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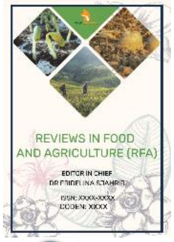

REVIEW ARTICLE

AN OVERVIEW OF AZOLLA IN RICE PRODUCTION: A REVIEW

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ABSTRACT

Azolla is a free-floating water fern which in symbiotic association with cyanobacterium *Anabaena azollae*fixes the atmospheric nitrogen. Nitrogen fixing ability of cyanobacterialsymbiont varies between 30 and 60 kg N ha^{-1} which designates *Azolla* as an important biological nitrogen source for rice ecosystem. Inoculation of *Azolla* is an alternative and sustainable source of nitrogen to increase the rice productivity and it also can decrease the use of synthetic fertilizer. A number of past researches prove that *Azolla* has been used as apotential biofertilizer for rice production. *Azolla* is either incorporated in the soil before rice transplanting or grown as a dual crop along with rice. The objective of this paper is to provide a brief account of importance as well as developments in the utilization of *Azolla-Anabaena* system in agriculture, mainly rice production.

KEYWORDS

Azolla, nitrogen,*Anabaena azollae,*rice crop.

1. INTRODUCTION

Bio-fertilizers are formulations of beneficial microorganisms that help the growth of plants by increasing the quantity and availability of nutrients through their biological activities (El-Hawary and El-Kholy, 2019). They are the preparations containing living cells or latent cells of efficient strains of microorganisms that help crop plants for the uptake of nutrients by their interactions in rhizosphere. Bio-fertilizers are mostly used in sustainable farming for increasing soil fertility and crop productivity. They are the most important component of integrated nutrient management. **(**Itelima et al., 2018). The mostly used microorganisms as bio-fertilizer are: nitrogen fixers (N-fixer), growth promoting rhizobacteria (PGPRs) like *Azotobacter*, *Azospirillum* and Phosphorus Solubilizing Bacteria (PSB) viz; *Pseudomonas sp.* and *Bacillus sp.* endo and ectomycorrhizal fungi, cyanobacteria and other useful microscopic organisms (Yasin et al., 2012). The use of bio-fertilizers makes the soil environment rich in all kinds of micro- and macro- nutrients also helps the release of plant growth regulating substances, production of antibiotics and biodegradation of organic matter in the soil (Sinha et al., 2014). Bio-fertilizers, contradict to synthetic fertilizer, are more economical and helpful in improving the soil structures and restoration of environment for leveraging agriculture (Yasin et al., 2012). Bio-fertilizers are thus considered the alternative of chemical fertilizers.

Rice (*Oryza sativa L.*) is a major cereal crop in Nepal. Plant nutrition is very important for the healthy growth of crops. Nitrogen fertilization is most important for the yield of grain in rice plant (Chaturvedi, 2006). The demand and synchronization of Nitrogen differ with inbred and hybrid rice varieties. Hybrid varieties having genetically high yield potential require higher amount of nitrogen as compared to inbred varieties (Ravi et al., 2007). The use of chemical fertilizers generally urea $[CO(NH_2)_2]$ or ammonium sulphate $[(NH_4)\ 2.SO_4]$ is very common in case of Nepalese rice farming system. The Nitrogen fertilizer efficiency is less than 40% and is very costly for farmers to purchase (Joshy, 1997). Recovery of Nitrogen by rice is very low as 10% and never exceeds 50% (Vlek and Byrnes, 1986). Loss from urea ranges from 11 to 54% when it is broadcasted in rice field after transplantation (Schnier, 1995).Thus,*Azolla*biofertification could be a good approach to increase the nitrogen use efficiency (NUE) in rice fields (Yao et al., 2018).

*Azolla*is a heterosporouspteridophyte that lives in a place having plenty of water. It is a free-floating water fern. It's most common utilization is the co-cultivation with rice, as water-filled rice paddies provide a perfect habitat for the water fern to propagate. *Azolla* has been used as green manure due to its rapid reproduction and high nitrogen content(3-6% N by dry weight) (Watanabale et al.,1981). The Nitrogen content is similar to that of legumes. It is an excellent source of bio fertilizerswhich fixes the atmospheric nitrogen in symbiotic association with a cyanobacterium, *Anabaena azollae* (Carrapico, 2002). *Anabaenaazollae*, a blue green algae can photosynthesize independently. The entire nitrogen requirement of the *Azolla* frond is supplied by the algal symbioant. An *Anabena-Azolla* relationship is ideal for the cultivation of rice under tropical conditions because of its ability to fix atmospheric nitrogen and capacity to multiply at faster rates.

