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AZOLLA: A UNIQUE ORGANISM THAT CAN SUBSTITUTE NITROGENOUS FERTILIZERS IN RICE CULTIVATION IN NORTH EASTERN INDIA

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ABSTRACT
The successful production of rice depends upon an economic and efficient supply of nitrogen, an element required in the largest quantity in comparison with the other essential nutrients. It is mostly grown on residual soil, fertility with minimum inputs, and ameliorative whereas for raising a good crop and for a good harvest the crop needs an adequate amount of nutrients. Small and marginal farmers have a low purchase capacity of good seed materials and other inputs. For these people agricultural practices should be affordable. Fertilizers can contribute to more than 40% of rice grain yield and dry matter production. There is a practice to supply some quantities of organic manure, farm yard manure, composted plant residues, and other wastes to provide essential plant nutrients. Chemical fertilizers which are a major source in supplying nitrogen to rice, increasing the yield but have adverse effects on overall soil and environmental health in the long term. *Azolla* is a small moss like floating plants, forming a dense covering on the surface of ponds. It also helps in the suppression of weed, enhances organic matter and improves efficiency of the inorganic fertilizers. Rice is a major food in North Eastern States of India. Due to the increase in population there is an increase in the demand for rice so *Azolla* is a better option to be used as a substitute for nitrogenous fertilizer. Thus, an intensive review of literature on *Azolla* that improves rice yield is carried out with the objectives to know the various other factors which contribute to the increase in the production of rice.

Keywords: *Azolla, Anabaena, Nitrogenous Fertilizer, Rice Crop, Nitrogen fixation, North Eastern States*

INTRODUCTION
Agriculture is the main occupation of the people of Northeast. Almost two third of people are involved in agriculture in the entire region (Hazarika, 2007). The North Eastern Region of India comprises eight states viz., Assam, Arunachal Pradesh, Meghalaya, Manipur, Mizoram, Nagaland, Sikkim and Tripura (Janaiah, 2020). This region has geographically, demographically, culturally, socially, economically and politically distinct features from the rest of India. It is geographically dominated by hilly areas and ethnic tribes who contribute to its economy and is driven by a rice based agriculture system. Total geographical area of the region is 262,180 km2 which is about 8% of the country's total area, sheltering about 4% of India's total population. The net sown area is highest in Assam (34%) whereas plains account for 84% of its total geographical area, followed by Tripura (24%), while Arunachal Pradesh has lowest net sown area (only 2%) (Janaiah, 2020). About 80% of NER's population lives in rural areas, depending on rice-based agriculture as the key source of livelihood. Rice is the major cereal and staple food for the people of North-Eastern Region occupying an area 3.41 million hectare with an average productivity of 13.95 q/ha. With the present level of productivity of rice in this region, per capita availability of rice is only 331 g/day while a subsistence requirement is 450 g/day. Further, local ethnic tribes with diverse cultural backgrounds account for two thirds of the population in NEHR (North East Council, 2018). Jhum cultivation is a common practice in hilly areas of NER. Nearly half million tribal farm families belonging to 38 major ethnic tribes practice jhum cultivation in 35 districts of NER. This traditional practice covers

about 2.5 million ha. of land but the productivity of Jhum lands is very low (about 1 ton/ha). Further, out of 4 million ha of net cultivated area in NER, one third suffers from soil erosion and degradation problems mainly due to widespread practice of Jhum or shifting cultivation by tribal communities and because of the topography of the land (Bujarbaruah, 2004).
Agronomically there are two general ecological types of rice; the lowland or irrigated and the upland or non-irrigated. Lowland rice is grown on lands that remain flooded with water from the time of transplanting to the harvesting. It is either grown under a rain fed or an artificial system of irrigation and is the most important. Its yield is much higher compared to the upland rice. Upland rice is entirely dependent upon seasonal rainfall and is, therefore, relatively less important. (Kochar, 2011)
Rice can be grown on many types of soil, from sandy loams and shallow lateritic soils to heavy clay, but heavy alluvial soils of river valleys and deltas are preferable. The crop prefers acidic soils. During the growing season swampy soils are required where the land remains submerged underwater for 60-90 days. A minimum of 60-120 cm of rainfall is required for upland varieties; while lowland types require 180-240 cm. It responds extremely well to nitrogenous (ammonium sulphate) and phosphate fertilisers (super-phosphate or bone meal) (Kochar, 2011).

