Azolla as a source of biofertilizer for sustainable crop production – a review

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Abstract - *Azolla* has been exploited widely as biofertilizer for rice and some crop plants, and it fixes atmospheric nitrogen due to the presence of a symbiotic cyanobacterium, *Anabaena azollae*. It has several other uses such as food, feed, biogas producer and hyper accumulator of heavy metals, etc. Agricultural fertilizers are essential to enhance proper growth and crop yield. Recently, farmers have been using chemical fertilizers for quicker and better yield. But these fertilizers endanger ecosystems, soil, plants, and human and animal lives. The use of bio-fertilizers like *Azolla* not only increases the crop productivity but also improves the long term soil fertility. This multidimensional uses of *Azolla-Anabaena* system would be ideal and environment friendly in sustainable agriculture.The current study critically reviewed the Azolla's potential capacity to be used in sustainable agriculture.

Keywords: *Azolla*, Sustainable Agriculture, Biofertilizer, Nitrogen

INTRODUCTION

Chemical fertilizers have been widely used to achieve maximum productivity in conventional agricultural systems. The continuous and excessive utilization of chemical fertilizers plays a major role, directly and/or indirectly, in changing environmental conditions (Ali *et al.*, 2021). To overcome this problem and achieve food security for the rising population, a new sustainable approach is needed for agriculture (Glick, 2018). This has made the environmentalists to switch over to organic farming. Organic farming has emerged as an important priority area globally in view of the growing demand for safe and healthy food and long term sustainability and concerns on environmental pollution associated with the indiscriminate use of agrochemicals. Organic farming not only ensures food safety but also improves the biodiversity of soil (Megali *et al.*, 2014).

Biofertilizers are an important component of organic farming, which contains live microorganisms in carrier-based formulations that can be applied directly in the soil, seed, or seedling stage. On application, it improves the nutrient status of the plant by efficient nitrogen fixation or phosphate solubilization or by increasing the number of microorganisms that assist in the availability of nutrients which are easily assimilated by the plants. Biological control of various phytopathogens is also another facet of using biofertilizers in agriculture (Singh *et al.*, 2021; Santhiya and Jeeva, 2022).

The aquatic fern *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 (Yadav *et al.*, 2014).It has the property to multiply faster at very high rates and covers the surface of water bodies; thus, it forms a thick mat and helps in reducing the volatility of ammonia in the fields. *Azolla* can be used as a biofertilizer in various crops such as rice, wheat, taro, banana and tomatoes (York and Garden, 2016). It is an environmentally safe, non-toxic and preferable fertilizer resource for household gardening and organic farming (Indrani*et al.*, 2019). The application of *Azolla* as a biofertilizer provides natural source nutrients and has tremendous potential to improve soil health and boost yield sustainability (Pereira, 2017; Akhtar *et al.*, 2020). It can be an alternative to improve rice yield without degrading the environment. It provides a natural source of many nutrients, especially N, improves the availability of other nutrients, plays a critical role in weed suppression, enhances soil organic matter, and improves efficiency of the inorganic fertilizers while maintaining the suitable soil pH condition (Thapa and Poudel, 2021).

Sustainable development refers to a mode of human development in which resource use aims to meet human needs while preserving the environment so that these needs can be met for both present and future generations (Mishra and Dash, 2014). *Azolla* is one of the hyperaccumulator plants which can absorb heavy metals 50–500 times faster than conventional plants; it has significantly helped the revolutionary progress of the phytoextraction technique (Subedi and Shrestha, 2015).This aquatic fern is used as a basis of green manure and decomposed organic material (Razavipou *et al.,* 2018). Since *Azolla* has various contributions in increasing crop yield without degrading the environment.

Azolla

Azolla sp. is a tiny free-floating fresh water fern (Plate 1) of tropical and sub-tropical Asia, Africa and America (Mishra and Dash, 2014). The name *Azolla* is derived from Greek word *azo* (to dry) and *allyo* (to kill) meaning that plant dies when it dries (Svenson, 1944).

