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Review Article

Azolla Plant Production and Their Potential Applications

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Globally, the human population is growing at an alarming rate, reducing land coverage over time. In the modern world, lifestyle changes, the nature of work, and food habits increase the incidence of serious diseases in animals, and human activity influences the environment, such as the use of chemical fertilizers for agriculture, large terrestrial ecosystems all over the biosphere, such as deforestation of plants, which could not sink from atmospheric CO_2, and the production of fuels for energy, which would increase the need for fossil fuels but would also deliver low energy fuels at a high energy cost. To overcome the above problems, Azolla plants perform well since they can be grown at low labor costs, on small plots of land, and for a variety of purposes including animal feed/livestock, poultry and fish production, environmental remediation, biofertilizer and biocontrol for mosquito repellents, carbon sequestration of CO_2, and bioenergy effectiveness all year. As a result of their low impact on the environment and human health, Azolla plants are becoming increasingly important. The purpose of this review is to provide evidence of Azolla plant production and its potential role in various applications for a greener, more sustainable approach. This review was progressive in that it assessed and produced peer-reviewed papers related to Azolla plant production and its potential role in different applications for a sustainable greener approach. Based on the findings of reputable educational journals, articles were divided into three categories: methods used to produce the nutritional composition of Azolla, environmental factors that affect the efficiency of Azolla plants, and strain improvement of Azolla for enhanced multipurposes and techniques that are currently being used to meet Azolla plants production and its prospective applications for different sustainable greener approaches. The present findings indicate that Azolla is a rich source of protein, which has a clear benefit in offsetting a portion of the nutritional needs of animal/livestock, poultry, and fish production with what is effectively a low-cost dietary supplement, biocontrol of mosquito repellent, environmental bioremediation, biofertilizer, carbon sequester of CO_2, and bioenergy for the potential need of Azolla plant applications. Azolla plants have long been recognized for their benefits in greener and more sustainable lifestyles, as well as quality enhancement and bio-based economy over traditional approaches. Because it relied on natural resources and utility green production, this review's recovery was chosen as an appropriate and environmentally sound solution for a long and healthy lifestyle.

1. Introduction

Population growth and urbanization could result in more pollution. Natural resources are being used up more and more as a result of an expanding human population and increased per capita consumption. It has frequently been used to combine biotechnological strategies to improve environmental sustainability in plant areas. With a concern for environmental sustainability, the aquatic fern Azolla is a well-established biotechnology product with the potential for global use [1, 2]. The name Azolla is derived from the Greek words azo (to dry) and allyo (to kill), and it refers to the plant's inability to thrive in dry conditions. It is a free-floating aquatic fern in the *Salviniaceae* family, genus Azolla [3–6]. The distribution of the water fern (Azolla) varieties includes *Azolla pinnata*, *Azolla nilotica*, and *Azolla*

mexicana. The most important variety is *Azolla pinnata*, which can be grown easily and with a low initial investment. It is commonly found and grows naturally in the stagnant water of canals, ponds, drains, rivers, and marshy lands in the tropics and subtropics [7]. One of the promising alternatives is Azolla, a free-floating aquatic fern, which under ideal conditions grows exponentially and doubles its biomass every three days. It is synonymously called a green gold mine due to its high nutritive value and super plant due to its fast growth [8].

Azolla is a genus of seven to nine species of small floating aquatic ferns that are found worldwide, from tropical to temperate zones. All species belong to two subgenera: the *Euazolla* subgenus, which includes *Azolla filiculoides*, *Azolla mexicana*, *Azolla caroliniana*, *Azolla microphylla*, *Azolla rubra*, and the *Rhizosperma subgenus*, which includes *Azolla pinnata*, *A. nilotica*, and *A. imbricate* [9].

Azolla caroliniana, *Azolla circinata*, *Azolla japonica*, *Azolla mexicana*, *Azolla microphylla*, *Azolla nilotica*, *Azolla pinnata*, and *Azolla rubra* are the eight species found worldwide. However, *A. pinnata* is common in India. Its protein content is 4 to 5 times that of lucerne and hybrid Napier. Furthermore, its biomass production is nearly 4 to 10 times that of hybrid Napier and lucerne, respectively [10–13]. There are 21 species of Azolla in the world, with seven of them being popular: *Azolla anabaena*, *Azolla caroliniana*, *Azolla cristata*, *Azolla feliculoides*, *Azolla mexicana*, *Azolla microphylla*, and *Azolla pinnata*. Fresh Azolla is available in four varieties: duckweed fern, fairy moss, mosquito fern, and water fern. Azolla cultivation is common in China, Vietnam, and the Philippines [13].

Azolla gradually spread to most parts of the world, including Africa, Asia, Europe, North America, Oceania, and South America, either accidentally or as an ornamental plant [12]. It was first discovered in Europe, North and sub-Saharan Africa, China, Japan, New Zealand, Australia, the Caribbean, and Hawaii [14].