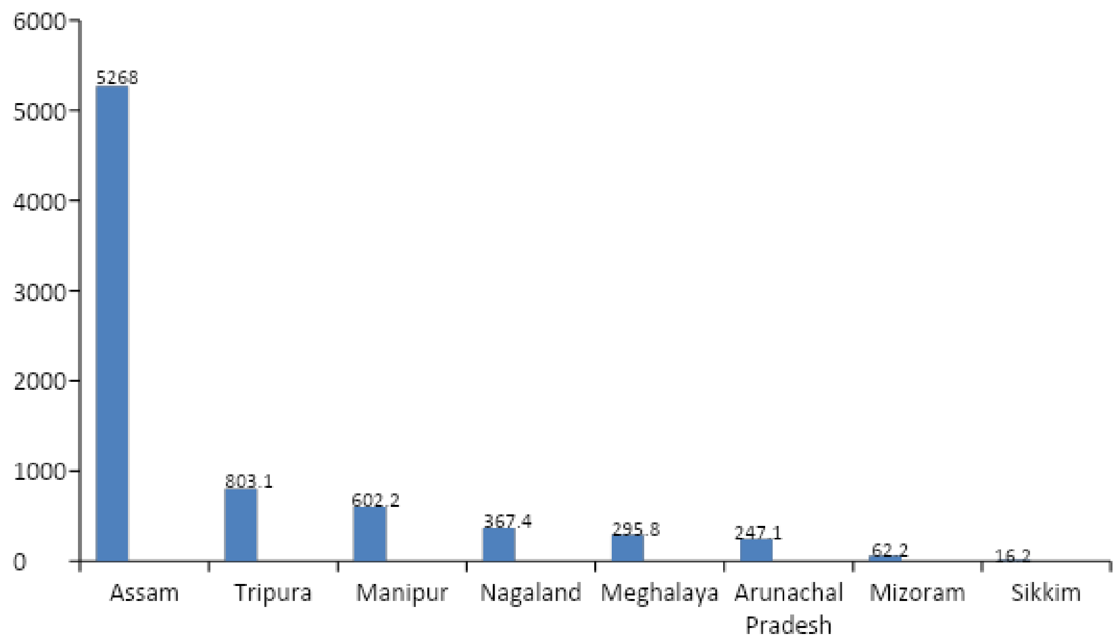

Figure 1: Rice production in North Eastern States (thousand tonnes)
Source: https://www.nfsm.gov.in/ and RBI Statistic (1920-1921)

2. MORPHOLOGY AND DISTRIBUTION OF AZOLLA

Azolla is a small elegant water fern with worldwide distribution. The genus Lamarck is not only abundantly found floating on water surface in tropics and subtropics but also found in temperate regions (Moore, 1969). Lamarck established this genus in 1915. *Azolla salvinia* is a separate genus. However, both *Azolla lam* and *Azolla sal were* kept in the Salviniaceae family (Moore, 1969). There are six species of Azolla that were identified by Hills and Gopal (1967). These are *Azolla caroliniana (wild), Azolla mexicana, Azolla pinnata, Azolla rubra, Azolla microphylla. Azolla pinnata* is found almost in all the districts of Assam and other states of NEH Region.

Table 1: Distribution of *Azolla* spp. in Asia

S.N	*Azolla* Species	Distribution
01.	*Azolla caroliniana, wil*	Eastern USA, Mexico, Spain, France
02.	*Azolla filiculoides, Lam.*	USA, U. k., Germany, Australia, New Zealand Czechoslovakia and Japan
03	*Azolla Mexicana, Persl.*	Mexico and USA
04.	*Azolla nilotica*	African Countries
05.	*Azolla pinnata*	India, Bangladesh, China, Indonesia, Japan, Philippines, Sri Lanka, Thailand, Vietnam and Laos

Source: (Hazarika, 2007)

The *azolla* plant, measuring up to 1.5 cm during the growth period, consists of floating, branched rhizomes with small, alternately overlapping leaves and simple roots (2-5 cm) and is submerged in the water. Each of the bilobed leaves consists of dorsal and ventral lobes. The dorsal lobe is green and purple, fleshy, aerial, chlorophyllous, and contains algal symbionts within the central cavity. The thinner ventral lobes contain spongy tissues, white or pink, and remain partially submerged in the water. Plants contain three types of roots with absorbent hairs and older brownish roots. Roots contribute 30% of the total plant weight. The older roots get detached from the plant. The fern multiplies mostly vegetatively, but reproductive structures known as sporocarps are formed during winter, when the fern is left unharvested for a fortnight. The temperature below 20°C or above 35°C has an important role in the formation of sporocarp. The ventral part of the plant contains a large number of paired spherical micro sporocarp or one microsporocarp and one mega sporocarp up to 25 numbers in the breeding plots. Each of the microsporocarp contains 50-200 stalked microsporangium with chloroplasts. Each microsporangium contains about 25 microspores (Hazarika 2007; Singh *et al.*, 2016).

