



An overview on the aquatic fern *Azolla* spp. as a sustainable source of nutrients and bioactive compounds with resourceful applications

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ABSTRACT

The present work was conducted to explore *Azolla*'s perspective as a multifaceted aquatic resource to maintain the preservation of the environment. *Azolla* is a small floating aquatic nitrogen-fixing fern with distinctive composition. In both developing and developed nations, *Azolla* is a pteridophyte of agronomic significance. In a comparatively short time frame, it generates full biomass. Hence, an overview is presented of *Azolla* spp. including classification, distribution, and important applications in various fields in many countries, particularly in Egypt. *Azolla* is beneficial as a source of several crucial nutrients and bioactive compounds. The study highlights *Azolla*'s effective use as a biofertilizer, animal feed, water purifier, and biological herbicide. *Azolla*'s impact on the concentrations of floodwater nutrients and heavy metals was also supervised. The presented data offer a scientific context that could be fruitful feedback for researchers who work in this field regarding *Azolla* in many countries, especially Egypt.

INTRODUCTION

At present, there is intensive attention for using natural materials as sustainable resources for bioactive compounds. An interesting aquatic fern that is extensively studied is "*Azolla* spp.". *Azolla* is a genus of aquatic ferns and small- leafed floating plants, native to the tropics, subtropics, and warm temperate regions of Africa, Asia, and the Americas (Mosh, 2018). It is a floating pteridophyte that is symbiotic with the cyanobacterium *Anabaena Azollae* (Yang *et al.*, 2020).

Azolla species were successfully used as a biofertilizer, animal feed, water purifier, biological herbicide and for concentration of nutrients and heavy metals from flood waters (Selvaraj *et al.*, 2014). *Azolla* was also shown to be a source of bioactive secondary metabolites such as phenols, tannins, sugar, anthraquinone glycosides and steroids, and also rich in protein, vitamin and minerals (Selvaraj *et al.*, 2013).

Concerning *Azolla* spp. the current study overviewed: classification, distribution and important applications in different fields in many countries and in Egypt in particular.

Origin, species and distribution of *Azolla*

Azolla is a water fern that floats on the surface of fresh water ponds, rivers, and flooded fields in the tropics and subtropics of both the New and Old Worlds. Its size is typically 1-2.5 cm in diameter, but some species can achieve the size of 15 cm or more (Qiu & Yu, 2003).

In 1783, Lamarck first introduced the word *Azolla*. The *Azolla* genus belongs to the division Pteridophyta, the class Polypodiopsida, and the order Salviniiales. It is also a member of the family Salviniaceae and has two subgenera and six living species (Roy et al., 2016). Two subgenera are grouping the species of the genus *Azolla*: subgenus *Azolla* (*Euazolla*) with 4 species, *A.caroliniana*, *A.mexicana*, *A. microphylla* and *A.filiculoides* Lam., all are native of America, while subgenus *Rhizosperma* with 2 species *A.nilotica* and *A. pinnata* are native of Africa (Al-Saadi et al., 2016).

In tropical, subtropical, and warm-temperate regions of the world, *Azolla* typically grows in freshwater. *Azolla caroliniana* is found in eastern North America and the Caribbean, *A. filiculoides* in the Americas from southern South America through western North America to Alaska. Moreover, *A. microphylla* is detected in tropical and subtropical America, *A. mexicana* in the Americas from northern South America through western North America, *A. nilotica* in East Africa from Sudan to Mozambique, and *A. pinnata* in most of Asia and the coast of tropical Africa (Moshia, 2018).

Environmental growth conditions

Azolla frond varies in length, with ranges from 1cm to 2.5cm. It has a main rhizome that is divided into secondary rhizomes, all of which have alternately arranged small leaves. Optimum temperature for *Azolla* growth has been shown to range between 18 and 28°C. The optimal growth and biomass production of *Azolla* in the Anzali wetland might be related to water depth. Notedly, it is able to grow on a wet mud surface or wetted peat litter, but it prefers to grow in free-floating conditions on calm water surfaces. It can grow very quickly, and thus cause severe problems mainly in tropical and subtropical regions (Sadeghi et al., 2013).

With a distinctive macromolecular composition, aquatic plants may be used as food or feed for special applications. However, depending on the form and analytical method, their composition greatly varies. *Azolla filiculoides* is a floating aquatic fern, belonging to the family Salviniaceae and has a high growth rate and annual productivity of 39 t dry weight per hectare as recorded in an outdoor experiment. The first fern for which a nuclear genome has been completely sequenced is *Azolla* (Dohaie et al., 2020).

Phytochemicals and bioactivity of *Azolla* spp.

Azolla includes several valuable phytochemicals such as flavonoids, hormones, alkaloids, phenols, triterpenoid derivatives, amino acid and fatty acid types (secondary metabolites). These bioactive components contribute to a broad variety of useful and therapeutic properties, such as antioxidant, anticarcinogenic, anti-inflammatory,

antidiabetic, hepato- and gastro-protective, antiviral, neuro-protective, cardio-protective and anti-hypertensive (Maswada *et al.*, 2020).

