Advancements in the Utilization of Azolla-Anabaena System in Relation to Sustainable Agricultural Practices

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The nitrogen fixing aquatic pteridophyte Azolla has the ability to fix atmospheric nitrogen at cheaper and faster rates due to the presence of a symbiotic cyanobacterium Anabaena azollae. Because of this property it has been exploited widely as biofertilizer for rice plants. In addition to this it has several other uses such as food, feed, biogas producer and hyper-accumulator of heavy metals etc. Because of the multifaceted uses the promotion and use of Azolla-Anabaena system would be ideal and environment friendly in sustainable agriculture. This article provides a brief account of the importance as well as developments in the utilization of Azolla-Anabaena system in agriculture and allied sectors.

Key Words: Azolla-Anabaena; Biofertilizer; Nitrogen; Multifaceted Uses; Sustainable Agriculture

Introduction

The aquatic pteridophyte Azolla is an excellent biofertilizer and green manure having global distribution. Ability of Azolla-Anabaena system to fix atmospheric nitrogen at faster rates makes it an outstanding agronomic choice for the cultivation of rice under tropical conditions. Nitrogen fixation potential of the Azolla-Anabaena system has been estimated to be 1.1 kg N ha⁻¹ day⁻¹ and one crop of Azolla provided 20-40 kg N ha⁻¹ to the rice crop in about 20-25 days (Watanabe et al., 1977). The ability of nitrogen fixation is due to the presence of a heterocystous cyanobacterium Anabaena azollae which is confined to the dorsal leaf cavity of the fern (Moore, 1969). This cavity is formed by the enfolding of the adaxial epidermis in the dorsal leaf lobe (Peters, 1976). Azolla has multifaceted uses and has gained considerable importance in the recent times as biofertilizer, green manure and as poultry feed and cattle fodder (Singh and Subudhi, 1978). Increase in body weight of the chicks upon feeding with Azolla was observed. Azolla is a rich source of protein and essential amino acids and contains several vitamins such as vitamin-A, vitamin B-12 and beta carotene. It is also rich in minerals such as Calcium, Phosphorous, Potassium, Magnesium, Copper and Zinc etc. The protein composition of Azolla is 25-35% on dry weight basis and is easily digested by poultry (Parashuramulu et al., 2013). Azolla has many uses such as animal feed, human food, medicine, production of biogas, hydrogen fuel, water purifier, weed control, reduction of ammonia volatilization and is aptly referred it as green gold mine (Wagner, 1997). Azolla plants in huge numbers sequestered significant quantities of atmospheric CO₂ and converted it directly into biomass of Azollë (Speelman et al., 2009). According to them the plants died and sank to the bottom of the Arctic Ocean and were deposited as sediments that now form a layer beneath the Arctic Ocean. Thus these plants had a significant impact on...
Earth’s climate 50 million years ago (Bujak, 2007). The plant has the ability to multiply fast which is a remarkable aspect in its exploitation as biofertilizer. Agronomic potential of Azolla has been demonstrated and successful exploitation of this organism in the agriculture context of Asian countries has also been highlighted (Singh, 1977a; Singh, 1989). Besides this there are several reports on its potential and agronomic significance (Watanabe, 1982). Rice ecosystems are characterized by the pattern of rain fall, depth of flooding and drainage and by the adaptation of rice to these agro-ecological factors and rice is grown in flooded conditions in more than 128 million hectares and is suitable for the cultivation of Azolla (IRRI, 1994, Gillar, 2002). Increase in yield due to application of Azolla was demonstrated by several studies conducted in the past at several locations in the country. Similar results have been obtained with Azolla along with the application of chemical nitrogen fertilizers (Singh et al., 1992). Highest grain yield in rice plants is observed when a comparison of Azolla application is made with other biofertilizers. Suppression of weeds and reduction in the volatilization of ammonia in rice fields due to the formation of a thick mat in rice fields by Azolla is observed (Singh, 2000). Use of Azolla is very popular in India and the most widely distributed species of Azolla include A. pinnata, A. futiluloides, A. rubra, A. microphylla, A. mexicana and A. caroliniana, respectively (Hills and Gopal, 1967). There are also reports on its successful use in crops other than rice. Trials have been conducted using Azolla successfully in wheat also as biofertilizer to enhance the yield (Marwaha et al., 1992). Mahapatra and Sharma (1989) observed beneficial effects of Azolla on subsequent wheat crop with increase in grain yield. Application of 20 tonnes of Azolla along with 60 Kg nitrogen recorded highest yield of wheat (Sharma et al., 1999). The plant is also used in phytoremediation programs to clean up the polluted and contaminated waters. Phytoremediation is fast emerging as an excellent option to ward off the pollutants from aquatic ecosystems (Arora et al., 2003).

However, the full potential of the plant is still underutilized despite its advantages and concerted attempts are required for the popularization of the system. Therefore, the present review takes stock of the developments related to the successful exploitation and application of Azolla-Anabaena system and its multifaceted uses for sustainable agricultural practices.