It contains almost all essential good protein sources rich in crude protein, essential amino acids, vitamins, growth promoter intermediaries, and minerals such as calcium, phosphorus, potassium, ferrous, copper, and magnesium. On a dry weight basis, it contains 25–35% protein, 10–15% minerals, and 7–10% of amino acids, bioactive substances, and biopolymers, and 15% of total ash. Animals easily digest it because of its low lignin content; the animals can quickly grow accustomed to it; it is economical to cultivate; and it contains an ether extract, which is essential for ruminant diet [4, 15–17].

The manufacture of Azolla helps farmers to reduce the expense of supplementing feed for livestock. In return, *Anabaena azollae* fixes atmospheric nitrogen and supplies Azolla with ammonia. So, lowland rice fields are a cost-effective, ecofriendly biofertilizer. Additionally, for animals, poultry, and fish, feeding supplements are beneficial. The twin potential as a biofertilizer and animal feed makes the water fern Azolla an effective input to both the vital components of integrated farming, agriculture, and animal husbandry [1, 18, 19].

The biomass produced by Azolla spp. phytoremediation can also be used in novel ways to recover energy and fertilizer. Large amounts of biomass can be used as feed for anaerobic digestion, which generates biogas and is digested for use as fertilizers [20, 21]. Azolla's nutritional value includes high protein content. The essential amino acid content, particularly lysine, was 0.42% higher than that of corn, bran, and broken rice concentrate [22, 23]. Azolla is used in agriculture for a wide range of purposes, including biofertilizer, human food, cow and poultry feed, weed and mosquito control, and human food. It has been demonstrated that Azolla inoculation enhances rice yield and growth under a variety of agroecological conditions [24]. They are easy to grow in a short period, are produced in a small area, have high nutritional composition, play a great role in agriculture, produce bioenergy, are of low cost, are environmentally friendly, and so on. Azolla plants as a sustainable green approach and their potential role in different applications are intriguing topics for review. The goal of this review was to attempt, to begin with, an overview of the Azolla, its nutritional composition, environmental factors that affect the efficiency of Azolla, strain improvement of Azolla for enhanced multiple purposes, and the green approach of Azolla for different applications.

2. Nutritional Composition of Azolla Production

Azolla's nutritional value is well documented, and it is promoted as a good source of protein. It is also found to contain essential minerals such as iron, calcium, magnesium, potassium, and significant amounts of vitamins A and B-12 [4, 25, 26]. Probiotics and biopolymers are found in Azolla plants (Table 1). Sandy soil has a low level of nitrogen content and low water retention capacity, so utilizing organic stuff, particularly from local sources, is one way to get around this. Sandalwood soils' capacity to retain water and serve as a source of nutrients can be enhanced by the addition of organic matter [42].

2.1. Environmental Factors of Azolla Production. Azolla production mainly depends on the chemical and biological properties of water and irrespective of water quality; the consistent availability of water year-around is very much important to produce the required quantity of Azolla for regular use [40]. Azolla can grow quickly, doubling in 2–5 days, and form very dense mats in favorable habitats, causing many difficulties for boat transport, water animals, and native plant species, as well as becoming a source of eutrophication. Water availability is critical to its growth. Optimal light intensity (15–18 lux), temperature (18°–28°C), and relative humidity (55–83%), all help to promote growth. Azolla can be fragmented and killed by wind and turbulent water [43].

In general, the ideal temperature ranges from 20 to 30°C. Temperatures above 37°C harm Azolla multiplication. The ideal relative humidity ranges from 85 to 90%. The ideal pH range is from 5.5 to 7. This fern is harmed by pH levels that are too acidic or alkaline [11].

Table 1: Nutritional composition and applications of different Azolla species.