Azolla extracts contain phyto-constituents that are responsible for antioxidant activity, such as tannins, phenolic content and flavonoids (Mithraja *et al.*, 2011).

Azolla is one of the high-biomass and protein-producing aquatic plants that can be used as a direct fish feed or food component as an alternative protein source. Due to higher crude protein content (13%: 30%) and critical amino acid composition (rich in lysine) compared to other green forage crops and aquatic macrophytes, *Azolla* has gained its significance in aquaculture (Mosha, 2018).

The leaves of *Azolla microphylla* were found to contain alkaloids, tannins, saponins, hormones, terpenoids, flavonoids, and phenols. In ethanol, methanol and water, almost all the phytochemical constituents were present. Both leaf extracts have been tested for their antimicrobial activity against species of bacteria (Sathammpriya *et al.*, 2018).

Phytochemical compounds extracted from *A. pinnata* crude extract could be an innovative application for the conception of a bio-insecticidal product. *A. pinnata* crude extracts could be more effective than a single active compound due to the synergism of its active ingredients. The extracts may be effective in managing the resistant population of mosquitoes. Those compounds are used for insecticidal, pestilential, anti-parasitic, nematicide, antimicrobial and antioxidant activities (Ravi *et al.*, 2018).

There are several bioactive compounds present in *Azolla pinnata*, such as essential amino acids, vitamins, beta-carotene, minerals, saponin, and flavonoids. It is often known to be a good source of high-quality protein. Extracts of *Azolla* were shown to ameliorate lead acetate-induced hepatotoxicity by reducing oxidative stress (Shaaban *et al.*, 2020).

Nutrition benefits of *Azolla*

Numerous studies have shown that *Azolla microphylla* resulted in increased milk production, increased weight of dairy cattle, pigs, ducks and broiler chickens when fed on it (Selvaraj *et al.*, 2014).

Azolla can be used either directly or indirectly in a fish pond due to a larger percentage of nutrient composition on a dry weight basis and other elements such as nutrients, chlorophyll, carotenoids, amino acids, and vitamins. When introduced into the diet, most species of Tilapia (*Oreochromis niloticus*, *Tilapia mossambica*, *Tilapia zillii*) and the Cyprinidae family (*Labeo rohita*, *Catla catla*, *Labeo calbasu*, *Labeo fimbriatus*, *Ctenopharyngodon idella*, *Barbonymus gonionotus*) have been reported to use *Azolla* (Mosha, 2018).

The *Azolla pinnata* aquatic fern was reported to have a substantially high nutritional content, with a large proportion of calcium, vitamins and minerals. The effect of fresh *A. pinnata*, as a substitute for commercial fish feed (CFF) for *Barbonymus*

gonionotus, was explored. The results showed that 25 percent of CFF could be replaced with *A. Pinata*, without significantly decreasing their development and product quality (Das *et al.*, 2018).

Azolla pinnata is as a source of protein of high quality with almost all the essential amino acids, vitamins, beta-carotene, minerals and large amounts of bio-polymers. A farm trial was carried out on sheep, goats and chicks that were fed on *Azolla pinnata*. The study revealed a substantial increase in milk production (10-15%) and meat by weight (8-10%) in dairy animals, goats and chicks. Following the findings of this study, *Azolla* was recommended in rain-fed areas as a possible unorthodox protein source for livestock during the lean time of the year (Kumar and Chander, 2017).

An experiment was conducted to observe the effect of substitution of concentrate mixture with *Azolla pinnata* on growth performance. For a 90-day feeding trial, twelve heifers were randomly allocated into two treatment classes. The concentrate mixture was replaced by *Azolla pinnata* in the treatment group at a 5 percent stage (DM basis). Furthermore, improved weight gain, average daily gain (ADG), and feed conversion performance (FCE) replacing concentrate with *Azolla pinnata*, were recorded. Blood creatinine and BUN concentrations were 53 percent, and 17 percent lower in the *Azollapina* fed heifer than in the control group, respectively. Higher concentrations of serum ALT and AST were recorded in *Azolla Pinnata*-fed heifers (Roy *et al.*, 2016).

The findings of Rieta and Cabaral (2018) showed that feeding *Azolla* increased the feed conversion ratio for organically grown broilers in a great extent. Findings also showed that all panellists are willing to pay higher prices for confirmed lower cholesterol levels and better meat quality of the new organic produced broiler chicken. 70% of the panellists suggested that social media is the best way for the consumer public to disseminate knowledge to be aware of the natural development and nutritional benefits of growing *Azolla*-fed broilers.

It has been documented that *Azolla* is a good source of protein, critical mineral elements and vitamins. For tropical climates and livestock feeding, it is ideally adapted. The high amounts of carbohydrates, protein, crude fat, complete digestible nutrients and lower content of crude fiber are present in *Azolla nolotica*. Remarkedly, due its high levels of protein, carbohydrates and crude fat, concentrated *Azolla* protein (ACP) may be used as a human feed supplement. It has a gross energy content of 434.67 kcal/100 g and concentrations of 55.48 and 1.51 percent, respectively of calcium and phosphorus (Mohamed *et al.*, 2018).