**Distribution, Habit and Habitat and Morphology**

Azolla is found in both temperate and tropical regions. It grows luxuriantly in ditches, fresh water ponds and paddy fields. The Azolla plants are delicate, small and triangular or polygonal in shape (Fig. 1). It is free floating and aquatic but can grow on moist soils as long as the moisture persists in the soil. The sporophytic plant has a horizontal rhizome of 0.5 to 7 cm in diameter with branches having densely arranged and overlapping leaves. A leaf consists of a thick dorsal lobe and a thin ventral lobe. The symbiotic Blue Green Alga is confined to the dorsal lobe (Peters and Mayne, 1974). An epidermis covers the surface of the dorsal lobe and the epidermis has vertical rows of single celled stomata and trichomes of one or more cells. The ventral lobe which helps in floating due to its convex surface touching water has a few stomata and trichomes (Eames, 1936).

**Growth and Multiplication**

Azolla is found in both temperate and tropical regions and it grows luxuriantly in ditches, fresh water ponds and paddy fields. The pH requirement of the soil is 7.2 for an ideal growth coupled with a temperature of 32°C. However, studies conducted elsewhere in
India have shown that the region specific choice of the species for their use as biofertilizer. A study was conducted at Centre for Conservation and Utilization of Blue Green Algae, Indian Agricultural Research Institute, New Delhi to compare the biomass production and nitrogen fixation potential of different species of Azolla (Arora and Singh, 2003). Based on the experiments A. microphylla was found to perform better and hence selected for mass multiplication. This strain is maintained round the year as it is able to withstand both high as well as low temperature conditions.

**Taxonomic Status of Azolla**

The Genus *Azolla* was established by Lamarck in the year 1783 and placed in the family Salviniaeeae under the order Salviniales. However, *Azolla* is placed in the monotypic family *Azollaceae* and there are seven extant Species of *Azolla* (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 megasporecarps 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 Rhizosperma 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**

Mode of reproduction in *Azolla* is mainly vegetative and because of this reason the biomass has to be maintained round the year. The fern is heterosporous and produce both mega and micro sporocarps and their germination is influenced by several factors.

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

Singh *et al.*, (1990)
However, production of sporocarps is under the influence of the environment and no uniformity has been observed regarding their time of production by the plants. For example *Azolla microphylla* sporulates throughout the year (Kar *et al*., 1999). Density of population of the plants especially high density has been found to induce the efficiency of sporocarp production (Watanabe, 1982). Production of sporocarps was generally favoured by relatively short day and cool nights at Cuttack (Singh *et al*., 1987). Increase in the sporocarp germination is influenced by combined nitrogen sources. The germination of sporocarps in *Azolla caroliniana* varies in response to light, amino acids, sugars and abscissic acid (Singh *et al*., 1990). Megasporocarps of *Azolla* and their germination in varied paddy soils has also been reported (Nayak *et al*., 2004). Enhanced sporulation in *Azolla microphylla* and *Azolla pinnata* due to application of Gibberellic acid has also been reported (Kar *et al*., 2002). However, these methods using chemicals besides being uneconomical

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**Table 2:** Effect of *Azolla* and various organic treatments on rice (*Pusa Basmati 1*) grain yield during kharif (2003-08)