Species	Composition	Amount of Azolla in %	Applications	Reference
Anabaena azollae	N-uptake and DM	91–215 and 74–125	Improves soil and water retention	[24]
Azolla pinnata	DM, CP, OM, EE, and total ash	90.00 ± 0.77, 22.05 ± 0.72, 81.05 ± 0.44, 3.25 ± 0.76, and 18.94 ± 0.31	Azolla can be used for feeding livestock	[27]
Azolla pinnata	CP, minerals, and amino acids	25–35, 5–15, and 7–10	It is easily digestible due to low lignin content	[28]
Azolla pinnata	DM, OM, CP, EE, CF, NFE, and ash	90.70, 84.41, 22.93, 2.82, 11.63, 47.03, and 15.59	For effect on growth performance	[29]
Azolla filiculoides	CP, EE, Cfib, ash, and NFE	23.2, 3.30, 13.6, 17.9, and 41.9	Milk production of indigenous cattle-fed supplements	[30]
Azolla pinnata	DM, CP, and lipid	4.65, 28.52, and 4.15	Used for plant growth and metabolism	[31]
Anabaena azollae	DM, CP, CF, ash, Cfib, carbohydrate, and energy	6.38, 27.1, 6.37, 14.29, 34.29, 45.86, and 349.17 kcal/100 g	Used as producing animal feed and bioenergy	[32]
Azolla pinnata	CP, EE, Cfib. NFE, and ash	26.4, 3.42, 15.96, 41.06, and 14.86	As a feed ingredient in commercial broiler production	[33]
Azolla pinnata	Protein, lipids, and fibers	30.6 and 4.5	For beneficial dietary supplement of grass carp	[34]
Azolla filiculoides	Water content, CP, CL, ash, Cfib, and NFE	89.69, 28.28, 1.52, 12.17, 10.84, and 47.17	Positive effects on fish immunological indicators and growth performance	[35]
Azolla pinnata	CP and amino acids	25–30% and 7–10%	Increases the milk production	[36]
Azolla pinnata	DM, OM, CP, EE, NDF, and ADF	9.90, 79.7, 26.50, 3.90, 39.4, and 44.28	As a potential protein supplement in the feed of livestock	[16]
Azolla pinnata	DM, OM, CP, CF, EE, ash, NFE, NDF, and ADF	4.7, 82.66, 22.48, 14.7, 4.5, 17.34, 40.98, 54.85, and 36.57	Azolla has effect on growth performance of broiler rabbits	[37]
Azolla caroliniana	DM, CP, Cfib, EE, OM, NDF, and ADF	89.00 ± 0.52, 23.59 ± 0.28, 14.03 ± 0.57, 3.23 ± 0.15, 78.39 ± 0.20, 42.14 ± 0.61, and 36.03 ± 0.29	A good source of protein for livestock feeding	[38]
Azolla pinnata	CP, EE, CF, ash, calcium, and phosphorus	26.6, 3.3, 13.86, 19.6, 2.28, and 0.22	Incorporated at 5 percent level in poultry broiler feed	[3]
Azolla circinata, *Azolla pinnata*, *Azolla rubra*, *and Azolla mexicana*	Protein	14, 13.2 ± 0.7, 20, 12.0 ± 0.5, 26, 11.5 ± 0.8, and 32, 15.4 ± 0.3	The best substitute of protein from expensive sources and low-cost Azolla species are the livestock feed for Ompok pabda	[39]
Azolla pinnata	DM, CP, EE, ADF, NDF, lignin ash, and nitrogen	5.70, 25.86, 4.86, 21.51, 45.60, 11.33, 19.37, and 4.48	Used as a supplementary feed to animals	[40]
Azolla pinnata	CP	25	For improving Azolla production and crude protein content	[41]

DM, dry matter; CP, crude protein; CF, crude fat; CFib, crude fiber; OM, organic matter; EE, ether extract; ADF, acid detergent fiber; NDF, neutral detergent fiber; NFE, nitrogen-free extract.

Low light intensity, 90% humidity, no nitrogen, and no pH control were the growth conditions that resulted in the slowest growth rate of 0.064 days. High biomass yields could be obtained by utilizing the optimal growth conditions explored. This biomass could then be used in phytoremediation processes, among other things [44].

Azolla is a macrophyte that grows well in Malaysia's climate. Its ideal growing conditions were discovered to be a water depth of 20 cm, a nutrient concentration of 812.5 ppm, a pH level of 7.0, an average daily temperature in Malaysia ranging between 21°C and 32°C, and 100% sunlight exposure [32]. The maximum growth rate of *Azolla filiculoides* is achieved in 2.1 days at a temperature of 22°C, a light intensity of 20 lx, a humidity of 75%, and a pH of 6.4. Furthermore, a model was proposed that can predict the Azolla growth rate by taking into account the effects of key variables [45].

Temperature (°C) 28.94–33.46°C, the influence of light intensity (Lux) 1413.29–1561.57 Lux, and the influence of relative humidity (%) on biomass 47.28–64.85 (%) were the optimum environmental conditions for Azolla (*Azolla pinnata*) production. This resulted in a weekly vegetative mass yield of 3.73 kg and a dry matter yield of 172 g per pit. When compared to pig manure, cattle, sheep, and poultry manures are comparable and superior [46].

The most important factor influencing the Azolla growth rate is pH. The fastest rate of growth occurs at a pH of 7. Azolla grows significantly worse at pH levels higher and/or lower than 7; however, a slightly acidic medium is better for Azolla development than a neutral one. Because N_2 fixation was inhibited, adjusting the other parameters to optimal conditions had a positive effect on Azolla growth and multiplication in acidic pH [45].

2.2. Strain Improvement of Azolla for Enhanced Multiple Purposes. If Azolla production is to reach a scientifically feasible level, strain improvement is inevitable. It is frequently necessary to engineer the Azolla to improve its properties to meet the demands of demanding engineering applications. Azolla is a wonder fern with numerous benefits. Despite being considered an invasive species, farmers in several countries around the world use it as a high-quality livestock feed supplement and biofertilizer. It has a high protein and mineral content, which is essential for livestock feeding, lowering feed costs, and increasing productivity [12].