The megaspores are smaller in size, oblong in shape with nine floats, a characteristic of *Azolla pinnata*. The antheridia develop within the massula, which used to hold together microspores and produce antherozoids, which escape through the mucilage of the massula. Each megasporangium produces one megaspore, and during release, its wall possesses long hairs with which the glochidia of the massulae are attached, and both micro and megaspore are held in proximity. The megaspore divides to form the prothallus, which produces archegonia with one egg cell in each, and after fertilization, the zygote divides and produces the sporophytes (Moore, 1969; Singh *et al.*, 2016). During vegetative reproduction, a lateral branch gets separated from the main rhizome following the formation of an abscission layer at its base. The dispersal of *azolla* occurs due to water currents, aquatic birds and human beings (Hazarika, 2007; Singh *et al.*, 2016).

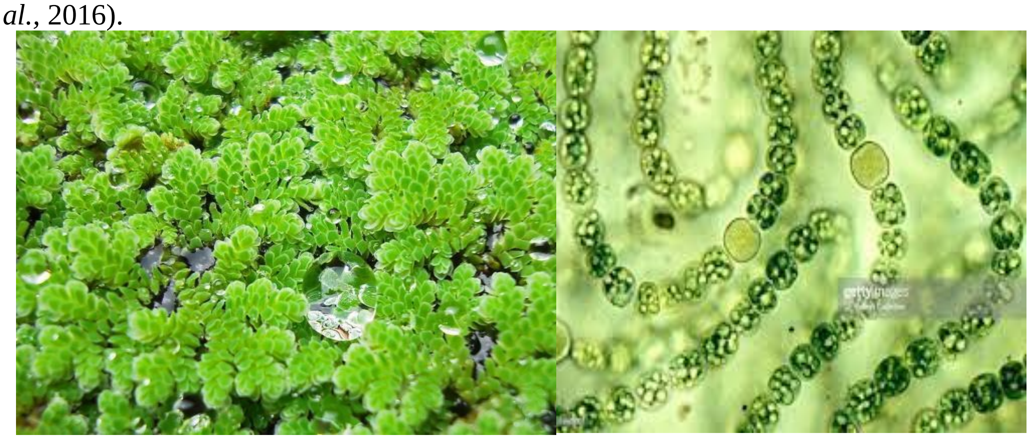

Figure 2: (Under CC): (A) *Azolla pinnata* **(B) *Anabaena***

3. CULTIVATION OF AZOLLA

Azolla is easily cultivable under the agro climatic conditions of the North Eastern states, and its beneficial effect on rice crops is evaluated through various field experiments. It is widely found almost in entire North-Eastern states, and it is being multiplied in rice fields as dual cropping. It grows well along with undisturbed paddy stubble, which remains in harvested paddy fields under low moisture conditions. Under this situation, *azolla* gets shade from the paddy stubble and multiplies slowly under the low moisture content of the soil. The stubble provides warm and marshy conditions to the fern, and thus it multiplies and produces 19.3 q of green biomass per hectare. Similarly, mulching of the harvested paddy field with grasses and paddy straw also produced encouraging *azolla* biomass. However, when stubble is cut down at 2 inch above the soil, it does not grow well (Hazarika, 2007; Sathe, 2016).

Azolla inoculums at a rate of 100-300 g are recommended for multiplication in small ponds. The pond should be rectangular and of small size, preferably 2 x 3 m. It should be maintained with at least 10-15 cm of continuous water. The water level should be maintained by inlets and outlets from ponds. It multiplies under favourable conditions (25-27 C) in 3-4 weeks' time and can then be further shifted to other ponds for further multiplication or directly transferred to paddy fields. A dose of 20 kg P_2O_5, and 5 kg nitrogen/ha is necessary for the growth of *azolla* (Hazarika, 2007).