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azolla (A)</td>
<td>2.87</td>
<td>2.54</td>
<td>2.43</td>
<td>2.36</td>
<td>2.29</td>
<td>2.06</td>
<td>2.43</td>
</tr>
<tr>
<td>BGA (B)</td>
<td>2.70</td>
<td>2.46</td>
<td>2.35</td>
<td>2.29</td>
<td>2.19</td>
<td>1.90</td>
<td>2.32</td>
</tr>
<tr>
<td>FYM (F)</td>
<td>2.69</td>
<td>2.24</td>
<td>2.26</td>
<td>2.12</td>
<td>2.17</td>
<td>2.04</td>
<td>2.25</td>
</tr>
<tr>
<td>Vermicompost (V)</td>
<td>2.90</td>
<td>2.66</td>
<td>2.53</td>
<td>2.60</td>
<td>2.39</td>
<td>2.18</td>
<td>2.54</td>
</tr>
<tr>
<td>A+B</td>
<td>3.35</td>
<td>3.25</td>
<td>3.03</td>
<td>3.16</td>
<td>3.02</td>
<td>2.68</td>
<td>3.08</td>
</tr>
<tr>
<td>A+F</td>
<td>3.70</td>
<td>3.42</td>
<td>3.38</td>
<td>3.29</td>
<td>3.18</td>
<td>2.57</td>
<td>3.26</td>
</tr>
<tr>
<td>A+V</td>
<td>4.08</td>
<td>3.85</td>
<td>3.67</td>
<td>3.57</td>
<td>3.43</td>
<td>2.64</td>
<td>3.54</td>
</tr>
<tr>
<td>B+F</td>
<td>3.33</td>
<td>3.26</td>
<td>3.43</td>
<td>3.48</td>
<td>3.37</td>
<td>2.60</td>
<td>3.25</td>
</tr>
<tr>
<td>B+V</td>
<td>3.91</td>
<td>3.50</td>
<td>3.47</td>
<td>3.52</td>
<td>3.48</td>
<td>2.82</td>
<td>3.45</td>
</tr>
<tr>
<td>F+V</td>
<td>3.75</td>
<td>3.58</td>
<td>3.64</td>
<td>3.56</td>
<td>3.61</td>
<td>3.02</td>
<td>3.53</td>
</tr>
<tr>
<td>A+B+F</td>
<td>4.05</td>
<td>3.66</td>
<td>3.79</td>
<td>3.68</td>
<td>3.82</td>
<td>3.34</td>
<td>3.72</td>
</tr>
<tr>
<td>A+F+V</td>
<td>4.08</td>
<td>3.70</td>
<td>3.81</td>
<td>3.89</td>
<td>3.93</td>
<td>3.45</td>
<td>3.81</td>
</tr>
<tr>
<td>B+F+V</td>
<td>4.10</td>
<td>3.82</td>
<td>3.88</td>
<td>3.93</td>
<td>3.91</td>
<td>3.64</td>
<td>3.88</td>
</tr>
<tr>
<td>A+B+F+V</td>
<td>4.19</td>
<td>4.35</td>
<td>4.38</td>
<td>4.16</td>
<td>4.48</td>
<td>3.68</td>
<td>4.20</td>
</tr>
<tr>
<td>N&lt;sub&gt;80&lt;/sub&gt;P&lt;sub&gt;40&lt;/sub&gt;K&lt;sub&gt;30&lt;/sub&gt;</td>
<td>4.93</td>
<td>4.68</td>
<td>4.21</td>
<td>4.34</td>
<td>4.61</td>
<td>3.46</td>
<td>4.37</td>
</tr>
<tr>
<td>N&lt;sub&gt;0&lt;/sub&gt;P&lt;sub&gt;0&lt;/sub&gt;K&lt;sub&gt;0&lt;/sub&gt; (Control)</td>
<td>2.02</td>
<td>1.84</td>
<td>1.76</td>
<td>1.78</td>
<td>1.89</td>
<td>1.68</td>
<td>1.82</td>
</tr>
<tr>
<td>C.D (at @5 %P)</td>
<td>0.95</td>
<td>0.48</td>
<td>0.31</td>
<td>0.41</td>
<td>0.32</td>
<td>0.26</td>
<td>0.46</td>
</tr>
</tbody>
</table>

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

Auxins in combination with Gibbrellic acid has also been reported (Kar *et al*., 2002). However, these methods using chemicals besides being uneconomical
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Influence of fertilizer application on the sporocarp production has been observed (Singh et al., 1987) and induction of sporulation under phosphorous deficient conditions was recorded (Kannaiyan et al., 1988). An increase in the sporulation frequency and sporocarp number without compromising the biomass yield is observed due to the change in schedule of phosphorous application in Azolla (Kar et al., 2001). The sporocarps germinate after 3-5 months of dormancy period and percent germination is higher during August to October (Singh et al., 1984b). Farmers have to carry large quantity of the fresh inoculums to the field for application every time which involves transportation and many times and this leads to perishing of the culture. This is also one of the reasons responsible for the poor adoption of Azolla by the farmers. Hence to make the Azolla biofertilizer technology more effective and serious attempts have to be made to successfully propagate the fern through sporocarps.

Production Technology for Azolla Biofertilizer and Modes of Application

The propagation of Azolla is generally carried out in soil-based nurseries. However, to maintain the germplasm medium based cultures is advisable.

Effects of nutrient status of the medium on the productivity and nitrogen fixation in Azolla are reported (Kushari and Taheruzzaman, 1990). A comparative study on the growth, pigments, nitrogen fixation and nutrient status of Azolla maintained as soil and nutrient based cultures showed that the soil based cultures are as good as those raised on nutrient media (Dawar and Singh, 2002). Azolla production can be carried out in nursery plots, ponds, ditches, canals, concrete tanks and polythene lined ditches. The field selected for Azolla cultivation needs to be thoroughly prepared and leveled uniformly. Generally 20 m x 2 m size plots are made in the field with suitable bunds and irrigation channels with a water depth of at least 10 cm and in each plot water (20 liters) is added and inoculated with Azolla (8-10 Kg). Single super phosphate (100 g) in 2-3 split doses is applied at an interval of 4 days to each plot. Furadon or carbofuran (3 % active granules) can be applied in the plots (100 g plot\(^{-1}\)) with or after a week of inoculation. 100-150 Kg fresh Azolla can be harvested from each plot after 15 days from each plot. The same method can be used to produce Azolla in bigger plots. The quantities of in-puts need to be varied as per requirement. Depending on the availability and need of the inocula, Azolla can also be maintained in nursery in trays or earthen or cemented pots of any dimension. If the production is carried out in a pond or canal fertilizers and insecticides are not applied. Cattle slurry and animal dung for the production and utilization of Azolla as biofertilizer for rice has been reported (Singh et al., 1993). The cattle slurry and animal dung are effective as phosphorous fertilizers and application of Azolla is found to enhance the C, N and available P content of the soil.