Because the reproductive structure of Azolla is rarely available, molecular tools are required for identification. A vegetative structure can be used, but it is unreliable [47]. As a result, removing Cyanobacteria from Azolla is the most important step in molecular marker techniques for DNA extraction. *Azolla filiculoides* cyanobiont (AzCy) was extracted, and its gene expression pattern was compared to that of the wild type (AzCy+) using several molecular techniques [48]. According to Das et al. [49], the optimal replacement of Azolla in the diet of Jalauni lambs was 25% mustard cake protein with no differences in nutrient digestibility and utilization. Replacing 5% of the concentrate mixture in the heifer diet with dried Azolla increased average daily gain (ADG) by 15.7% and feed conversion efficiency (FCE) by 20% [50].

The average daily gain of white New Zealand rabbits fed diets containing 0, 10, and 20% Azolla as a protein replacement was higher for 10% than for 0 and 20%, and the same trend was observed with feed conversion ratio (FCR), which was higher for 10% than for 0 and 20% [51]. Sihag et al. [29] studied whether a concentrated mixture of goat diets could be replaced with sundried Azolla up to 15% without affecting economic feeding. Furthermore, when 10% protein of the concentrate mixture was replaced by Azolla meal on a DM basis, there were no significant differences in DM intake, ADG, or feed efficiency of the Mecheri lamb groups [52].

Bhatt et al. [53] found that replacing 15% of the protein content of concentrate with Azolla increased female calves' average daily gain. Sharma et al. [54] concluded that green Azolla supplementation of 150, 250, and 350 gm to concentrate diet improved the growth performance of male children, with ADG increasing by 21.13, 29.34, and 22.59%, and FCR increasing by 13.3, 17.38, and 12.82%, respectively, when compared to 0 Azolla supplement, and the best green Azolla supplement was with 250 gm. Supplementing dried Azolla with up to 20% of DM intake improved lamb carcass characteristics [36].

3. Green Approach of Azolla Plants for Different Applications

The aquatic fern, Azolla, has long been recognized for its importance in ensuring ecosystem sustainability through soil fertilization, bioremediation, and its role in greenhouse gas mitigation [55]. Azolla is used in agriculture as a biofertilizer, a source of food for human consumption, cattle and poultry feed, and a weed and mosquito control agent. It also has several applications as a biocontrol agent and improves water quality [56].

3.1. Azolla in Animal Feeds/Livestock Feeds. The use of *Azolla pinnata* as an alternative feed source for crossbred dairy cows revealed that replacing commercial feed with 15–25% *Azolla pinnata* can increase fat percentage and milk production by 7–13%. The low-cost supplementation of Azolla can also improve the mean economic returns from a single cow in the village by increasing milk yield per month [57–59], as shown in (Figure 1). The growth performance and nutrient digestibility of *Azolla pinnata* feeding in Sahiwal Calves (Bos indicus) by replacing concentrate protein content at 15% and 30% could significantly improve the growth of Sahiwal female calves during the winter season. The crude protein content of *Azolla pinnata* concentrate at 15% and 30% could significantly improve the growth performance of Sahiwal female calves [53].

The production of Azolla helps farmers in lowering the cost of supplementing livestock feed. Azolla's technology will be widely adopted by dairy farmers, particularly those with limited land for fodder production. It contains 25–30% protein, which makes it digestible for many animals, and has

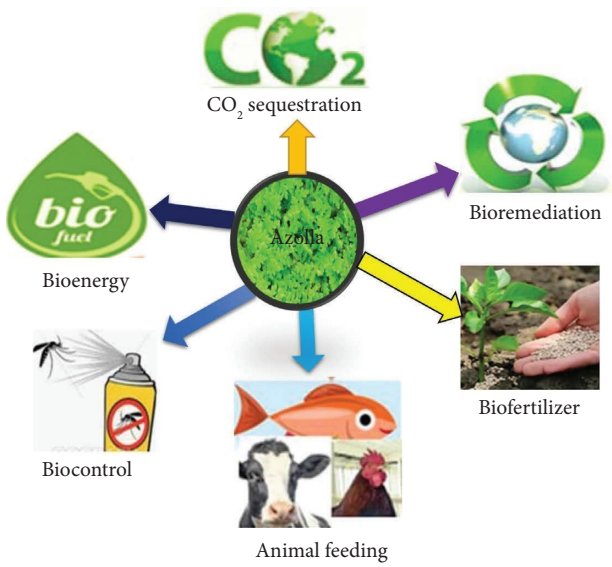

Figure 1: The green approach of Azolla for different applications [24].

low lignin content. It is high in essential amino acids, vitamins (vitamin A, B12, and beta-carotene), growth promoter intermediates, and minerals such as calcium, phosphorus, potassium, iron, copper, and magnesium [18].

Due to climate change and other factors, there is an acute shortage of feed and fodder for dairy animals. Green fodder shortages in Himachal Pradesh's rain-fed districts of Bilaspur, Hamirpur, and Una have been estimated to be 30–35% during the lean season. Due to its high palatability and enhanced yield under rain-fed conditions during the lean period, *Azolla pinnata* has been observed as an alternative to green fodder and as a supplementary protein diet in the current studies [30, 60]. The Azolla cultivation technology is considered as an intervention to meet their dairy farmers' needs. About 40% of the project respondents were included in the study, and it was concluded that 65% of them adopted the cultivation of Azolla and 35% did not adopt the new technology for various reasons [11].