Factors Affecting Growth of *Azolla*

3.1. Role of Temperature on Biomass Yield

Temperature is an important factor in the production of *azolla*. With the increase in temperature and humidity, its growth and productivity increase. Maximum growth and biomass production were recorded from June to August (28-29 q/ha) and minimum growth was observed from November to January (12-14 q/ha). During winter months when *azolla* ponds were shaded with straw/weed cover (20.6 q/ha), the growth of fern was its maximum with the maximum green biomass yield, followed by polythene covered ponds (Hazarika, 2007).

Azolla caroliniana (wild) is a cold tolerant isolate and survives under acute moisture stress situations, even in barren ponds, fields, and canals. However, for the maintenance of parental stock, the fern needs to be protected under straw and weed cover shade (Singh, 1981). It is reported that *Azolla pinnata* L. perform well under tropical conditions (Hazarika, 2007).

3.2. Role of Phosphorus and Water Level

The water depths of ponds as well as the dose of phosphate fertilizer have greatly influenced the biomass yield of *azolla*. Water level in ponds of about 15 cm is optimum for good biomass yield, however, the fern grows well at 5-10 cm of water level. Under optimum congenial climatic conditions, the fern produced 172 g/ha of biomass per harvest at 20 kg P_2O_5 (as SSP) and 15 cm water level maintained in the ponds (Hazarika, 2007).

3.3. pH of Water

The fern prefers to grow under partial shade (dual cropping with rice), at a pH of 5 along with lime at the rate of 50 kg/ha for one season. However, as dual cropping with rice, the fern should be released 7-10 days after transplant of rice. Fresh *azolla* releases nitrogen due to its rapid mineralization. For insect pest control, Furadon smokes at 100 g/plot are applied 7 days after inoculating *Azolla*. After 15 days of inoculation, it was harvested and introduced into the main field as a source of primary inoculum (Hazarika, 2007, Sathe 2016; Adzman *et al.*, 2022).

4. AZOLLA ANABAENA SYMBIOTIC RELATIONSHIP

The eukaryotic water fern *azolla* and the prokaryotic endosymbiont *anabaena*-*azolla* are actively involved in the symbiotic association and fix atmospheric nitrogen. Fern provides the carbon source sucrose to the *anabaena* while the *anabaena* fixes atmospheric nitrogen and transfers it to *azolla*, which in turn multiplies very rapidly. The *anabaena* is associated with the dorsal lobes of the leaf from the onset of their development and is never in direct contact with the external environment. Its shoot tips are supported above the water surface by the stem, and the colony of *anabaena* filaments is associated with every apical meristem. The site of biological nitrogen fixation is the heterocyst of the *anabaena* while vegetative cells are involved in photosynthesis. The nitrogen requirement of the *azolla* is fulfilled by the *anabaena*. The ammonia from nitrogen fixation by the cyanobiont is released by the fronds ammonia assimilating enzymes (Samal *et al.,* 2020).

High levels of nutrients, particularly phosphorus, iron, nitrogen, and manganese, are present in water of rice fields with *azolla* dual cropping due to their liberation from decaying *azolla* plants after chemical and enzymatic action. The release of these nutrients stimulates further growth of the fern as well as the rice crop, and the occurrence of an anaerobic and nutrient-rich environment below the *azolla* mats might not be suitable for the survival of aquatic weeds and aquatic insect populations.

5. APPLICATION OF AZOLLA

Two methods of application of *azolla* have been developed in India, as a green manure before planting rice and with rice when the fern grows with the rice crops for some time. It is recommended for submerged paddy fields, within a maximum temperature of 38°C. It has been proven that it can increase the average yield of crops within the range of 15% to 20 % (Sathe, 2016).

Table 2: Release of Nitrogen as Ammonia

Sl. No.	Days	Percentage of Ammonia released
1.	7	33.5
2.	14	40.5
3.	21	56.0
4.	28	67.6
5.	35	71.3
6.	40-42	81.5

Source: (Hazarika, 2007)

6. FACTORS CONTRIBUTING TO THE INCREASE IN RICE YIELD

6.1. Contribution of Azolla in Biological Nitrogen fixation

Nitrogen is the single most limiting factor in rice cultivation, which affects the crop yield. Nitrogen fixation is a process in which molecular nitrogen (N_2) in the atmosphere is converted into inorganic nitrogenous compounds such as nitrates or ammonia. An estimated 190 x 1012 gm per year of nitrogen is fixed by natural processes (both non biological and biological). Biological fixation of nitrogen is about 90%, and non-biological nitrogen fixation (by lightning, 8%) and UV radiation (2%) contribute about 10% of the total N_2 fixed by natural processes (Jain, 1974).