Methods of Application of Azolla

The most common mode of application of Azolla in the field is as green manure or as a dual crop along with rice. In case of application as green manure Azolla collected directly from ponds/ditches is applied in the field. It may be grown in nurseries as specified earlier and can also be applied in the field. A thick mat of Azolla will be formed after application in about 2-3 weeks time and can be incorporated in the soil. Rice can also be transplanted in the field.

Table 3: Chemical composition of Azolla

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Dry matter (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>24-30</td>
</tr>
<tr>
<td>Crude fat</td>
<td>3.3-3.6</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>4-5</td>
</tr>
<tr>
<td>Phosphorous</td>
<td>0.5-0.9</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.4-1.0</td>
</tr>
<tr>
<td>Potassium</td>
<td>2-4.5</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0.5-0.65</td>
</tr>
<tr>
<td>Manganese</td>
<td>0.11-0.16</td>
</tr>
<tr>
<td>Iron</td>
<td>0.06-0.26</td>
</tr>
<tr>
<td>Soluble sugars</td>
<td>3.5</td>
</tr>
<tr>
<td>Crude fire</td>
<td>9.1</td>
</tr>
<tr>
<td>Starch</td>
<td>6.54</td>
</tr>
</tbody>
</table>

Source: Singh and Subudhi (1978a)

also lead to environmental contamination. Influence of fertilizer application on the sporocarp production has been observed (Singh et al., 1987) and induction of sporulation under phosphorous deficient conditions was recorded (Kannaiyan et al., 1988). An increase in the sporulation frequency and sporocarp number without compromising the biomass yield is observed due to the change in schedule of phosphorous application in Azolla (Kar et al., 2001). The sporocarps germinate after 3-5 months of dormancy period and percent germination is higher during August to October (Singh et al., 1984b). Farmers have to carry large quantity of the fresh inoculums to the field for application every time which involves transportation and many times and this leads to perishing of the culture. This is also one of the reasons responsible for the poor adoption of Azolla by the farmers. Hence to make the Azolla biofertilizer technology more effective and serious attempts have to be made to successfully propagate the fern through sporocarps.

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subsequently. Single super phosphate (25-50 kg ha\(^{-1}\)) is applied in split doses. After analyzing the soil P-status the dosage of the same can be reduced. Cattle dung or slurry may also be used instead of single super phosphate. 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\(^{-1}\).

In dual cropping, *Azolla* is grown along with rice and each crop of *Azolla* contributes on an average 30 kg N ha\(^{-1}\). After 7-10 days of transplantation fresh inoculums of *Azolla* is applied in the field at the rate of 0.50-1.0 ton ha\(^{-1}\). Single super phosphate is applied at the rate of 20 kg ha\(^{-1}\) in split doses. In about 15-20 days time a thick mat of *Azolla* is formed. *Azolla* thus incorporated decomposes in about 8-10 days time and release the fixed nitrogen. Another crop of *Azolla* can be raised in a similar way during the crop cycle of rice. *Azolla* production technology is simple and not very expensive and at the same time it is very efficient in terms biomass accumulation and nitrogen fixation. 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 ability to fix atmospheric nitrogen at substantially higher rates has led to the exploitation of the organism as biofertilizer. Application of *Azolla* in rice paddy fields has a positive role in improving the soil fertility index. The ability of nitrogen fixation is due to the presence of the symbiotic cyanobacterium *Anabaena* that occurs in the dorsal leaf cavities of the fronds (Peters and Meeks, 1989). The symbiont is able to meet the entire nitrogen requirement of the association. Calvin cycle operates in both the partners and the primary end product of photosynthesis is sucrose (Van Hove, 1989). A strong interaction exists between nitrogen fixation and the photosynthesis and the source of ATP and NADPH is photosynthesis. The capacity of *Azolla* to fix nitrogen in the field has been estimated to be 1.1 kg N ha\(^{-1}\) day\(^{-1}\) and this fixed nitrogen is sufficient to meet the entire nitrogen requirement of rice crop within a few weeks (Lumpkin and Plucknett, 1980). The rapid and substantially higher rates of nitrogen fixation coupled with production of high biomass have made the organism an outstanding agronomic choice.

