavy metals from aquatic settings has been extensively studied using Azolla (Rai 2008; Rai and Tripathi, 2009; Rai 2010a; Rai, 2010b). However, this is an area where no serious efforts have been attempted despite the potential of the organism for bioremediation. Capability of the organism to reduce these ions in to metallic particles is possible since the plant itself could act as a strong reducing agent. Recently, a hydroponic system was created to purify water that contains copper and cadmium (Valderrama *et al*., 2012). Azolla's potential for phytoremediation was recently examined. These findings demonstrate that Azolla may be used to effectively phytoremediate contaminated waterways. The wastewater from municipal sewage systems is highly contaminated with nitrogen and phosphorus. To mitigate this, bioremediation initiatives utilizing amoeba may be implemented. If the utilized biomass doesn't contain any heavy metals, it can be used as green manure (Sood *et al*., 2011). Nevertheless, the biomass may be burned to stop the recycling of the heavy metal in the environment or dried and extracted for the purpose of recovering the metal when utilized for bioremediation of waste water containing heavy metal load.

Table 3: Bioaccumulation potential of heavy metals by various *Azolla* spp.

***Azolla* spp**	**Heavy metal**	**Concentration of heavy metal accumulated (µg metal g^{-1})**
A. pinnata	Cadmium Mercury	2759 450
A. caroliniana	Lead Chromium	416 964
A. filiculoides	Nickel Chromium	28443 12383
A. microphylla	Nickel Cadmium	21785 1805

Research Perspectives for Future Research

Maintaining sustainability will be a difficult undertaking in light of the diminishing productivity and health of the soil as a result of rising human activity. Azolla-Anabaena system has several applications and is a great biofertilizer for rice crops. Focused effort is needed to increase its utility in the agricultural and related areas. Therefore, it is imperative to fix a few major difficulties with Azolla in order to effectively utilize and exploit it. These include the challenges with identification, sporulation observation, sporocarp storage, and abiotic stress tolerance, among other things. Priority attention must be given to these problems in order to increase the organism's usefulness in agriculture and related fields. Given the advancements in morphological and genetic approaches to taxonomical study on Azolla, an integrative strategy is required to address classification issues involving many accessions in accordance with the International Rice study Institute's list in the Philippines.

Therefore, in order to identify species and produce molecular fingerprints, an integrated method including morphological and molecular characteristics must be taken into account. One common issue with many germplasm collections is contamination. Hence reliable and reproducible fingerprints must be developed for the precise identification of the plants.

Other than the standard morphological diagnosis, we lack any practical techniques to identify situations where hybrids may arise as a result of contamination. Because Azolla reproduces mostly by vegetative means, the biomass needs to be maintained year-round. Every time they need to apply fresh biomass on the field, farmers have to haul significant amounts of it. Occasionally, it also entails transporting people across great distances, which causes civilizations to disappear. This is also one of the factors behind farmers' low Azolla adoption. In order to increase the efficacy of Azolla biofertilizer technology, efforts need to be undertaken to effectively spread the organism through sporocarps. Understanding the timing of sporulation in various Azolla species in relation to the parameters regulating it in an ecological niche is therefore a crucial prerequisite for using sporocarp technology. Strategies for sporulation induction and storage might be devised if we comprehend the pattern of sporulation and the circumstances unique to it.

It is crucial to screen plants against abiotic stressors such salt, high temperatures, pesticides, UV-B rays, and heavy metals.

An important barrier to plant growth and development in general is rising soil salinity. Studies done elsewhere, however, revealed that Azolla's reaction to salt increases differed. Hence, research on salt tolerance in Azolla demonstrates the possibility of salinity tolerance in many species; nevertheless, no more progress has been achieved in this area via the application of sophisticated molecular biology techniques.

Given that the organism is subjected to both high and low temperatures in its surroundings, it is worthwhile to use sophisticated methodologies to comprehend the tolerance mechanisms. Understanding of the common proteins involved in stress tolerance will result from this approach. To increase stress tolerance, it is essential to identify new proteins and genes, as well as the patterns of expression and roles they play in stress adaption. This may provide to a deeper comprehension of Azolla's abiotic stress tolerance mechanism. Given the impending threat of a climate change scenario and the rising levels of pesticide and heavy metal pollution, these kinds of techniques may prove useful in understanding the tolerance mechanism.

Proteomics and genomes are significant in this aspect. Azolla has the potential to be an effective plant-based remedy for contaminated water sources. Despite the organism's potential for bioremediation, no significant attempts have been made in this field. Azolla bioremediation procedures may be used to clean up the high levels of phosphorus and nitrogen present in municipal sewage water waste. If the utilized biomass doesn't contain a lot of heavy metals, it can be used as green manure. However, the biomass can be burned to stop the heavy metal from being recycled in the environment or dried and removed for the purpose of recovering the metal when used for bioremediation of waste water containing heavy metals.

Insect and pest infestation is a highly prevalent issue in the Azolla outdoor mass multiplication tanks during the humid seasons. These insect pests, which are members of the Lepidopterous and Dipterous orders, can totally destroy plants in two to three days (Singh, 1977). Recently, October and November two months that normally encourage healthy Azolla growth have also been reported to have a higher pest prevalence at the Centre for Conservation and Utilization of Blue Green Algae, Indian Agricultural Research Institute, New Delhi (unpublished observations). The possibility that such attacks are caused by changes in the climatic situation warrants more investigation. Currently, biological and chemical controls are in place to limit the number of insects and pests. Therefore, species that are able to fend off insect attacks might be screened. Finding out the phytochemical makeup of various strains is crucial, particularly since Azolla is a key component of organic rice cultivation and the nutrition of cattle and poultry.

Conclusions

Effective management of soil health is essential to the sustainable intensification of agriculture, and Azolla has been shown to affect the dynamics of the whole soil and the microbial population of bacteria that fix nitrogen. The buildup of soil enzymes will result in an improvement in the soil fertility index. Thus, in order to preserve the fertility of the soil, we require effective strains of Azolla. Azolla strains with high nitrogen fixation efficiency from various agro-ecological zones should be screened, and their responses to a range of abiotic stressors, including salinity, heavy metals, and UV-B radiation, should be assessed. It is necessary to try improving strain applying molecular biology advancements. Studies on soil fertility index that make use of soil enzymes must be carried out with Azolla either by itself or in collaboration with other microorganisms.

Effective extension techniques are also urgently needed to encourage the application of this beneficial biofertilizer. The organism has a great deal of potential, which has not yet been completely realized in terms of enriching soil organic matter, soil enzymes, and the soil microbial community. Policymakers, scientists, and farmers must work together to promote Azolla as a potential bioinoculant for sustainable crop development and production.

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