Azolla can flourish well in flooded rice fieldsthus, it is also extensively used as a most suitable bio-fertilizer for the rice fields to improve the nitrogen content within few weeks of its incorporation (Bhuvaneshwari and Singh, 2015). *Azolla* biomass can be used in rice fields as partial or complete replacement of synthetic fertilizers because of its ability to provide 1.5-2.0 million tons of nitrogen whereas the requirement of urea for same amount of crop production is 3.3-4.0 million tons (Raja et al., 2012). Along with the supply of nitrogen, *Azolla* also decreases the soil pH and water temperature, inhibit NH_3 volatization, and prevents weeds and mosquito prolification (Pabby et al., 2004). The nitrogen fixation ability of one crop of*Azolla* to the rice crop in about 20-25 days is found to be 20-40 kg Nha^{-1} and the ability of *Anabaena azolla* system has been estimated to be 1.1 kg N ha^{-1} day^{-1} (Watanabe et al., 1977). The integration of *Azolla* with urea has increased urea use efficiency (Cisse 2001; Vlek et al., 1995).

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2. Source of Information

This paper was prepared by collecting the information from all available resources i. e. journals, annual reports, articles, proceedings, etc. published by different authors, researchers, professors and scientists as well as research Centre and stations. The information was gathered and systematically arranged into different heading and subheading, by reviewing more than 40 research papers intensively.

3. Research Highlight

The complete study reviewing different paper was done and collected information's were systematically arranged into different subheadings namely:*Azolla* biology, its symbiont nature with *Anabena*,Biological Nitrogen fixation by *Azoll*a, Production of *Azolla*biomass and application to the rice crop,Nitrogen Yield by *Azolla* in rice field and Contribution of *Azolla* to yield of rice

3.1 Genus *Azolla*

The genus *Azolla* was firstly introduced by Lamarck in 1783 (Lumpkin and Plucknett, 1980). For several years *Azolla* belonged to family Azollaceae but now taxonomist has assigned *Azolla*to a monotypic family Salviniaceae (Al-saadi and Abdullah, 2016). The genus has several species. *A.pinnata* and *A.nilotica* belonging to the section Rhizosperma and *A. caroliniana*, *A. filiculoides*, *A. mexicana*, *A. microphylla* and *A. rubra* belonging to section *Azolla* (Rothwell, 1999). The sporophyte of *Azolla* has a main rhizome that branched into secondary rhizomes, both bears the alternatively arranged small bilobed leaves. The adventitious root, which absorbs nutrients directly from water or from the soil in shallow water, arises from ventral sides of rhizome (Roy et al., 2016). The areal dorsal lobe is chlorophyllus, where Anabena- azollae inhibits, and a ventral lobe is partially submerged and hyaline (Calvert et al., 1985; Peters et al., 1982; Singh, 1977).

*Azolla*is a free water fern widespread on tropic and subtropic and warm temperate regions (Raja et al., 2012). The delicate, small triangular or polygonal shaped plants grows luxuriantly in ditches, fresh water and paddy fields (Yadav et al., 2014). Its size is generally 1-2.5 cm in diameter, but some species can achieve the size of 15 cm or more (Lumpkin and Plucknett, 1980). *Azolla* reproduce both sexually and asexually but vegetative reproduction is more common. (Qiu and Yu, 2003). It can multiply vegetatively through fragmentation and reproductively through spore production. Only*A. Mexicana*forms spores throughout the year, and for the other species of *Azolla*sexual reproduction occurs during limited periods of the year (Singh et al., 1987). Oslen reported that*Azolla* strains are capable of growth from pH 3.5-10 with its best optimum between pH 5 and 8 (Oslen, 1970).

3.2 Anabaena Azollae symbiosis

Azolla (mosquito fern, duckweed fern, fairy moss, water fern) lives in symbiosis with a filamentous, heterocyst- forming cyanobacterium called*Anabaena,* occurs in the dorsal leaf cavities of the fronds, with the ability of atmospheric nitrogen fixation (Pereira and Vasconcelos, 2014). The algal symbiontAnabaena azollaeStrasburger, belongs to the phylum Cyanophyta, order Nostocales and family Nostocaceae (Moore, 1969). After the sporophyte breaks out through the apical membrane, initial contact between *Anabaena* cells and *Azolla* plant takes place (Lin and Watanabe, 1988). Theendosymbontshows the synchronous growth and development (Shi et al., 1984; Hill 1975). *Anabaena azollae*possesses sinuous trichomes (threads) composed of bead like or barrel-shaped cells without a sheath (Shen, 1960). Fjerdingstad claimed that the cyanobacterium is the ecoform of *Anabaena variabilis* (Fjerdingstad (1976).