*Azolla* strains have been successfully exploited as an efficient biofertilizer for rice paddy fields because of the nitrogen fixing potential. Dr. P K Singh and coworkers from the Central Rice Research Institute, Cuttack successfully popularized the use of *Azolla* as a promising biofertilizer in the Eastern parts of India. *A. pinnata* fixes 75 mg N g\(^{-1}\) dry weight day\(^{-1}\) and produces a biomass of 347 ton fresh weight ha\(^{-1}\) in a year. This biomass contains 868 kg N which is equivalent to 1900 Kg urea. A wide variability regarding growth and nitrogen fixation among different strains of *Azolla* is observed by a study conducted at Central rice Research Institute, Cuttack (Singh, 1988). Among the several factors that influence the growth and nitrogen fixing potential of *Azolla* are nutrient availability, rate and the time of inoculation etc (Kannaiyan, 1993; Singh and Singh, 1995). In addition to sustaining rice yields, inoculation of *Azolla* has been reported to enhance the soil biological health. It is important to optimize use of organic, inorganic and biological inputs in an integrated manner taking into consideration the ecological and soil conditions to sustain crop productivity. Soil enzyme activity is considered as an index of microbial activity and fertility of the soil. *Azolla* decomposes rapidly in soil and supply nitrogen to the crop plants. It contributes significant amounts of phosphorus, potassium, sulfur, zinc, iron and molybdenum in addition to other micronutrients besides addition of nitrogen. The biological health of the soil due to application of *Azolla* has resulted in improving mineralization and consequent increase in the microbial status of the soil. In low land rice cultivation mineralization of organic nitrogen to ammonia is an important process (Sahrawat, 1983). The rate of mineralization is influenced by factors such as C: N. *Azolla* species with a low C: N mineralized in 2 days while the species with high C:N mineralized in 5 days (Wang et al., 1987). The decomposed organic matter plays an active role in the development of microbial population irrespective of the time taken for mineralization. Soil fertility is
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also influenced by the humic substances formed during the decomposition of Azolla (Bhardwaj and Gaur, 1970). For wetland rice cultivation Azolla and Sesbania are used as green manure (Ventura and Watanabe, 1993). Continuous application increased the organic nitrogen content of the soil significantly. Increased cellulolytic and urea hydrolyzing activities in addition to significant increase in the population of heterotrophic bacteria were recorded (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).

Combined incorporation of nitrogen fixing green manures such as Sesbania and Azolla shows significant enhancement in the activity of soil enzymes such as dehydrogenase, phosphatase, cellulose and amylase (Kumar and Kannaiyan, 1992). Similar enhancement in the microbial population, total bacterial, cellulolytic, phosphate solubilising and urea hydrolysing bacteria was observed (Gopalaswamy and Kannaiyan, 2000c). Azolla helps to sustain soil nitrogen supply by returning N to the soil in quantities roughly equal to those extracted from soil by the rice plants (Cisse and Vlek, 2003). Maximum population of bacteria, fungi and actinomycetes and high urease and dehydrogenase activities due to organic farming using Azolla as one of the components was reported (Krishnakumar et al., 2005). Field experiment studies have been conducted at the Centre for Conservation and Utilization of Blue Green Algae, Indian Agricultural Research Institute, New Delhi. In these studies optimum yield in organic Basmati rice was recorded and improvement in grain and soil quality noticed. The inputs used in this study included Azolla, blue-green algae, farmyard manure and vermicompost as organic amendments (Singh et al., 2007). Experiments conducted at Banaras Hindu University, Varanasi showed beneficial effects of Azolla in the cultivation of rice (Bhuvaneshwari, 2012). Application of Azolla has been found to significantly improve the physical and chemical properties of the soil especially nitrogen, organic matter and other cations such as Magnesium, Calcium and Sodium released into the soil (Bhuvaneshwari and Kumar, 2013).

Improvement in Crop Productivity Due to Application of Azolla

Productivity of rice is related to the availability of nitrogenous fertilizers. Nitrogen can also be supplemented to the rice crops through biofertilizers such as Azolla and cyanobacteria. Application of Azolla to rice plants is ideal as the growth requirement for both the organisms are similar. The organism is able to multiply fast which is a remarkable aspect in its exploitation as biofertilizer. Successful exploitation of this organism in the Asian context has also been highlighted (Singh, 1989). Field experiments conducted at Central Rice research Institute, Cuttack using high yielding varieties of rice showed that application of 10 tons ha⁻¹ of fresh Azolla is as efficient as basal application of 30 kg of N (Singh, 1977; Singh, 1978). Their experiments resulted in increase of height, tiller, dry matter, number and weight of panicles, grain and straw yield etc. Increase in yield due to application of Azolla was demonstrated by several studies conducted at several locations. A single crop of Azolla provides 20-40 kg N ha⁻¹ (Singh, 1977b). Similar results have been obtained with integrated application of Azolla and chemical nitrogen fertilizers. When a comparison of Azolla is made with other biofertilizers highest grain yield in rice is observed (Singh et al., 1992). After the decomposition of the organism nitrogen content is made available to the rice plants. It was found that the plant decomposed 8-10 days after incorporation into the soil and rice plants are benefitted after 20-30 days (Singh, 1977d). The release of N by Azolla is slow and its availability to the first crop of rice is about 70% to that of ammonium sulphate. Moreover, the release of nitrogen is faster as compared to nitrogen fixing cyanobacteria (Saha et al., 1982). Fresh Azolla releases its N faster as compared to dried Azolla due to rapid mineralization and mineralization of N is faster at room temperatures (Singh, 1979a; Singh, 1979b). A comparative study was conducted at Cuttack on the N release efficiency of chemical N fertilizers, Azolla and BGA and other organic manures. It was found that the release of N by Azolla
was comparatively slower and 87% of N in chemical fertilizer is released within 10 days. Hence the application of Azolla in combination with chemical fertilizers offers an excellent choice for rice cultivation (Singh et al., 1981). Increase in grain yield with application of Azolla alone and chemical nitrogen fertilizer (150 kg N ha⁻¹) is reported (Singh et al., 1992). At Central Rice Research Institute, Cuttack, field trials demonstrated that use of Azolla enhanced crop yield and crop N uptake significantly as compared to treatments without Azolla (Manna and Singh, 1989). Utilization of Azolla pinnata as biofertilizer in acidic soils of Kerala has also been reported (Sevichan and Madhusoodanan, 1998). Suppression of weeds and reduction in the volatilization of ammonia in rice fields due to the formation of a thick mat in rice fields by Azolla is also observed (Singh et al., 1981). Comparison of Phosphorous enriched and un-enriched A. caroliniana on the performance of Azolla dual cropping with rice showed higher biomass and N yield by applying phosphorous to P-enriched inocula (Singh and Singh, 1995). Potential exploitation of the system as phosphorous biofertilizer to improve the nitrogen and phosphorous balance in polluted environments was attempted (Singh et al., 2010).