Plate 1. A) *Azolla* sp. habit; B) surface of the paddy field covered by *Azolla* sp.; C) *Azolla* species associated with wetland plants

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 *Azolla*ceae 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). There are now seven extant species of the family Salviniaceaeceae – *Azolla caroliniana* Willd.,*Azolla cristata* Kaulf., *Azolla filiculoides* Lam., *Azolla imbricata* (Roxb. ex Griff.) Nakai, *Azolla mexicana* C. Presl, *Azolla microphylla* Kaulf. and *Azolla pinnata* R. Br (IPNI version 1.1). The Sub-Genus *Euazolla* is characterized by the presence of three floats of megasporocarps and In contrast, the Sub-Genus *Rhizosperma* consists of nine megaspore floats. The trichomes are important in the identification of the organism at the Species level (Lumpkin and Plucknett, 1982; Nayak and Singh, 1988).

Distribution

Azolla occurs naturally in freshwater ditches, ponds, lakes and sluggish rivers of warm-temperate and tropical regions (Fig. 1). Its absence from regions that have prolonged freezing or aridity (Small and Darbyshire's, 2011).

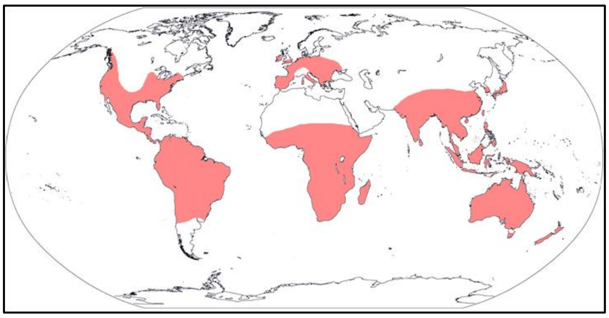

Fig. 1. Distribution map of *Azolla* (Source:Small and Darbyshire's, 2011).

Habit and Morphology

The *Azolla* plants are delicate, small and triangular or polygonal in shape. 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).Its ventral surface has a multi-branched rhizome that bears small leaves. It contains handing roots into water to absorb the nutrient contents directly (Roger, 1999). The leaves consist of chlorophylls and a colorless lobe to supply buoyancy. Each lobe contains a cavity that provides a microcosm environment with self-developed and defined. It behaves as a symbiotic unit association when an energetic and metabolic reaction occurs (Adhikari *et al.,* 2020).

***Azolla* as Green manure**

Azolla was primarily grown as a green manure for rice, but it is also grown with water bamboo (*Zizanica aquatica*), arrowhead (*Sagittaria sagittifolia*) and taro (*Colocasia esculenta*) (Anonymous, 1975). The positive effect was recorded with*Azolla* green manure on the number of shoots, length of longest leaf, fresh weight, and dry weight of rice plants (Ngo, 1973). The *Azolla* cultivation increased the nitrogen content of the soil to a level equal to that produced by a crop of soybeans (Shen *et al.,* 1963). *A. filiculoides* could provide one half of the nitrogen requirement for rice if it were grown as a green manure before rice seeding (Taller *et al.,* 1977). Two successive *Azolla* layers, incorporated into the soil before rice transplanting, can supply 50 percent of the nitrogen necessary to produce 5 tons of rice per hectare (Tran and Dao, 1973).

The application of *Azolla* as green manure, it can be collected directly from ponds/ditches. 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 week time and can be incorporated in the soil. Rice can also be transplanted in the fieldsubsequently. 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} (Yadav*et al.,* 2014). Fresh *Azolla* into the soil significantly increased water holding capacity, organic carbon, ammonium nitrogen, nitrate-nitrogen and its available phosphorus, potassium, calcium and magnesium, while it decreased pH and bulk density, such incorpation significantly raised the yield of mung beans (Raja *et al.,* 2012).

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 (Yadav *et al.,* 2014).