Anabaena filaments associated with the apical meristem of each main and lateral branches is undifferentiated and non- nitrogen fixing (Subudhi and Singh, 1978b). The epidermal hairs called the primary branched hairs (PBH) grow from the axil of dorsal lobe, as leaf cavities develop in the form of depressions on the adaxial epidermis of the dorsal leaf lobe. Its terminal cells have an ultrastructure of transfer cells and remain in interaction with Anabaena filaments (Calvert and Peters, 1981). The algal filaments along with PBH get partitioned into the leaf cavities,as the developing leaves are displaced from the meristem. Concominant with the onset of leaf cavity closure, Anabaena cells enlarge and the alga develops heterocysts and starts nitrogen fixation. Simple hairs emerge from the cavity wall and more than 20 simple hairs develop near the photosynthetic mesophyll zone (Calvert and Peters, 1981). To the periphery of cavity in the matured dorsal leaf lobe, Anabaena Azollae are localized (Shi et al., 1984).

To isolate the hair cells from *A. filiculoides* containing a packet of *Anabaena*, aprocedure has been developed. (Uheda, 1986).The routes by which metabolic exchange between the two partners of the association is not clearly understood. However, distribution, morphology, and transfer of cell structures of the two populations in the cavity trichomes suggests that the branched hairs of the cavity are involved in ammonium uptake and /or metabolism (throughout the development) and simple hairs are involved in facilitating the transfer of sucrose from the mesophyll to the endophyte in mature cavities (Konar and Kapoor, 1972; Duckett et al., 1975a; Calvert and Peters, 1981; Peters et al., 1985a). The interior surface of a mature leaf cavity is lined with an envelope and largely filled with gases. The mucilage is secreted by cyanobacterium (Shi *et al*., 1987)and those cavities free from endophyte do not contain mucilage (Lumpkin and Plucknett, 1980; Duckett et al., 1975a).

It is believed that only one kind of *Anabaena Azolla*is found in the leaf cavities of different *Azolla* species.*Anabaena azollae* from several*Azolla* species shared similar antigens (Ladha & Watanabe, 1982). The similarity of *Anabaena Azollae* in different *Azolla* species is also verified by 'nif' gene studies (Franche *et al*., 1985). However, *Anabaena azollae* is observed not to be uniform through out genus *Azolla*, through the use of using monoclonal antibodies technique (Plazinski *et al*., 1988).

3.3 Biological Nitrogen fixation by *Azoll*a

The site of Nitrogen fixation is the heterocyst of the symbiont *Anabaena*. Nitrogenase, the nitrogen fixing enzyme, is present on the heterocysts (Peters, 1975). It is found that the nitrogenase activity in fresh isolated symbiont is 6-10 than that of association and 12-10 times higher than that of free living cyanobacteria (Becking, 1976). The endogeneous source of fixed nitrogen gives *Azolla* a good advantage over other floating hydrophytes in many environments. Nitrogen fixation along with the high growth rate enables *Azolla* to accumulate greater than 10 kg of nitrogen/ha/ day under optimum conditions (Lumpkin, 1985).

The nitrogen fixation in *Anabaena azollae* is closely related and dependent on photosynthesis (Peters, 1976). The source of all the ATP and reductant required to convert nitrogen to ammonia in the symbiosis is photosynthesis. Simultaneous measurement of the rates of photosynthesis, respiration and C2H2 reduction in *A. imbricata* revealed that nitrogenase activity is primarility dependent on the photosynthetically captured radiation energy, and secondarily on C02 fixation (Shi et al., 1981). The close interactionbetween photosynthesis and N2-fixation has been demonstrated by measurement of the action spectra for nitrogenase- catalyzed C2H2 reduction in the association and in the isolated endophyte (Tyagi et al., 1981). Glutamine synthetase, Glutamate synthetase, Glutamate dehydrogenase are the three enzymes involved in assimilation of ammonium (Ray et al., 1978).

Enzyme glutamine synthetase (GS), present in heterocyst, incorporates the newly fixed nitrogen into glutamine. Glutamate synthetase (GOGAT) is found to be active in the vegetative cells and glutamate is transported from these cells to the heterocysts (Ladha and Watanabe, 1987). The fixed N2 is transported to the host by the symbiont where it is incorporated into the amino acids and these amino acids along with reductant and photosynthate are provided to the symbiont. *Anabaena azollae*not only fixes the nitrogen, but also excretes ammonia. Some researcher used N2 to find the distribution of nitrogen compounds produced by *Anabaena azollae* symbiosis (Peters,1976; Peters 1977). He found that N2 distributed as extracellular ammonia (49.9%), intracellular ammonia (6.4%), extracellular organic nitrogen (5.6%) and intracellular organic nitrogen (38.1%). Kannaiyan and Goyal observed ammonia excretion in four algal symbiosis isolated from different species of *Azolla* (Kannaiyan and Goyal, 1988).