Nitrogenous fertilizers may be subdivided into three categories: nitrates, ammonium salts, and simple amides. These are soluble in water; hence they are taken directly by the plants through their roots. Soil microorganisms convert ammonium salts into nitrates, which are then absorbed by the roots. However, in acidic soil, this conversion is extremely slow. Nevertheless, like nitrates, the ammonium salts are also soluble in water, and in acidic soil environments. Plants are adapted to absorb them directly (Sodhi, 2002). Cyanobacteria, which fix atmospheric nitrogen, and relatively thin ventral leaves provide buoyancy that remains partially submerged in water. The symbiont liberates a substantial amount of biologically fixed nitrogen as the host absorbs ammonia through branched hairs present in the cavity. Unbranched hairs transport fixed carbon from the host to the cyanobiont (Peters et al., 1982). An average of 35-50 % ammonia fixed by the cyanobacterium is released to the field, and for this reason, a*zolla* is used as a biofertilizer in the rice fields. Nitrogen fixation and a high growth rate can enable *azolla* to

accumulate more than 10 kg N ha^{-1} day^{-1}. This aquatic fern is used as a basis for green manure and decomposed organic material, widely known as compost (Hazarika, 2007).
A layer of *azolla* covering a hectare of land of rice weighed about 10 tons of green biomass, which would ensure 30 kg N after decomposition. Growing a second crop of it could double this amount after decomposing or incorporating the previous layer. It decomposes in 8-10 days after incorporation in soil, and rice seedlings could be transplanted after 10 days of *azolla* incorporation. In rice growing areas, it survives for a long period through its spores and regenerates and spreads naturally (Hazarika, 2007).

6.2. Soil organic matter content

Azolla compost contains nitrogen content of 1.51% P content 1.27-2.66% and K content 6.0-7.85% and which thus improves organic matter of the soil (Hazarika, 2007). It releases the content very slowly, thus maintaining the reserve for a longer time (Gupta and Potalia, 1990). This is an advantage over raw organic matter present in the soil. 90% of *azolla* was decomposed in 4 weeks (Watanabe et al., 1989). The *azolla* when absorbed into the soil would generate humic substances due to the mineralization process, which would also yield soil organic carbon (Bhardwaj and Gaur, 1970; Thapa and Poudel 2021).

6.3. Soil pH

Soil pH influences many of its physical, chemical, and biological properties and processes, which affect plant growth and biomass yield (Neina, 2019). A slightly acidic to neutral pH of the soil is a favourable environment for plant development since all nutrients are available at this pH. Soil pH 6 is considered a suitable condition for rice growth (Abdul Halim *et al.*, 2018). It was reported that in flooding conditions, soil pH also increased simultaneously. It was found that the incorporation of *azolla* reduced the soil pH (Asghar, 2018).

6.4. Increase in the availability of nutrients

Before flooding and planting rice seedlings, the fields are fertilized with farmyard manure or compost. Green manuring with a leguminous crop is also widely recommended. Rice responds extremely well to nitrogenous (ammonium sulphate) and phosphate fertilisers (super phosphate or bone meal). Addition of fertilizers just before panicle formation greatly increases the yield. The fern grows under partial shade as a dual crop with rice in lowland. Every 100 g of live Azolla contributes 0.5 kg N, 0.4 kg Ca, 0.5 kg Mg, and 0.6 kg Fe (Singh, 1981). Generally, 6 tons of it is comparable to 50 kg of N (Hazarika, 2007; Thapa and Poudel 2021).