***Azolla* - Soil nutrient availability**

Azolla has a remarkable ability to accumulate K in its tissues in a low K environment; it decomposes rapidly and releases nutrients N, P and K into the field after field water is drained (Bhuvaneshwari and Singh, 2015). It solubilizes Zinc (Zn), Iron (Fe), and Magnesium (Mg), making them available to the rice crop, and releases plant growth regulators and vitamins that promote the crops to grow faster (Bhusal and Thakur, 2021). Its continuous application increased the soil nutrient availability (Subedi and Shrestha, 2015). In general, the use of *Azolla* improves soil nutrient availability through biological activity, which also helps to build up the micro flora for mineralization. When *Azolla* decayed, it released soil-available P into the soil (Watanabe *et al.,* 1989). There was no significant difference at the beginning of available soil P in *Azolla* added paddy soils (Rivai*et al.,* 2013). However, there was an 89% increase in *Azolla* added available soil P at rice panicle initiation. Similarly, found that P and Ca contents were also higher in *Azolla*, averaging 124.83 ppm and 345.3 mg/100g (Halder and Kheroar, 2013). *Azolla* treated soil showed a 29.12 % increase of K (Dey *et al.,* 2018). The *Azolla* show the positive results of integrated soil nutrient management practices, which are lacking in many Asian countries.

Azolla helps in addition of organic matter and release of cations such as Magnesium, Calcium and Sodium. The total N, available P and exchangeable K in the soil and N-uptake by rice can be improved (Subedi and Shrestha, 2015).Application of *Azolla* also plays a definite role in enhancing soil fertility by increasing available nitrogen, organic carbon, phosphorous and potassium (Mandal *et al.,* 1999). Their utilization also helps curb NH_3 volatilization, prevent rise in pH, reduce water temperature, build up organic matter, and influence the transformation and availability of iron, manganese, zinc, and copper, which improves the infiltration and movement of water in soil (Mandal *et al.,* 1999, Pabby *et al.,* 2004). Therefore, *Azolla* application is considered as a good practice for sustaining soil fertility and crop productivity irrespective of some limitations (Subedi and Shrestha, 2015).

Effect of *Azolla*on soil organic matter

Azolla compost impact plant growth and yield positively and improve the organic matter in the soil (Gupta and Potalia, 1990). It maintains its reserve for a long time by releasing its content materials slowly, which provides advantages over raw, unrotted organic matter and chemical fertilizers (Kandel *et al.,* 2020). The high organic C content of *Azolla* contributes to the increase in organic Carbon. According to 90% of *Azolla* was degraded in 4 weeks (Watanabe *et al.*, 1989). The *Azolla* that had been absorbed into the soil would shortly be mineralized. It would generate humic substances as a result of the mineralization process which would also yield soil organic C (Bhardwaj and Gaur, 1970). The inoculation of *Azolla* built up a considerable soil organic carbon content (Setiawati *et al.,* 2018). *Azolla* and cow manure equal combination increased the soil organic C content ranging from 1.3− 1.7 % (Setiawati *et al.,* 2018). Similarly, it was reported that *Azolla* treated soil oxidizable organic C increased 25.51% (Halder and Kheroar, 2013). The higher soil microbial populations of bacteria, fungi, actinomycetes, and higher enzyme activities in *Azolla* incorporated soil, increasing nutrient recycling in the soil (Krishnakumar *et al.,* 2005).

Biological nitrogen fixation

Application of *Azolla* in rice paddy fields has a positive role in improving the soil fertility index. The ability to fix nitrogen 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. The Calvin cycle operates in both the partners and the primary end product of photosynthesis is sucrose (Van, 1989). A strong interaction exists between nitrogen fixation and photosynthesis and the source of ATP and NADPH is photosynthesis. The capacity of *Azolla* to fix nitrogen in the field has been estimated at 1.1 kg N ha^{-1} day^{-1} and this fixed nitrogen is sufficient to meet the entire nitrogen requirement of the rice crop within a few weeks (Lumpkin and Plucknett, 1980). *A. pinnata* fixes 75 mg N g^{-1} dry weight day^{-1} and produces a biomass of 347 tonnes fresh weight ha^{-1} in a year. This biomass contains 868 kg of nitrogen, which is equivalent to 1900 kg of urea. A wide variability regarding growth and nitrogen fixation among different strains of *Azolla* is observed (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).

