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EXPLORING USE OF AZOLLA AS THE POTENTIAL LIVESTOCK FEED RESOURCES - A REVIEW

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ABSTRACT: Azolla is considered a wonder fern with multiple benefits. It is adopted as a high-quality livestock feed supplement, and as a bio-fertilizer by farmers in several countries across the world, despite being considered it as an invasive species. This paper reviewed literatures on the factors contributing to the invasiveness of Azolla in a controlled environment. In total 53 references were referred which includes 11 books, 26 peer reviewed journal papers and 16 grey literatures from 1997 to 2022. It is synthesized that Azolla becomes invasive if allowed to escape into water bodies that are favorable for its growth. It grows faster in conducive environments such as favorable light intensity and temperature with easily available nutrients, leading to problem of clogging drainage system, reduces light penetration to the deeper part of the water bodies and reduces oxygen for other aquatic organisms. Conversely, Azolla can be easily cultivated in a controlled environment and reap its benefits as livestock feed supplement. It has high protein and mineral content which is vital for livestock feeding, and help reduce feed cost and enhance productivity.

Keywords: Azolla; controlled environment; growth; invasiveness

1. INTRODUCTION

Investment on feed and fodder production and enhancing year-round access at an affordable price is the government priority strategy to achieve livestock products self-sufficiency in Bhutan. Ensuring optimal nutrient supply and its bioavailability is key to enhancing livestock production. However, limited land available for fodder production has resulted in the shortage of livestock feeding resources. Mitigating fodder shortage issue particularly during lean season remains a challenge. Farmers have resorted to feeding commercial feeds, even for ruminants, which has translated into higher cost of livestock production. Therefore, it has become imperative for the public sector to explore alternative high quality and cheap livestock feed resources to help address feed shortage and maximize profit for farmers. Azolla (*Azolla pinnata*) a floating water fern with its roots submerged in the water commonly called as the "Water Fern" (Rahman and Hasegawa 2011) is accepted as a sustainable source of livestock feed (Pillai et al. 2005) in many countries. It is also known by other common names - large mosquito fern and red water fern (Hussner 2010). It is recorded as one of the fastest growing plants on the planet and it grows without soil (Azolla foundation 2022). There are at least eight species of Azolla worldwide: Azolla caroliniana, Azolla circinata, Azolla japonica, Azolla mexicana, Azolla microphylla, Azolla nilotica, Azolla pinnata and Azolla rubra (Raja et al. 2012). Amongst the species, Azolla pinnata is the most common species. Azolla gained popularity as an unconventional livestock feed ingredient due to high quality protein content, besides being used as a high nitrogen containing bio-fertilizer (Singh 1980). A unique symbiotic association of Azolla with the blue-green algae - Anabaena azollae make it a wonder plant (Immanuel 2019; Hossain 2021) with high protein content of over 20%. It also contains almost all essential amino acids, minerals and vitamins such as β-Carotene (Vitamin A precursor) and B12 but carbohydrate and fat content are very low (Tamuly and Alam 2018). Further, it also contains probiotics and biopolymers (Bhaskaran and Kanappan 2015) making it very suitable for use as livestock feed. Azolla could be a potential source of nutrients and has a considerably high feeding value (Anitha et al. 2016). Azolla has enormous potential as a livestock feed due to high content of protein, essential amino acids, vitamins, growth promoter intermediaries and minerals

(Mathur et al. 2013). However, Azolla was reported as an invasive alien species in several European countries, especially in the Atlantic-Mediterranean regions (Espinar et al. 2015), and in this context, propagation of Azolla in the field is restricted in Bhutan. For example, Azolla filiculoides, which was deliberately introduced in mainland Britain in the late 19th century (Sculthorpe 1967; Preston and Croft 1997) caused serious weed problems (Mansoori 1995; Janes 1998) - impeded navigation, water flow and angling, causing killing of fishes and threatening wetland nature reserves with thick floating mats. Therefore, this review work was aimed to investigate the benefits and possibilities of cultivating Azolla as a potential livestock feeding resources in Bhutan; and understand factors contributing Azolla to proliferate and become invasive in the wild.

2. MATERIALS AND METHODS

Information on invasiveness of Azolla is limited nor any attempts were made to understand its habitat needs in the Bhutanese context. An extensive desktop literature review was carried out from 1979 to 2022 keeping in mind the potential of Azolla as livestock feed resources and its invasiveness. The reviewed articles consisted of published journal papers, books and grey literature on Azolla. A total of 53 references were reviewed which includes 11 books, 26 peer reviewed journal papers and 16 grey literatures. The reviewed information was consolidated and articulated in order to address the objectives of this study.