Azolla units were established for cultivation of Azolla, and then, it was fed to select cattle, goat, and chicks as a protein supplement for two months regularly. There was an appreciable increase in milk production (10–15%), meat by weight (8–10%) and egg-laying capacity (10–15%), in milch animals, goats, and chicks, respectively [60]. Egg production, egg weight, feed conversation ratio, performance efficiency index, and shape index were all improved with the addition of fresh Azolla (*Azolla pinnata*) at 200 g/duck/day in place of 20% of the conventional duck layer diet, along with enrichment of yolk color [28].

According to Kamaruddin et al. [4], Azolla is more suitable to be used as a source of fodder mixture to ruminants because it has a higher nutritive value in terms of high crude protein, easier digestion, low lignin content, the animals can quickly grow accustomed to it, it is economical to cultivate, and it contains ether extract, which is essential for ruminant diet. Azolla can be easily grown in a controlled environment and used as a livestock feed supplement. It has a high protein and mineral content, which is essential for livestock feeding, lowering feed costs, and increasing productivity [12].

The effect of fresh Azolla supplementation in lactating buffaloes was studied in an early lactation period of 90 days. The average milk yield (kg/d) was found significantly higher ($P < 0.05$) in T1 (7.4Â ± 0.08) than T0 (6.5Â ± 0.13). On an average, milk yield increased by 0.9 L/day over control group animals [61]. After 60 days of feeding 1.5 kg of Azolla per day with conventional feed cottonseed cake, the milk yield increased to 9.30 L/day from 8.0 L/day. Milk yield increased by 1.30 L/day on average. A 16.25% increase in milk yield is a significant improvement [8, 62]. With feed costs rising across the country, it would be prudent for pig farmers to investigate the use of alternative cheaper feed resources. The literature on feeding Azolla to livestock contains promising results [13].

3.2. Azolla in Poultry. Azolla is high in protein and essential amino acids, and it also contains vitamins such as A, B-12, and beta-carotene. It also contains minerals such as calcium, phosphorus, potassium, magnesium, copper, and zinc. Azolla has a protein content of 25–35% by dry weight and is easily digested by poultry [59, 63]. The addition of dried Azolla to the ration of Vencobb broilers can be used safely up to 5% with no adverse effects; however, a 2.5% Azolla addition level is effective in improving both growth and biochemical parameters [64].

As a result, high feed costs, high bird mortality rates during the summer, off-odor, and house fly menace can be reduced by growing Azolla in and around poultry farms and feeding it to the poultry birds. The use of 30% Azolla (w/w) along with 70% commercial feed in poultry broiler diets increases body weight gain, improves FCR, reduces mortality due to heat stress during the summer, and lowers broiler production costs [33]. Azolla feeding reduced body weight and increased feed conversion ratio in broilers; however, adding DFM to an Azolla-based diet can enhance the positive effect of Azolla feeding at a 2.5% level, which shows a positive effect on breast muscle yield and gizzard weight of broilers, which can be enhanced by adding DFM to an Azolla-based diet [65], as shown in Figure 1.

Additionally, substituting low quantities of aquatic plants in poultry diets has improved performance, particularly when they provide a portion of the overall protein or serve as a source of egg coloration [66]. Globally, consumers have a wide range of preferences for yolk color, while darker shades typically command higher prices. Darker yolks instead of artificial coloring additives are preferred by baking operations and the food processing industry. The maximum computed Haugh units were found at a partial substitution level of 10%, but they gradually decreased as the partial substitution level climbed from 10% to 15% [67].

Chickens are one of the most widely consumed poultry species, making poultry one of the most prevalent livestock species in animal husbandry [68]. According to Lakshmanan

et al. [69], adding Azolla to layers of diets increased egg production, improved nutrient value, and reduced the need for concentrated feed. The high calcium content of Azolla was cited as having a beneficial impact on eggshell strength. Numerous researchers all around the world support the usage of alternative high-quality protein source feed. But there is not any scientific research on using locally grown Azolla in laying chicken under Ethiopian conditions [70].

Seasonal effects of azolla powder supplementation on Giriraja poultry bird mortality and deep litter contribute significantly to meat and it is a substantial source of protein for poultry. During the 2020-21 fiscal year, the poultry exported 255686.92 MT of poultry goods to the global market for a cost of Rs. 651.21435.53. These breeds have quick growth rates, increase egg production, require little in the way of input (feed, management, health care, housing, etc.), and can withstand a variety of climatic and environmental changes [71]. The current study's findings showed that Azolla meal up to a level of 7.5% in the diet increased body weight gain, feed conversion ratio, and nutrient retention without having any negative effects on carcass quality or slaughter features or increasing the cost of production [72].