3.4 Production of *Azolla* biomass and application to the rice crop

3.4.1 Production of Azolla biomass

Soil based nurseries are made for the propagation of *Azolla*. However, nursery plots, ditches, ponds, canals, small creeks, concrete tanks, polythene lined ditches can be used for *Azolla*production. Generally, in the rice field, small plots bounded by strong mud bunds are used. (Kannaiyan, 1982). Azolla to be produced and used as biofertilizer for rice, cattle slurry and animal dung are effective. (Singh et al., 1993). Single Super Phosphate as a source of inorganic fertilizer is used for higher biomass yield. In controlled conditions and availability of below mentioned factors *Azolla* can grow and provide sufficient amount of biomass.

3.4.2 Factors affecting growth and Nitrogen requirement of Azolla

All the essential macro and micro-nutrients, except combined nitrogen is required for optimum growth of *Azolla*. *Azolla* has been successfully grown in different N-free inorganic media (Huneke, 1933). Deficiency of Calcium and Phosphorous adversely affect the growth and nitrogen fixation by *Azolla* (Singh, 1979a). The quality and intensity of light also have pronounced effect it's on growth and nitrogen fixing ability. Various observations suggest that *Azolla* prefers an environment with certain degree of shading. The highest N content of *Azolla*at 25 K lux (Lumpkin and Plucknett, 1980).

The optimum temperature requirement for *A. mexicana*, *A. pinnata* and *A. caroliniana* are 30 °C and for *A. filiculoides*is 25°C (Peters et al., 1980). *Azolla* biomass increased by 14-16 times in 14 days at 30/25°C, 38/30°C and 40/32°C day/night temperatures, whereas nitrogenase activity was the highest on the 7th day at 30/25°C (Chapman et al., 1981). The optimum pH requirement for proper growth and nitrogen fixation *in Azolla* is 5-7. *Azolla* plants require N2 for N2-fixation, C02 for photosynthesis and 02 for respiration (Watanabe et al., 1977). The growth of *A. pinnata, A. mexicana*and *A. filiculoides* was found to be better in open containers due to free diffusion of gases (Becking,1979). At optimum light intensity of about 49 K lux with a day length of 12 hours, the growth rate of *Azolla*of 150-175 mg/g fresh weigh/day (Ashton ,1971).

3.4.3 Application of Azolla to Rice crop

The application of *Azolla*is donein the field is either as a green manure before transplanting or as a dual crop with rice after transplanting (Singh, 1979a,b; Rains and Talley, 1979). Both the methods are in practice under Asian conditions, but dual cropping is more in practice as it is reported to benefit the rice crop (Talley et al., 1977; Singh, 1979a,b). For application as green manure, *Azolla* collected from nurseries, ponds or ditches is applied in the field. After 2-3 weeks of application, a thick mat of Azolla can be seen and it is incorporated in the soil. Then, rice can be transplanted in the field. Cattle dung, Slurry or Single Super Phosphate (25-50 kg/ha) can be applied in the field. Pest control measures are to be undertaken in case of pest infestation (Yadav et al., 2014). About 20-40 kg N/ha is provided by one crop of *Azolla* application as green manure (Watanabe et al., 1977).

Azolla green- manuring along with increasing the grain and straw yield, also enhanced the number and weight of panicles and reduces the sterility in grains. (Kulasooriya and De Silva, 1977; Singh, 1979a,b). In case of dual cropping, fresh inoculum of *Azolla*is applied in the field at the rate of 0.5-1 ton/ha after the establishment of rice seedling. Single Super Phosphate (20 kg/ha) can be applied in split doses. A thick mat of *Azolla* is formed in about 15-20 days of time. After the decomposition of *Azolla* in the field, in about 8-10 days' time, it releases the fixed nitrogen. during the crop cycle of rice, another crop of *Azolla* can be raised in a similar way. Each crop ofAzolla contributes on an average 30 kg N ha–1in case of dual cropping (Yadav et al., 2014; Watanabe et al., 1977). Azolla dual cropping also helps in suppressing the weeds in the rice fields. (Moore, 1969; Lumpkin and Plucknett, 1980)