3. Findings from the review

3.1 Origin and distribution of Azolla

Azolla under the Azollaceae family is found in many countries (Tamuly and Alam 2018) and reported it as a native to the United States, including the western states (DiTomaso et al. 2013). *Azolla filiculoides*, one of the Azolla species reported as native to warm temperature and subtropical temperatures of South America (Lusweti et al. 2011). Whereas, Hussner (2010) reported that the *Azolla filiculoides* was native to Europe in the past but died and vanished during the last Ice Age. Gradually, Azolla spread to most parts of the world - Africa, Asia, Europe, North America, Oceania and South America (Kay and Hoyle 2001; CABI 2014) either accidentally or as ornamental plant (FAO 2015). It was introduced and found in Europe, North and sub-Saharan Africa, China, Japan, New Zealand, Australia, the Caribbean and Hawaii (GBIF Secretariat 2015).

3.2 Importance of Azolla in livestock farming

With global human population estimated to surpass nine billion by the year 2030 (Brouwer et al. 2019), the dietary protein requirement from animal source is expected to increase in tandem. As such, to meet the increasing demand of dietary protein, exploration for alternative sources of plant protein for livestock feeding is imperative. Azolla is proven to be a wonderful plant that can be grown in small plot of land (Gaffer 1984) producing high protein content (Pillai et al. 2005), containing favorable amino acid profile comparable to soyabean (Brouwer et al. 2019). According to Becerra et al. (1990), Azolla was used successfully as a protein supplement to replace soybean meal in the diets of growing pigs. Besides protein content, Azolla is very rich in essential amino acids (Sanginga and Van Hove 1989), vitamins (vitamin A, vitamin B12 and Beta-carotene), growth promoter intermediaries and minerals like calcium, phosphorus, potassium, ferrous, copper and magnesium (Saraf 2017). The milk production of indigenous cattle and buffalo in India was reported to increase by 15-20% after feeding Azolla within a period of 60 days (Meena et al. 2017; Khare et al. 2014). Saraf (2017) found higher annual biomass production and protein content in Azolla as compared to several fodder species in India (Table 1).

Table 1: Comparison of biomass and protein content of Azolla with different fodder species

Fodder Species	Annual biomass production (T/ha)	Dry matter content (T/ha)	Protein content (T/ha)
Hybrid Napier	250	50	4
Lucerne	80	16	3.2
Cowpea	35	7	1.4
Sorghum	40	3.2	0.6
Azolla	730	56	20

Source: Saraf (2017)

Azolla has enormous potential as livestock feed (Lumpkin and Plucknett 1982) and it was used for many years throughout Asia (Sajeda and Khatun 1983) and in parts of Africa to feed pigs, ducks, chickens, cattle, fish (Santiago 1988), sheep and goats and rabbits (Azolla foundation 2022). Besides the use as livestock feed, Azolla was reported to have heavy metal tolerance (Sarkar and Jana 1986) and removal effects (Kitoh et al. 1993; Khosravi et al. 2005 and Ferdoushi et al. 2008) from waste water (Pabby et al. 2004) if supplemented with salt (Hosravi et al. 2005). Viajante and Heinrichs (1985) reported that a rice field when cropped with Azolla reduces rice whorl maggots. Clark W (1980), reported that the People's Republic of China has 3.2 million acres of rice paddies planted with Azolla. This provided at least 100,000 tons of nitrogen fertilizer per year, worth more than $50 million annually.

3.3 **Factors affecting Azolla growth**

3.3.1 Habitat factors

Availability of water is the key factor to survival and growth of Azolla (Mousa 1994). Sadeghi et al. (2013) reported that for optimal growth, light intensity of 15-18 Kilo Lux (Klux), temperature from 18°- 28°C and relative humidity between 55-83% is required. Chapman et al. (1981) observed that Azolla grown under controlled environment increased 14 to 16 folds over 14 days at 12h day and night temperatures of 30°C/25°C, 38°C/30°C and 40°C/32°C. Similarly, the literature from recent studies showed that temperature above 30°C and below -4°C inhibited the growth of Azolla. Pabby et al. (2003) found that photosynthetic activity, growth and nitrogen fixation of Azolla and its symbiotic characteristics were all affected by light intensity. Further, they also noted that sporulation was regulated by the interacting effects of light intensity, photoperiod, temperature and other factors such as pH, nitrogen and phosphate supply. When light intensity was high and the amount of nutrients in water low, Azolla turned red. During hot summer or cold winter, it also turned red or brownish-red when grown under shaded conditions, whereas in nutrient-rich conditions, it became green (Sajeda and Khartum 1983). The literature from Sadeghi et al. (2013); Arora and Singh (2002), Carrapiço et al. (2000) and Katony et al. (1996) confirmed the need for both macro (e.g. phosphorus, nitrogen, potassium, calcium and magnesium) and micronutrients (e.g. molybdenum, cobalt, etc.) for the survival and proliferation of Azolla. Tuan and Thuyet (1979) pointed out that at pH 5, high light intensity increased Azolla growth; at pH 6 and 7, it inhibited growth and high light intensities (above 90 Klux) inhibited Nitrogen (N_2) fixation (Cary and Weerts 1992). However, lower light intensities or shading had a good effect on Azolla growth and multiplication. Sadeghi et al. (2013) reported that turbulence from wind and waves affected the growth and production of Azolla by breaking the fronds thereby negatively affecting the N_2 fixation capacity.