Azolla as an Input in Organic Agriculture

Recently there is a surge in the interest in organic farming and Azolla is used as an important and potential component in the organic farming of rice. Positive effect on the BGA and Azolla on rice yield has been reported earlier (Singh and Mandal, 1997; Singh and Mandal, 2000). Azolla has been used successfully as a component of organic cultivation in rice based cropping system. Field experiments have been conducted at Indian Agricultural Research Institute, New Delhi during 2003-2009 to find out suitable organic amendments for sustainable productivity of Basmati rice-wheat-green gram cropping system (Singh et al., 2011). Different treatment combinations comprising of organic amendments such as Azolla @ 1.0 ton ha⁻¹, Blue Green Algae @ 2.0 kg ha⁻¹, vermicompost and farm yard manure @ 5.0 ton ha⁻¹ applied alone or in combination were tested. Results revealed significant enhancement in grain yield of rice over absolute control due to the application of organic amendments like Azolla applied alone or in combination (Table 2). Optimum yield of Basmati rice (cv. Pusa Basmati 1) can be obtained in all the years with the application of four amendments (Azolla, BGA, vermicompost and FYM) together. Besides, enhancing and sustaining the productivity of organic rice-wheat system, higher productivity of vegetables like cauliflower, broccoli, cabbage and carrot grown after organic rice under organic nutrition were recorded (Singh et al., 2012). There was no serious incidence of any insect pest or disease in organically grown rice crop during these years. Microbial populations (Actinomycetes, Bacteria, Fungi and BGA) were found to enhance over the years due to the application of organic amendments in comparison to total control and recommended fertilizer application that accordingly result in a notable enhancement in dehydrogenase enzyme activity. The treatments have led to changes in the composition of microbial communities due to fertilization treatment and application of organic matter (Irisarri et al., 2001; Jha et al., 2004; Singh and Dhar, 2011; Singh et al., 2012). Rice grain analysis for Iron, Zinc, Manganese and Copper contents showed a significant increase in these essential ion contents in the treatments having two or more organic amendment added altogether over control. Similar results have been obtained by Bhattacharya and Chakraborty (2005). Organic nutrient management including Azolla inoculation showed considerable built up in soil organic carbon content. The values of soil physical parameters like available water content (AWC) and water retention capacity (WRC) are higher under organic management compared to INM and chemical fertilization. The increase in AWC was related to the increase in micro-and macro-porosity. Lower bulk density (BD) was observed in organic treatment as compared to INM and chemical fertilizer treatments (Singh et al., 2012). Highest net return is also recorded with integrated nutrient management (INM) of rice that included use of Azolla in addition of chemical fertilizer and it is followed by organic management with four inoculants including Azolla. These results clearly show how Azolla could be
successfully integrated with other bio-inoculants in rice as well as vegetable based organic cultivation.

Use of Azolla as Livestock Feed

The importance of Azolla as a sustainable feed for livestock and poultry is reviewed recently (Gouri et al., 2012). According to them Azolla can be used as an ideal source of feed for cattle, sheep, goats, pigs, rabbit and fish (Fig. 3). Because of the high nutrient content Azolla can be used as fodder for cattle and fish as well as poultry feed. It has Proteins, Vitamins, Calcium, Phosphorus, Iron, Copper, Magnesium, Beta carotene and Amino acids (Table 3). Azolla can be maintained throughout the year due to proper planning and cultivation practices and this in turn will ensure round the year availability of the organism for fodder purposes. Azolla in dried form is not preferred by the birds and hence the use of fresh biomass is an excellent poultry feed with no side effects (Singh and Subudhi, 1978a). About 20-25% of the commercial feed can be replaced by the incorporation of fresh Azolla biomass (Subudhi and Singh, 1978b). Utilization of Azolla as animal feed is suggested to be advantageous for the better productivity of livestock which comprise an integral part of the agriculture (Banerjee and Matai, 1990). Studies conducted on Azolla pinnata indicate that the level of Amino acids in the leaf protein compare favorably with the standards of FAO reference pattern and chick requirements (Dewanji, 1993). Integration of Azolla powder in the fish meal and feeding experiments is found to enhance the weight of the carp Osteobrama belangeri and resulted in better feed conversion efficiency and protein efficiency ratio (Basudha and Vishwanath, 1997). Use of Azolla protein supplement for the fish Tilapia mossambica and observed increase in feeding, absorption and growth rate (Sithara and Kamalaveni, 2008). They further observed that mixing of the Azolla biomass with other agricultural byproducts such as wheat and rice bran will lead to improvement in digestibility and protein quality.