Dry *Azolla pinnata* meal was substituted for the basic diet on a dry matter basis, and this had no negative effects on the growth or carcass traits of commercial broilers. Additionally, in commercial broilers, *Azolla pinnata* exhibits interesting immunomodulatory potential. *Azolla pinnata* meal may therefore be substituted for 5.5% of the baseline diet on a dry matter basis to increase immunity in commercial broilers [73]. Numerous studies have already examined the use of *Azolla fliculoides* as a compost or soil supplement in green manure for improving crop production. Crude protein, growth-promoting cytokinins, jasmonic acid, salicylic acid, and vitamins are all abundant in Azolla [74].

3.3. Azolla in Fish Production. Due to its omnivorous feeding habits, rapid growth, endurance to stressful situations, and high market demand, Nile tilapia (*Oreochromis niloticus*) is a well-documented candidate for BFT technology [76–78].

The effects of aquatic fern (*Azolla caroliniana*) (AQF) on Nile tilapia grown in a biofloc system are as follows: growth performance, skin mucus and serum immunities, and disease resistance According to the findings, dietary aquatic fern (AQF) can be employed as a novel tactic to provide a workable diet for sustainable aquaculture [79, 80].

Due to its higher percentage of nutrients on a dry weight basis and other components including minerals, chlorophyll, carotenoids, amino acids, and vitamins, Azolla can be utilized directly or indirectly in a fish pond. *Oreochromis niloticus*, *Tilapia mossambica*, and *Tilapia zillii* are among the species of tilapia that are commonly found. Additionally, the Cyprinidae family includes *Labeo rohita*, *Catla catla*, *Labeo calbasu*, *Labeo fimbriatus*, and *Ctenopharyngodon idella* [15].

Investigation of the possible impacts of fresh plant material partially replacing prepared pellets in the fish feed on fish growth, physiological status, and responses to disease and stress is necessary for using Azolla in small-scale aquaculture. The fundamental concept is to use Azolla fresh in fish feeding management rather than as an ingredient in prepared pellets, which is a simpler and more practical strategy for fish producers [35].

3.4. Azolla in Mosquito Repellent/Control. Because a thick Azolla mat on the surface of the water can prevent mosquito egg hatching and adult emergence, Azolla can also be used to deter mosquitoes. An investigation of water bodies, including ponds, wells, rice fields, and channels, revealed that the breeding of *Anopheles* spp. was almost eliminated in water bodies that were completely covered with Azolla [24]. Additionally, cultivating Azolla in and around the poultry farm and feeding it to the poultry birds can minimize the odor, house fly population, and mosquito threat [33].

Ravi et al. [81] reported that *Azolla pinnata* extract's fatal concentrations range from 1000 ppm to 1500 ppm. Overall, all assays against Aedes mosquitoes can be done at the LC50 for Adulticidal, which is 2572.45 ppm. Therefore, *A. pinnata* plant extracts at a single concentration will be efficient for future field applications and might be used as a substitute for vector control methods, as shown in Figure 1.

Alternative applications for bio-insecticides will offer a more effective and long-lasting method of controlling *Aedes aegypti* and *Aedes albopictus* mosquitoes. The effect of *Azolla pinnata* liquid extracts against *Aedes adulticidal*, *ovicidal*, and *ovipositional* deterring activity and its liquid chemical compounds was in line with United Nations (UN) global sustainable goals [82].

3.5. Azolla in Environmental Bioremediation. Because of its advantages over established conventional cleanup techniques, phytoremediation is currently gaining global attention as a promising environmental restoration technology. These benefits include lower capital costs, more efficient remediation, and lower environmental risks than traditional technologies [21]. The biotechnological approach used for pollutant removal has produced good yields at a low cost, but it still has a long way to go before becoming the primary response to environmental contingencies [83].

Recently, several plant species such as *Eichhornia* sp., *Pistia* sp., *Lemna* sp., *Azolla* sp., and *Phragmites* sp. have been widely used for polluted water decontamination. As a result of growing these plant species in the commercial wastewater treatment process, a massive amount of lignocellulosic biomass is produced [57, 84].

The devastating impacts of emissions and other human influences on the environment can be mitigated by plants. Curiously, the water fern Azolla can act as a phytoremediator to clean up pollution in the environment [85]. However, once the phytoremediation process is completed,

proper management of the produced biomass is required because it may be contaminated with toxic elements and cause serious environmental issues [86, 87], as shown in Figure 1.

3.6. Azolla in Biofertilizer. Because of its high potential for biological N fixation, Azolla biofertilizer may be a promising approach to improving N use efficiency (NUE) in paddy rice fields (BNF). As a result, substituting Azolla biofertilizer for 25% of urea-N provides farmers with a financially attractive alternative for significantly improving NUE and yield while effectively reducing N loss in intensive rice cropping systems [56, 59]. Azolla should be used as a biofertilizer for rice production in Ethiopia because it produces high biomass, is easy to manage and establish, increases macro- and micronutrient availability (it scavenges K and recycles P and S), improves soil physical and chemical properties and fertilizers use efficiency, increases crop yield by 15–19% (by one incorporation) in Ethiopia, releases plant growth hormones and vitamins, and does not attract rice pests [88].