3.3.2 Nutrient and chemical factors

As long as Azolla had its upper lobes in free contact with air and had access to atmospheric nitrogen, availability of N_2 and to some extent, the dissolved oxygen were not the limiting factors of Azolla growth (Sadeghi et al. 2013). Free access to atmospheric air by the upper lobes fulfilled all of its N_2 requirements (Costa et al. 2009).

Unlike N_2, Phosphorus (P) in the form of phosphate, is the limiting nutrient which determined the survival of Azolla. Sood et al. (2005) found that in the outdoor controlled conditions, *Azolla pinnata* sustained its growth and metabolic activities in P deficient conditions only up to 8^{th} day of growth. Further prolongation of P starvation resulted in the appearance of P deficiency symptoms. This finding was in conformance with that of Sadeghi et al. (2013). Hossain et al. (2021) concluded from their study that supplementation of 10 ppm P to the water used for culturing *A. pinnata* was optimum under outdoor controlled conditions. Doubling time was the fastest when the culture medium was supplemented with 10 ppm of P. Phosphorus content of *A. pinnata* was proportional to the P supplementation in the culture medium. Phosphorus supplementation also improved the protein and lipid contents of *A. pinnata.*

It was reported that in addition to the N and P, other macronutrients such as potassium, calcium and magnesium were also needed for the growth of Azolla (Sadeghi et al. 2013; Serag et al. 2000; Costa et al. 1999; Biswas et al. 2005; Lumpkin 1987; Kitoh and Shiomi 1991). Further it was reported that, Azolla required micronutrients like Mo_6+, Mn_2+, Zn_2+, Cu_2+, Fe_2+ and Co_2+ for optimal growth. The growth of Azolla would be impacted by an increase in nitrate concentration in the water (Sadeghi et al. 2013).

3.4 Azolla as an invasive species

Azolla filiculoides is in the observation list of alien plants that have medium risk (European and Mediterranean Plant Protection Organization [EPPO] 2012). The invasive species compendium maintained by the Centre for Agriculture and Bioscience International (CABI) are listed in Table 2.

The compendium maintained by CABI showed that *Azolla pinnata* is commonly distributed in the Asian countries. The species is also considered invasive and a pest in most of the tropical countries. Particularly, in Kashmir, India and south Africa, *Azolla cristata* has been recorded as an invasive species that threatens to outcompete native aquatic plants and reduce the light and oxygen levels in water bodies (CABI 2014). Considering the similarities of agro-ecological such as topography, elevation and temperatures between Jammu & Kashmir and Bhutan, *Azolla cristata* species might be a concern for Bhutan. Further, CABI (2014) also indicated that *Azolla pinnata* had low risk of spreading to non-tropical and subtropical areas, and spread between water bodies within natural areas unless it was introduced deliberately or for agricultural purposes by humans. In the western countries, besides awareness for information on the invasiveness of *Azolla*, biological controls (Gassman et al. 2006) were studied and finding of the studies was applied to control the spread of these invasive plants' species in the western countries.

In Bhutan, *Azolla pinnata* was introduced for a research-trial (Chofil and Pulami 2020) to assess its adaptability and promote as bio-fertilizer and for animal feeding. However, the species could not be released by the variety releasing committee (VRC) of the Ministry of Agriculture and Forests (MoAF) for mass adoption due to inadequate research evidence to support that Azolla was not invasive under Bhutanese conditions. Due to lack of information pertaining to the invasive nature of Azolla under uncontrolled environment, it could not be promoted as potential source of animal feed.