Role of *Azolla* in reclamation of contaminated soil

Application of *Azolla* in rice paddy fields has a positive role in improving the soil fertility index (Peters and Meeks, 1989).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. *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 also influenced by the humic substances formed during the decomposition of *Azolla* (Bhardwaj and Gaur, 1970). The continuous application increased the organic nitrogen content of the soil significantly. Increased celluloytic and urea hydrolyzing activities in addition to significant increase in the population of heterotrophic bacteria (Kannaiyan and Subramani, 1992; Kannaiyan and Kalidurai, 1995) and increased soil urease and phosphatase activity (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, 2000). Maximum population of bacteria, fungi and actinomycetes and high urease and dehydrogenase activities due to organic farming using *Azolla* as one of the components (Krishnakumar *et al.,* 2005).

***Azolla*: potential biofertilizer for increasing rice productivity**

The application of free-floating aquatic fern *Azolla* as a biofertilizer can be an alternative to improve rice yield without degrading the environment (Plate 1) (Thapa and Poudel, 2021). *Azolla*, offers significant potential as an N source in rice production (Wagner, 1997). Farmers can manage around 30-60 kg by incorporating *Azolla* at the rate of 16000 kg ha^{-1} in rice crops instead of supplying through N fertilizers, given the sustainability of soil health (Samal *et al.,* 2020). *Azolla* application desirably affects plant growth, biological yield and enhancing nutrient quality (Gupta and Potalia, 1990). *Azolla* incorporation in paddy fields increased grain yield, straw yield, caryopsis, and dry matter (Anjuli *et al.*, 2004). Its incorporation increases the paddy yield by 8-14% (Yao *et al.,* 2018). The rice yield increases up to 13% when *Azolla* was used as a biofertilizer in rice crops (Watanabe, 1977). *Azolla* application increased the yield components of rice (Kannaiyan and Rejeswari, 1983; Islam *et al.,* 1984).An increase in grain yields of rice from 14 - 40% has been reported, with *Azolla* being used as a dual crop and by 15-20 % being monocropping during the fallow season (Samal*et al.*, 2020). The highest rice grain yield when the application of *Azolla* compost at 5.0% of soil weight, which was on average 13.8% higher than that of the non-amended control (Razavipour *et al.*, 2018). The incorporation of 8-10 t of *Azolla* ha^{-1} produced the exact rice yield, 47% increase in grain yield over control (Singh, 1977).The rice yield can be increased by 36.6 -38% by using *Azolla* as a dual crop (Barthakur and Talukdar, 1983). *Azolla* dual cropping increases rice yield by 14-40% and 6-29% higher grain yield by growing *A. pinata* as a dual crop with rice (Moore, 1969; Le Van, 1963).Utilization of blue green algae increase yield in organic Basmati rice and improvement in grain and soil quality (Singh *et al.,* 2007; Bhuvaneshwari, 2012). These findings showed that the application of *Azolla* as a biofertilizer has positive and significant improvement in the rice yield.

The *Azolla* can successfully be used for increasing crop yield in a rice-wheat cropping system (Ali *et al.,* 1998). *Azolla* has been considered green manures in several developing countries to fertilize the paddies and improve the yields, which fixes nearly 40–60 Kg N/ha of rice crop (Rai *et al.,* 2018).They are phototrophic in nature and produce auxin, indole acetic acid and gibberllic acid, and fix 20-30 kg nitrogen/ha in submerged rice fields. As they are abundant in paddies, they are also referred as 'paddy organisms' (Mishra and Dash, 2014).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, 1979).The utilization of *Azolla* as green manure has been extensively investigated and an increase in paddy yield ranging from 9-38% has been observed in field experiments when *Azolla* was incorporated to the soil (Singh, 1977). Incorporation of *Azolla* into the soil also enhances the release of other nutrients. A 50% reduction in chemical fertilizer use can be achieved without significant loss to the rice yield with the use of *Azolla* along with chemical fertilizer (Francisco *et al.*, 2000).