As a food grade plant, *A. caroliniana* could be taken. There are impressive antibacterial properties of the second most common chemical, 'phthalic acid, ethyl pentyl ester.' There was no cellular or nuclear toxicity of the methanolic extract to cultured human lymphocytes. If it was aesthetically cultivated, *Azolla* could be a healthy food-grade plant (Nayak and Padhy, 2017).

Agricultural applications

Azolla has been known for its applications in the field of agriculture due to its properties that enables it to boost crops production. The fern was used as a fertilizer for a long period of time. A symbiotic association with cyanobacteria is the fixation of nitrogen to be ingested by the fern. The symbiosis is exceptional among any plant-cyanobacteria relationship as it is continuous. Its development and symbiotic association can be modulated by phytohormones (**Abd Elrasoul et al., 2020**). Several studies, showing the applications of *Azolla* in agriculture, are summarized herein.

The physiological role of *Azolla* filiculoides Lam. Extract in improving growth, physiology, yield and N uptake ability of maize plant and its growth and yield, especially under irrigation deficits. The findings showed that *Azolla* extract is a powerful biofertilizer, and its application in conjunction with deficit irrigation prevented any substantial decreases in the yields of N-deficient maize plant grain and stover. In addition, *Azolla* is economically sustainable and cost-effective organic fertilizer that replaces more than 30 percent of urea fertilizers without affecting grain yield (**Maswada et al., 2020**).

The production of cowpea under greenhouse conditions was enhanced by application of *Azolla* Pinnata and *Azolla* caroliniana as biofertilizers (**Ismail, 2017**). Similarly, *Azolla* biofertilizers enhanced the biochemical parameters of commercial tomato (Alisa) when the seeds were soaked in the equivalent concentration 24 hours prior to sowing (Hanafy and El-Emary, 2018).

Azolla treatments substantially improved plant height, number of branches/plant, shoot fresh and dry weights of chamomile plant. Also, *Azolla* extract as foliar (spray *Azolla*) had a beneficial effect on chemical constituents and improvement of the yield production by increasing the active substances of chamomile plants in sandy soil (**Rabie et al., 2020**).

The increase in grain yield was 57.37 and 51.71 percent, and in biological yield was 37.66 and 21.57 percent for foliar spraying with *Azolla* extract at 60 percent relative to control application for wheat in the first and second seasons, respectively. As NPK-fertilizer, levels rose from 60 to 80 and 100 %. The spraying of Egyptian bread wheat with *Azolla* extract was recorded, in addition to mineral fertilization, to maintain high performance, simultaneously minimize production costs and reducing production costs (**Altai et al., 2019**).

***Azolla* in Egypt**

In Egypt, *Azolla* species were introduced and naturalized almost four decades ago. The earliest known records of *Azolla* species were those reported from the rice fields around Qantara, Ismailia and Tanta, and were identified as *A. caroliniana* and *A. filiculoides*. These water ferns had spread vigorously since 1990 blocking the network of small irrigation and drainage canals in the Nile Delta. Nowadays, all six *Azolla* species are

naturalized in the Nile Delta; the most dominant of them is *A. filiculoides* Lam. (Shaltout *et al.*, 2013).

Recently, several researchers in Egypt dedicated their work on the way to benefit from using *Azolla* in different applications. Ismail (2017) evaluated the use of *Azolla caroliniana* and *Azolla pinnata* as green manures for cowpea plants. The author reported that application of *Azolla* caused a remarkable increase in the cowpea growth noting that *A. pinnata* was more effective than *A. caroliniana*.

Mineral nitrogen and/or *Azolla* were added as fertilizers to chamomile plants grown in sandy soil, and concluded that dry *Azolla* treatments greatly increased plant height, number of branches/plant, fresh and dry shoot weights, and number of inflorescences/plant (Rabie *et al.*, 2020).

In their study, Maswada *et al.* (2020) reported that the application of *Azolla* extract was proven to be an eco-friendly and cost-effective organic fertilizer that reduced more than 30% of urea fertilizer without affecting grain yield of maize plants.

Mohamed *et al.* (2018) stated that because of its high levels of carbohydrates, protein, crude fat, total digestible nutrients and lower crude fiber content, concentrated protein of *Azolla* may be used as a food substitute for humans. *Azolla* concentrated protein contained 40.83 crude protein (DM percent), crude fiber (4.63), ether extract (5.06), ash (17) and carbohydrate (DM percent) (30.5) Moreover, it has a gross energy content of 434.67 kcal/100 g, calcium and phosphorus concentrations of 55.48 and 1.51 percent, respectively. Zaki *et al.* (2020) found that *Azolla pinnata* has a good potential for use in prawn diets at reasonable levels compared to other conventional diets.

CONCLUSION

In conclusion, *Azolla* with its distinctive composition, is a small floating aquatic nitrogen-fixing fern. It is a pteridophyte of agronomical importance in both developing and developed nations. *Azolla* has many advantages and is known as a source of many essential bioactive compounds and nutrients. More work for sustainable research could be conducted for better exploitation of this significant natural resource.

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