Feeding experiments conducted on buffalo calves suggest that Azolla meal is a potential and unconventional source of protein (Indira et al., 2012). Although most of these studies advocate the use of fresh biomass, studies show that heat treated Azolla meal is partially substituted for fish meal resulted in significant growth of Labeo rohita (Maity and Patra, 2008). An experiment was carried out to investigate the effect of dietary inclusion of sun dried and ground Azolla (Azolla pinnata) on production performance of broiler chicken (Balaji et al., 2009). The per cent giblet yield of birds fed with 4.5% Azolla is found to be significantly higher than control and other treatments. Based on the experiments it is concluded that dietary inclusion of dried Azolla up to 4.5% levels did not have any adverse effect on production performance of broiler chicken. It is observed that aquatic plant species especially Azolla offer a great potential as a source of protein for animals due to ease of cultivation, productivity and nutritive value (Prabha and Kumar, 2010). Therefore, keeping in view of the importance of organic aquaculture these days use of Azolla is found to be one of the cheapest ways to increase the fish production in organic fish farming. Increase in fish production was observed when Azolla is used in organic aquaculture and it is strongly suggested that this practice can be an effective tool to increase the farm income and living standards of low income farmers from the Meghalaya region (Majhi et al., 2006). Poultry fed on Azolla show significant increase in the body weights and consequently have resulted in an increase in the net return (Rai et al., 2012). These studies in general indicate the tremendous possibility of using the Azolla
biomass effectively in feed and also to bring down the cost involved in feeding. However, there is a need to address the protein quality as well the amino acid and nutrient composition of different 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**

Another interesting and applied aspect of utilization of *Azolla* which is gaining popularity in the recent times is its application in the process of bioremediation. Increase in population and rapid rates of industrialization has resulted in the release of huge quantities of pollutants into water resources. The pollutants released are toxic in nature and pose great concern due to their adverse impact on plant and animal health. Therefore phytoremediation offer an excellent option to ward off the pollutants from aquatic ecosystems. *Azolla* may be used to clean up the polluted and contaminated waters (Table 4). Efficient removal of gold from waste water solutions using the fern *Azolla filiculoides* is reported (Antunes et al., 2001). 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). *Azolla* biomass is also employed in the phytoremediation of toxic heavy metals. Recently the phytoremediation potential of three different species of *Azolla* such as *A. microphylla*, *A. pinnata* and *A. filiculoides* is studied and it is found that *A. microphylla* accumulated more metal as compared to the other species (Arora et al., 2006). The biomass of *Azolla* (both live and dead) has also been used in the bio-sorption of several heavy metals (Umali et al., 2006; Mashkani and Ghazvini, 2009). Extensive work has been done using *Azolla* in the removal of heavy metals from aquatic environments (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.

A hydroponic system is developed to decontaminate the water containing Cadmium and Copper was developed recently (Valderrama et al., 2012). Phytoremediation potential of *Azolla* was reviewed recently (Sood et al., 2011). All these results show that *Azolla* may be successfully employed in phytoremediation of polluted water bodies. The municipal sewage water waste contains heavy load of Phosphorous and Nitrogen and bioremediation programs involving *Azolla* may be planned to decontaminate them. The used biomass may be used as green manure in case it is not having any heavy metal load in it. However, when used for bioremediation of waste water having heavy metal load the biomass may be dried and extracted for the recovery of the metal or incinerated to prevent the recycling of the heavy metal in the environment.

**Research Perspectives for Future Research**

In view of the declining soil health and productivity due to increased anthropogenic activities maintaining the sustainability is a challenging task ahead. The *Azolla-Anabaena* system is an excellent biofertlizer for rice crop and it also has several other uses. In order to improve its utility in agriculture and allied sectors focused attention is required. Thus, there is an urgent need to address certain key issues in *Azolla* for its exploitation and better utilization. These

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**Table 4: Bioaccumulation potential of heavy metals by various *Azolla* spp.**

<table>
<thead>
<tr>
<th><em>Azolla</em> spp.</th>
<th>Heavy metal</th>
<th>Concentration of heavy metal accumulated (µg metal g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. pinnata</em></td>
<td>Cadmium</td>
<td>2759</td>
</tr>
<tr>
<td></td>
<td>Mercury</td>
<td>450</td>
</tr>
<tr>
<td><em>A. caroliniana</em></td>
<td>Lead</td>
<td>416</td>
</tr>
<tr>
<td></td>
<td>Chromium</td>
<td>964</td>
</tr>
<tr>
<td><em>A. filiculoides</em></td>
<td>Nickel</td>
<td>28443</td>
</tr>
<tr>
<td></td>
<td>Chromium</td>
<td>12383</td>
</tr>
<tr>
<td><em>A. microphylla</em></td>
<td>Nickel</td>
<td>21785</td>
</tr>
<tr>
<td></td>
<td>Cadmium</td>
<td>1805</td>
</tr>
</tbody>
</table>