Table 3: Chemical Composition of Azolla

Sl. No.	Constituent's	Percentage on dry matter basis
01.	Nitrogen	4-5%
02.	Phosphorus	0.5-0.9%
03.	Potassium	2.0-4.5%
04.	Calcium	0.1-1.0%
05.	Magnesium	0.5-0.65%
06.	Iron	0.16-0.26%
07.	Crude Fat	3.0-3.36%
08.	Crude Protein	23-30%
09.	Soluble Sugar	3.4-3.5%
10.	Starch	6.5%
11.	Chlorophyll a	0.34-0.55%
12.	Ash	10.5

Source: (Hazarika, 2007; Thapa and Poudel 2021)

Table 4: Nutrients composition in different species of Azolla

Sl.No.	Species	Crude protein (%)	Crude fat (%)	Crude fiber (%)	Crude ash (%)
01.	*Azolla pinnata*	*20.4*	3.33	15.5	17.2
02.	*Azolla Microphyll*	*20.2*	3.5	15.8	16.3
03.	*Azolla filiculoides*	19.7	4.2	10.3	18.5
04.	*Azolla rubra*	19.0	4.1	14.2	15.5
05.	*Azolla Caroliniana*	18.8	3.9	14.0	16.7
06.	*Azolla Maxicana*	18.6	3.8	15.1	17.2

Source: (Datta, 2011)

7. POTENTIALITY OF GROWING AZOLLA IN NORTH EASTERN REGION

The *azolla* can be raised most successfully and economically in the tropical, subtropical belts of the Eastern Himalaya and also in the north-eastern region. There are plenty of natural water sources such as ponds, bells, and canals, where water remains year round. The temperature and other climatic factors are also favourable for the multiplication of *azolla*. Some local isolates also occur in the entire region. The fern grows well under 10-15 cm of water level in mid hill lowland areas. *Azolla caroliniana* (wild), a cold tolerant isolate of USA origin survives on moist soil surfaces for a longer period of time. During the cold winter spell from December to February, the species can be preserved in ponds under straw and weed mulch covering 10-15 cm above the water surface. The *azolla* multiplies under such a covered situation to its full growth and on the onset of monsoon; the inoculums can be transferred from the preserve ponds to main multiplication ponds for large-scale production of green biomass. It grows well at 25-30°C during the summer months at a soil pH of 5 to 6 in the entire North East Himalayan and North-East region (Hazarika, 2007).

The temperature variations during the growing season from June to October is in the range of 29-24°C.The temperature rising from 22-30°C from the months of June to August has been found to be most desirable for the growth of both rice and *azolla* under lowland. This can be grown with rice during the main 'kharif' season for fixing nitrogen in the soil. It fixes nitrogen at the rate of 15 to 20 kg/ha/ month in paddy fields and enhances rice yield. In sloppy areas and in the hills where terrace farming is being practised, there is a practice for keeping water flowing from plot to plot and from one terrace to another. Such arrangements have to be made in order to arrange the bypass channel for flowing excess water. The terrace needs a constant water level of 5-10 cm to keep the *azolla* roots touching the soil. At higher altitudes when day temperature remains below 200C, application of 5-8 kg of N through urea/ha is desirable for the rapid growth of *azolla* (Nehra *et al.*, 2007; Hazarika, 2007).

CONCLUSION

Azolla is a unique organism because of its symbiosis with the nitrogen-fixing *Azolla-Anabaena*. The compost of *azolla* contains many nutrients, and therefore it improves the soil quality. It plays a significant role as dual cropping with rice, as a green manuring crop or as compost in lowland rice when applied alone as well as with other bioorganic or synthetic biofertilizers. The fern can be safely used as green manure before transplanting of rice. The production cost of the fern is cheaper it provides a dependable source of nutrients and it is easily affordable by farmers. The potentiality of *azolla* as a partial substitute for chemical nitrogen is very well evident from research experiments. In the North-Eastern Hill region, although there is an abundance of perennial water sources, the potential of *azolla* could not be exploited, mainly because during the winter months its biomass productivity is low. Water is a prerequisite for *azolla* multiplication. It cannot withstand desiccation. Hence, it cannot be used in upland rice.

The storage value of fresh inoculum is also low and hence the fern is difficult to transport over long distances. An irrigation network and a network for inoculum conservation, production and distribution are highly essential for *azolla* utilization. High temperature inhibits growth. Temperatures higher than 34 ±

1°C during the day are detrimental to azolla growth. Black rot disease caused by *Rhizoctonia solani* is a serious one affecting *azolla* in the multiplication plots. During the winter months of November to February, weather remains almost dry without any rain, and the temperature goes very low with low atmospheric humidity, which causes a moisture deficit in most of the agricultural fields, including wetland after harvesting upland rice. Under such situations, the potentiality of *azolla anabaena* symbiosis system cannot be harvested to its optimum, unless a suitable isolate of *Azolla* is identified for its adaptability to local climatic conditions.

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