3.5 Nitrogen Yield by *Azolla* in rice field

The nitrogen yield by *Azolla* species particularly depends upon agricultural practice, nature of fertilizer management, presence of soluble ion and the history of *Azolla* cropping in the field. (Watanable, 1982). *Azolla*biofertilizerincorporation increased the nitrogen recovery of the crop by 49–64% anddecreased nitrogen loss by 26–48% (Yao et al., 2018). *Azollafiliculoides* incorporated in paddy soil in pots has the nitrogen fixation ability of 128 kg N/ha in 50 days. (Tujimura et al., 1957). *Azollapinnata* incorporated in rice field has the average nitrogen fixing ability of 0.3-0.6 kg/ha/day (Beckling, 1976). Similarly, reported the nitrogen fixing ability of 2.3 ha/day in fallow paddy field (Singh, 1979a). Roger and Reynaud, found that the nitrogen fixation rate of *Azollaafricana* was 0.6-1.8 ha/day (Roger and Reynaud, 1979).

3.6 Contribution of *Azolla* to yield of rice

Azolla incorporation in paddy fields increased the yield of grain, straw yield, caryopsis and dry matter (Pabby et al., 2004). The nitrogen fixation ability of*A. azollae* and the nitrogen content of the fern make this symbiosis suitable for the use as bio-fertilizer (Pabby et al., 2004). Peters found that the use of *Azolla* as bio-fertilizer increased the yield of rice by 112% over unfertilized controls when applied as a monocrop, when applied as an intercrop with rice by 23%, and when applied both as a monocrop and an intercrop by 216% (Peters, 1978). Singh found that either the application of 30-40 kg N/ha through ammonium sulphate, or incorporation of 8-10 tons of *Azolla*/ha fresh produced the same rice yield, 47% increase in grain yield over control (Singh, 1977). Combination of *Azolla* with lower dose of Nitrogen in planted paddy fields gave higher paddy yield, thus a judicious combination of azolla and nitrogen provides a better yield (Singh 1979 a,b). A group researcher found that *Azolla*incorporation increases the paddy yield by 8-14% (Yao et al., 2018). Behera reported that the grain yield increased by34% due to the incorporation of 10 ton *Azolla*/ha (Behera, 1982). Kannaiyan and Bartharkur and Talukdar reported 36.6 to 38% increase in grain yield due to use of*Azolla* as dual crop (Kannaiyan, 1982; Bartharkur and Talukdar, 1983). Moore found the increase in rice yield by 14-40% due top *Azolla*dual cropping, whereas Le Van reported 6-29% higher grain yield by growing *A. pinnata* as a dual crop with rice (Moore, 1969; Le Van, 1963). A group researhcers reported the application of *Azolla* along with neem cake coated urea recorded the maximum grain yield of rice (Sukumar et al., 1988).

Benefits of using *Azolla* on the rice field are: Basal application of *Azolla* at the rate of 10-12 tones/ha enriches soil nitrogen content by 50-60 kg/ha and reduces 30-35 kg of nitrogenous fertilizer requirement of rice crop. Inoculation of green *Azolla*at the rate of 500 kg/ha increases the soil nitrogen content by 50 kg/ha and reduces the nitrogen fertilizer by 20-30 kg/ha (Roy et al., 2016). Inoculation of *Azolla* on flooded water decreases the NH_3 volatilization by 12–42% (Yao et al., 2018). Due to its high biomass production rate, *Azolla* improves the soil physical structure by supplying huge amount of organic matter on its incorporation to soil (Subedi and Shrestha, 2015. *Azolla* also solubilizes the, Zn, Fe and Mn and make them available to the rice crop. It releases plant growth regulators and vitamins that enhance the growth of the rice crop. It reduces the evaporation rate from the rice field, reduces disease occurrence, suppresses weed growth, enhances flowering fruiting, and increases plant establishment and survival at seedling or transplanting and so on (Biswa et al., 2005; Monajjem and Hajipour, 2010). Azolla also reduces the intensity of light penetration, water evaporation, and suppress different weeds like Echinochloa crus-galli, Cyperus spp., Paspalum sp. and so on and thus, lead to improved crop growth and productivity (Biswa et al., 2005; Monajjem and Hajipour, 2010).

4. Conclusion

The fundamental to successful rice production is the optimum amount of Nitrogen input and *Anabaena azollae* symbiosis has been known to increase nitrogen input in rice cultivation. The amount of rice production will be increased due to the inoculation of nitrogen fixing bacteria. Therefore, the effective strains of *Azolla*in terms of nitrogen fixation from different agro ecological zones are to be screened and their contribution to rice production needs to be evaluated. Better extension strategies are also the need of hour to promote the use of bio-fertilizer with other benefits.Concerted efforts are required from the part of policy makers, scientists and farmers to promote*Azolla* as a viable bio-fertilizer for sustainable rice production.

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