3.5 Azolla cultivation in Bhutan

Azolla pinnata was recently imported in Bhutan from Sikkim, India by the Agriculture Research and Development Center (ARDC), Bajo as bio-fertilizer, with prior approval from the Department of Agriculture, MoAF. Further approval for import of Azolla in Bhutan was sought from Bhutan Agriculture and Food Regulatory Authority (BAFRA). The use of Azolla as a new technology was then adopted by Regional Livestock Development Centre (RLDC), Wangdue for promotion as supplementary animal feed in the west-central region of the country. As promotion of a fodder technology, Azolla was provided to 40 members of Dairy Farmers Groups (DFGs) in Dagana and Tsirang Dzongkhags during the month of April, 2020 (RLDC 2021).

The Azolla was cultivated in the farmer's field under controlled environment. The farmers were trained to build troughs (5 x 3.5 m) from tarpaulin sheets, placed on dry land, away from free-flowing water bodies, as recommended by Pillia et al. (2005). Locally available soil was spread on the tarpaulin trough. On top of the soil, small amount of manure mainly cow dung, was used as fertilizer. Tap water was filled in the trough where Azolla was broadcasted and left to grow. Farmers noted that the production of Azolla doubled in 10-15 days after setting it up in the trough. The laboratory analysis of harvested Azolla contained 4.53 ± 0.73% dry

Table 2: List of Azolla as invasive in nature and their distribution in Asia

Azolla *spps.*	Datasheet type	Last modified	Distribution in Asia
Azolla pinnata	Invasive species, pest, host plant	16 Nov. 2021	Bangladesh, Brunei, Cambodia, China, India, Indonesia, Japan, Laos, Malaysia, Myanmar, North Korea, Pakistan, Philippines, Sri Lanka, Taiwan, Thailand, Vietnam
Azolla mexicana	Invasive species	10 Dec. 2019	China, India, Iran, Israel, Japan, Taiwan
Azolla filiculoides	Invasive species	20 Nov. 2019	India (Jammu and Kashmir) and Japan
Azolla cristata	Invasive species	10 Dec. 2019	India (Kerala)

matter (DM) and 25.42% crude protein (RLDC 2020). The farmers also reported improvement in milk production upon feeding Azolla to their cattle. It is expected that unless the farmers intentionally release the Azolla into the natural streams, ponds and rivers, there is less possibility for Azolla to escape in the wild and become invasive. Also considering the rugged terrain and high inclination of topography in Bhutan, the flow of water is turbulent where it is practically impossible for Azolla to settle and form thick mats. As reported by Lumpkin (1987) Azolla breaks its fronds and does not gather as mat on turbulent water. Additionally, the concentration of orthophosphate (Espinar et al. 2014) has to be adequate in the water bodies to enable Azolla to proliferate as weed or as pest. Since the concentration of orthophosphate in water bodies in Bhutan is unknown and no studies have been carried out, it is difficult to suggest the possibility of Azolla growing in the open conditions. However, as the soil fertility in general in Bhutan is poor with regards to Phosphorus content, it is unlikely that Azolla will grow in the freshwater bodies. On the contrary, as evident from Table 3, considering the temperature range in the country, Azolla could be grown favorably.

Table 3: The mean maximum and minimum temperature in the country for the year 2020

Month	Mean (Max.)	Mean (Min.)	Remarks
January	15.5	3.7	The maximum
February	17.3	5.8	temperature was
March	20.6	9.3	recorded 33.6ºC
April	22.1	11.6	during the
May	23.1	14.5	month of August
June	24.8	17.8	at Punakha and
July	24.1	18.6	the minimum
August	26.6	18.8	temperature was
September	24.6	18.1	recorded -6.5ºC
October	25.4	15.0	during the
November	21.6	8.3	month of
December	17.8	6.1	January in Haa

Source: Department of Hydromet Services, 2020

The minimum and maximum temperature range for the country is found highly favorable for the growth of Azolla. The use of Azolla for feeding animal could replace certain portion of imported plant proteins sources required for manufacturing animal feeds. The farmers may be encouraged to use tarpaulin method and green house to cultivate Azolla as livestock feed to reap benefits (Raja et al. 2012) for feeding the livestock in cold and dry winter months.

4. CONCLUSIONS & RECOMMENDATION

Azolla is exploited for its benefits in nitrogen fixation, phosphorus removal from wastewater, used as bio-fertilizer and supplemental protein in animal feeding globally. Concurrently, Azolla is considered as an invasive species and pests in many countries. However, growing Azolla under controlled environment, and using it to feed livestock will reduce feed cost, and enhance productivity. The Azolla cultivation technology is simple and could be promoted in Bhutan, with a caution to farmers not to drain the residual water from Azolla cultivation trough into the open water bodies. Nevertheless, there is a need to get more insight through applied research to understand and document the management aspects and benefits of this aquatic fern under Bhutanese environment as the potential protein source for livestock feeding in Bhutan.

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