In the first and second seasons, respectively, foliar spraying with Azolla extract increased the grain yield by 57.37 and 51.71%, and biological yield by 37.66 and 21.57% relative to the control application. NPK fertilizer levels increased from 60–80 to 100%. In addition to mineral fertilization, spraying Egyptian bread wheat with Azolla extract was recorded to maintain high performance while simultaneously minimizing production costs [89], as shown in Figure 1.

Azolla is regarded as one of the most promising biofertilizers due to its ability to fix approximately 30–60% kg N ha^{-1} from atmospheric nitrogen, which could replace up to 25% of nitrogen mineral fertilization [55, 90, 91]. The fixation of nitrogen by cyanobacteria is a symbiotic relationship with the fern. Because it is continuous, symbiosis is unique among plant-cyanobacteria relationships. Phytohormones can influence its development and symbiotic association [92].

Azolla extract is a powerful biofertilizer, and its use in conjunction with deficit irrigation prevented any significant decreases in N-deficient maize plant grain and stover yields. Furthermore, Azolla is a cost-effective organic fertilizer that replaces more than 30% of urea fertilizers without affecting grain yield [91].

Azolla contains nitrogen (N), phosphorus (P), iron (Fe), and magnesium (Mg). Liquid fertilizers made from Azolla have been shown to increase the population of these microalgae at a certain concentration. The application of *Azolla microphylla* fertilizers improved population density and chlorophyll content in *Spirulina platensis*. The optimal Azolla-based fertilizer concentration was 5 mL/L with a density of 321, 500 cells/mL [22].

Azolla significantly increases the number of nitrogen fertilizers available to growing crops, and it has been used as a "green" nitrogen fertilizer to increase production for thousands of years [93]. Azolla biomass can be used as a biofertilizer and soil amendment product to increase soil organic content, thereby increasing crop yield and quality [94, 95].

The decomposition of Azolla biomass releases a large amount of nitrogen into paddy soil for plant absorption (75–80% of the total collected). Green Azolla has grown twice as a dual crop in rice at 500 kg·ha^{-1}, enriching soil nitrogen by 50 kg·ha^{-1} and reducing nitrogen requirement by 20–30 kg·ha^{-1}. Azolla increases rice production by 20–30% [24]. Azolla is notable for its massive growth capacity, which is primarily due to its ability to fix atmospheric nitrogen via its symbiotic cyanobacteria *Nostoc azollae*, which lives within specialized leaf cavities of Azolla and performs a variety of significant ecological benefits [96].

Herbicide use in the aquatic environment is likely to be significantly reduced as a result of *Azola filiculoides* biocontrol. The biocontrol of *Azola filiculoides* is anticipated to result in substantial reduce in the usage of herbicides in the aquatic environment. In warmer climates like South Africa, where the plant is no longer seen as a hazard due to the introduction of the insect, climate change may lead to even more successful biocontrol of *A. filiculoides* by *S. rufinus* in the United Kingdom [14].

3.7. Azolla in Carbon Sequester of CO_2. Large terrestrial ecosystems all over the world, such as forests, where plants can act as a sink for atmospheric CO_2, which helps to reduce a significant percentage of anthropogenic carbon emissions, are where the majority of this assimilation takes place [59, 97]. Azolla biomass can also be used as a biofertilizer and a product for soil amendment to increase the organic content of some soils, which will improve crop output and quality while costing less, as shown in Figure 1. This is crucial because it lessens the need for the production and use of inorganic fertilizers, which have negative effects on the environment because of their high nitrogen concentration and ability to absorb nitrogen from the atmosphere [98].

CO_2 emissions decreased by 35%, N_2O by 22.32%, and CH_4 by 4.74% as a result of the 50% inclusion. The overall impact of this decrease in conventional feeding is mitigation of climate change equivalent to 28.47% of GWP kg CO_2/1,000 birds. The prospective effects show that *Azolla pinnata* can be a cheap and sustainable feedstock in backyard poultry farming, especially given that it only needs a straightforward multiplication technique [99].

The Eocene Azolla bloom episode, which made a large contribution to the capture of atmospheric CO_2 and the subsequent cooling of the Earth, will always serve as a reminder of Azolla's capacity as a quick CO_2 sequester. Due to Azolla's capacity for such rapid growth, it is possible to use it as a means of capturing large amounts of atmospheric CO_2 as biomass, which may then be stored away to entirely remove the carbon from the active carbon cycle. It was discovered that each 1-ha pond could hold 21,266 kg of CO_2 (C) annually. 1,018,023 km^2, or about a fifth of the Amazon forest area, was determined to be the necessary total area to reduce the overall annual rise [100].