Effect of *Azolla* in weeds

Weeds reduce the Rice yield ranging from 15 − 20% and up to 50% (Sureshkumar *et al.,* 2016). *Azolla* covering water surface reduces light penetration of soil surface, resulting in the depreciation in the germination of weeds (70% of the weed). Thus, the growth of *Azolla* reduces aquatic weeds in flooded rice fields like *Echinochloa crus-galli*, *Cyperus* sp., *Paspalum* sp. and so on and, this leads to improve crop growth and productivity (Biswas *et al.,* 2005). The degree of suppression increases with an increase in the percent of *Azolla* cover and water depth (Kalyanasundaram *et al.,* 1999). Application of presumed at 10 t ha^{-1} + *Azolla* at 1 t ha^{-1} recorded the least weed count and highest weed control index in rice crop, as the thallus growth formed a very thick mat on the surface of the water, shortening the interception of light by weed seeds and seedlings (Gnanavel, 2015). Weeds were suppressed by 69 – 100% at rice flowering and 86 – 95 % at harvest depending upon weed species due to the use of the *Azolla* (Janiya and Moody, 1984). The ability of a thick, light-proof *Azolla* mat to suppress weed development has long been observed in rice field (Shen *et al.,* 1963).

Other uses

Azolla used as fishfood and weed control (Edwards, 1974); Mosquito control (Ansari and Sharma, 1991; Benedict, 1923; King *et al.,* 1942; Cohn and Renlund, 1953; Shaver, 1954; Neai, 1965; Burkill, 1966); A fodder crop (Dao, 1973). *Azolla* was fed to pigs, ducks and chickens (Chevalier, 1926; Chevalier, 1926; Fujiwara *et al.,* 1947; Dao and Tran, 1966; Burkill, 1966; Anonymous, 1975); cattle (Le Van and Sobochkin, 1963; Dao and Tran, 1966; Sculthorpe, 1967); fish (Le Van and Sobochkin, 1963; Sculthorpe, 1967). *Azolla* has been found to help purify water (Cohn and Renlund, 1953) and to be an ingredient in soap production by some African tribes (Chevalier, 1926). It was chewed to cure sore throat in New Zealand (Usher, 1974). *Azolla* is used for preparing cough medicine (Raja *et al.,* 2012). *Azolla* as a component of Space Diet (Katayama *et al.,* 2008) and also used in the production of biogas (Das *et al.*, 1994).

Government Policies to implement *Azolla* as biofertilizers

Many Governments of Asian countries have implemented policies which have directly and indirectly supported in the biofertilizers implementation. The Government of India has been encouraging the use of biofertilizers in agriculture (Ghosh, 2004). State level governments are also emphasizing the biofertilizers usages. The government of Odisha, has trained farmers to utilize *Azolla* as a biofertilizers (Mishra and Dash, 2014). The government of Bangladesh has put forward the policies to support the production and implementation of bio-fertilizers. It has also supported the ongoing research on *Azolla* for wetland Boro rice (Goswami *et al.*, 2014).Similarly, Nepal's Agricultural Biodiversity Policy, 2006 has emphasized on use of biofertilizers Amendment in 2014 (Atreya, 2015). Countries; China, Myanmar, Lao PDR, Thailand, Cambodia and Vietnam government have shifted their focus in promoting sustainable agriculture, thus emphasizing the policies in biofertilizers promotion (Atieno *et al.*, 2020). Thailand Institute of Scientific and Technological Research (TISTR) have selected and commercialized blue-green algae for use as biofertilizers (Damrongchai, 2000). Philippines government has developed program to promote the use of *Azolla* incorporation instead of heavy incorporation of chemical fertilizer during rice production (Rosegrant *et al.*,1985).

Conclusion

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. The high growth rates of *Azolla* reduce the environmental risks, and it is a cost-effective method for developing wetlands. However, before using *Azolla*, the economics of using *Azolla* should be considered because technology is very labor-intensive, and it is suitable for adoption in locations where farm labor is affordable. Besides its utilization as biofertilizer and livestock feed, *Azolla*, the 'green gold mine' of the nature is also used as medicine, water purifier, human food and for production of biogas. 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. Concerted efforts are required from the part of policy makers, scientists and farmers to promote *Azolla* as a viable bioinoculant for sustainable crop production and development.

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