Source: Sood et al., (2011)
include the difficulties encountered in identification, observation of sporulation, storage of sporocarps and abiotic stress tolerance etc. These issues are needed to be addressed on priority basis to further enhance the utility of this organism in agriculture and allied sectors. In view of the progresses made using morphological and molecular approach in taxonomical research on *Azolla* an integrative approach is needed to solve the problems related to classification using several accessions as per the list of International Rice Research Institute, Philippines. Therefore, an integrative approach involving morphological and molecular characters must be taken in to consideration to assign species and create molecular finger prints. Contamination is a frequently encountered problem in many germplasm collections. The contamination can lead to the development of hybrids and we do not have any concrete tools to detect such a situation other than the conventional morphological identification. Hence reliable and reproducible fingerprints must be developed for the precise identification of the plants.

The mode of reproduction in *Azolla* is mainly vegetative and because of this reason the biomass has to be maintained round the year. The farmers have to carry large quantity of the fresh biomass to the field for application every time. At times it also involves transportation to long distances leading to perishing of the cultures. This is also one of the reasons responsible for the poor adoption of *Azolla* by farmers. Hence to make *Azolla* biofertilizer technology more effective, attempts must be made to successfully propagate the organism through sporocarps. Hence an important pre-requisite in the sporocarp technology is to have an understanding regarding the time of sporulation in different species of *Azolla* in relation to the factors controlling it in an ecological niche. Once we understand the pattern of sporulation and conditions specific to it, then strategies could be developed for induction of sporulation and its storage.

Screening of the plants against abiotic stress conditions such as salinity, high temperature, pesticide, UV-B and heavy metals is essential. Increasing soil salinity is a major deterrent for growth and development of plants in general. However, works conducted elsewhere showed varied response by *Azolla* to increase in salinity. Thus, the studies conducted on *Azolla* regarding salinity tolerance show the potential of salinity tolerance in different species but no further advancement has been made in this direction using advanced molecular biology tools. Since in the environment the organism is exposed to high and low temperature conditions, it is worth understanding the tolerance mechanisms using advanced techniques. Such an approach will lead to understanding of the common proteins involved in stress tolerance. It is imperative to identify novel proteins, genes and their pattern of expression and functions in stress adaptation in order to improve the stress tolerance. This could help in better understanding the abiotic stress tolerance mechanism in *Azolla*. With the imminent threat of climate change scenario and increasing contamination due to pesticides and heavy metals such approaches could be helpful in deciphering the mechanism of tolerance. In this regard, proteomics and genomics play an important role.

*Azolla* may be successfully employed in phytoremediation of polluted water bodies. However, this is an area where no serious efforts have been attempted despite the potential of the organism for bioremediation. The municipal sewage water waste contains heavy load of phosphorous and nitrogen and bioremediation programs involving *Azolla* may be planned to decontaminate them. The used biomass may be applied as green manure in case it is not having heavy metal load. However, when used for bioremediation of waste water having heavy metal load the biomass may be dried and extracted for the recovery of the metal or incinerated to prevent the recycling of the heavy metal in the environment.

The incidence of insects and pests is a very common problem encountered in the outdoor mass multiplication tanks of *Azolla* during the humid seasons. These insect pests belong to Lepidopterous and Dipterous orders and can damage the plants in 2-3 days completely (Singh, 1977). At the Centre for Conservation and Utilization of Blue Green Algae, Indian Agricultural Research Institute, New Delhi of
late the incidence of pests is also observed during the months of October and November which are otherwise months that support good Azolla growth (Unpublished observations). Changes in the climate scenario may be responsible for such attacks and may be investigated in detail. At present there are chemical as well as biological controls to check the incidence of pests and insects. Hence species which can repel the insect attack could be screened. Determination of phytochemical composition of different strains is also important especially when Azolla is used as an important input in organic rice farming and poultry/cattle feeding.

Conclusions

The fundamental to sustainable intensification of agriculture is effective soil health management and Azolla has been known to influence the dynamics of the total soil and microbial population of nitrogen fixing bacteria. The soil fertility index will improve due to the accumulation soil enzymes. Therefore we need to have efficient strains of Azolla to maintain the soil fertility. Efficient strains of Azolla in terms of nitrogen fixation from different agro-ecological zones are to be screened and their performance under various abiotic stress conditions such as salinity, heavy metals and UV-B need to be evaluated. Strain improvement must be attempted using innovations in molecular biology. Soil fertility index studies using soil enzymes need to be conducted employing Azolla alone or as a consortium with other micro-organisms. Sound extension strategies are also the need of the hour to promote the use of this biofertilizer with other benefits. The organism has tremendous potential and its real potential to enrich soil organic matter, soil enzymes and the soil microbial population has not yet been fully exploited. Concerted efforts are required from the part of policy makers, scientists and farmers to promote Azolla as a viable bio-inoculant for sustainable crop production and development.

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