In general, the annual rate of CO_2 emissions has slowed down to a steady linear growth of about 2 ppm/year since the early 2000s, especially when contrasted to the prior several decades, when the rate increased steadily from about 0.5 to 2 ppm [101]. If anthropogenic contribution were to be ignored, the carbon cycle would be in a stable state since it

involves both biotic and abiotic processes that transport the carbon between the natural carbon stores. It was anticipated that terrestrial ecosystems yearly absorb 20–30% of anthropogenic CO_2 emissions [102].

3.8. Azolla in a Source of Bioenergy. The production of biofuels for bioenergy, which would not only reduce the need for fossil fuels but would also deliver high-energy fuels at a lower cost compared to other forms of biofuels, could be the most significant possible use for the collected Azolla biomass [50]. The commercial production of biodiesel using certain materials is shown in Figure 1. Nowadays, the majority of researchers are focusing more on using microalgae as a useful source to provide green energy. However, the harvesting process is expensive and uses a lot of energy. Using these macroalgae for the production of biodiesel offers certain benefits, even though its oil content is not as high as that of microalgae due to Azolla's environmental problems. Azolla's potential as a source of oil for the manufacturing of biodiesel is being examined [45].

Additionally, one of the most current techniques to improve the amount of lipid, protein, and phenolic contents recovered from Azolla is the integrated biorefinery [103]. Population growth and the development of new technologies have increased the energy needs of the industrialized world, which are currently largely met by nonrenewable fossil fuels. Due to the widespread use of diesel fuel for transportation, industry, and agricultural output, fuel consumption has been steadily rising [104].

The fatty acid profile was used to estimate if Azolla biodiesel complies with the EN14214 standard's standards for fuel density, cetane number, and iodine value. However, due to the presence of the midchain (di)hydroxy compounds and the relatively high lignoceric acid concentrations, the cold filter plugging point (CFPP) is predicted to be too high [105].

4. Current Status and Future Perspective of Azolla Production

Azolla strains from various agroecological zones that are effective at fixing nitrogen need to be screened, and their performance under various abiotic stress conditions such as salinity, heavy metals, and UV-B exposure needs to be assessed. It is necessary to try to improve the strain using molecular biology advances [6]. Research efforts may be focused on creating Azolla cultures that are more resistant to environmental changes, such as variations in soil pH, temperature, salt, and heavy metal contamination. To address the abiotic stress tolerance of Azolla species in various agroclimatic zones for harvesting atmospheric N as well as bioremediation of heavy metals, genetic engineering techniques may be practical [106].

According to Hemalatha et al. [107], the structure of Azolla, which is a self-sustaining closed-loop, has a significant positive impact on the environment. The use of biotechnology and the usage of alternative feedstock generated from aquatic plants have both been statistically shown to support the sustainability of agricultural sectors. In genetic engineering research, methionine (MET) production has been modified to increase the amount of MET in plant proteins. Azolla cultivation in a bioreactor can speed up the process and save money [108]. According to a study by Sobhani et al. [109], using a disposable, low-cost bioreactor enables the mass production of *Hypericum perforatum* L. in just four weeks. A bioreactor can grow duckweed, an aquatic plant, on a huge scale [110, 111].

Aquatic fern (Azolla) use requires less effort and is associated with fewer risks. Since temperature is one of the key climatic factors in plant growth, temperature-tolerant species are recognized as an essential study area to be investigated in the context of a changing climate. More research is needed on the phytoremediation properties of dead Azolla biomass, but it seems more practical given how easily this fern can be grown in a suitable season, how easily dry biomass can be stored, how easily biomass can be transported for sustainable use, and how easily contaminated biomass can be disposed of after being turned into ash and buried deeply in wastelands [24]. Approaches using Azolla biotechnology are evolving rapidly and will do so in the future. The improvement of Azolla efficiency has been one of the key factors taken into account in the development of molecular genetics, cellular and genetic engineering, as well as genome editing. To improve the amount of secondary metabolites and nutrients in aquatic plants, basic genetics and genetic engineering studies must also be used [1].

5. Conclusion

The free-floating fern known as Azolla is found all around the world. The plant has roots that extend far into the water and is made up of a short, branching, floating rhizome with tiny leaf-like fronds. Azolla is one of nature's protein-rich wonder plants. It also includes nearly all of the necessary minerals, vitamins, and amino acids, including carotene (a precursor to vitamin A) and B12, although its carbohydrate and lipid contents are quite low. But cultivating Azolla in a controlled environment and feeding it to animals will cut feed costs and boost output. As a fish production, bioremediation, bioenergy, CO_2 sequestration, poultry feed, and a weed and biocontrol/mosquito control agent, biofertilizer and green manure are frequently utilized in value-added bioproducts. Numerous molecular methods must be investigated, including intergenic spacer, RAPD, SCAR marker, and RFLP.

Data Availability

All data presented or analyzed during this study are included in this article.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Authors' Contributions

Gamachis Korsa, Digafe Alemu, and Abate Ayele contributed equally to this review. Also, authors have approved the latest version of the manuscript and agree to be held accountable for the content therein.

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