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Potentials of azolla-cyanobacteria symbiosis as a biofertilizer in lowland rice production systems: A review

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Abstract

The reliance on chemical fertilizers seriously undermines ecosystem safety, soil health, and the essential reserves of organic matter. In contrast, biofertilizer Azolla significantly enhances rice yield while promoting soil fertility over extended periods. This literature review employs the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) approach, offering valuable insights for policymakers and researchers regarding Azolla's numerous advantages, potential limitations, and innovative applications as an environmentally friendly modification for rice production. Significant findings highlight Azolla's remarkable ability for effective nitrogen fixation, which can even surpass the symbiotic relationship typically found in legumes when conditions are favorable. Notably, it can produce an additional 30–120 kg of nitrogen per hectare, thereby significantly increasing overall rice yield. Acting as a green manure in wet soil, Azolla accelerates the process of nitrogen mineralization and skillfully regulates floodwater pH levels, which helps to reduce ammonia volatilization losses and enhances the efficiency of nitrogen fertilizers. Furthermore, it substantially improves the chemical and physical properties of the soil, greatly enhancing microbial activity and facilitating the release of essential cations such as sodium, calcium, and magnesium. This multifaceted process leads to increased soil nitrogen content, available phosphorus, exchangeable potassium, and total nitrogen, ultimately benefiting rice nitrogen uptake. Therefore, applying Azolla is strongly advocated as a beneficial practice for sustaining crop productivity, enriching soil fertility, and promoting overall environmental sustainability.

Keywords: Azolla, biofertilizer, biological nitrogen fixation, organic matter, symbiosis, environmental sustainability, increased yield, sustainable agriculture.

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Contents

Contribution of this paper to the literature
The paper's primary finding is that Azolla enhances nitrogen fixation, resulting in an increase of 30–120 kg per hectare in rice yield. As a green manure, it improves soil properties, regulates pH, and enhances nutrient availability, making Azolla an effective and sustainable alternative to chemical fertilizers in rice production.

1. Introduction

A biofertilizer is a natural entity that contains viable microorganisms such as bacteria, fungi, or algae. When applied to soil or plants, these microorganisms enhance the availability and absorption of nutrients by plants [1]. According to Kumar et al. [2], biofertilizers offer several advantages, including being viable, environmentally sustainable, and economically beneficial alternatives to synthetic fertilizers. By promoting soil health and reducing the reliance on chemical inputs, biofertilizers support sustainable agricultural practices [3]. Besides, biofertilizers enrich the soil over time, leading to improved soil fertility and structure [4]. This gradual enhancement supports long-term agricultural sustainability and resilience [5]. Unlike chemical fertilizers, which may yield immediate results, the benefits of biofertilizers become increasingly apparent over time [6]. Through processes such as nitrogen fixation and phosphorus solubilization, biofertilizers increase the availability of nutrients in the soil and promote plant growth. They capture atmospheric nitrogen and convert it into a form that plants can utilize, thereby reducing dependence on synthetic nitrogen fertilizers.

Moreover, biofertilizers stimulate root proliferation by releasing growth-promoting hormones, leading to stronger and healthier root systems [7, 8]. Additionally, biofertilizers are essential for the conversion and regulation of nutrients. The presence of beneficial microbes in biofertilizers guarantees that plants efficiently absorb and use nutrients, promoting the best possible growth and development. By providing nutrients and controlling physiological functions, they also support the resilience and health of plants [7, 9]. According to studies, using biofertilizers instead of traditional farming methods can significantly increase crop yields [10]. Increased plant vigor, nutritional availability, and soil fertility are the reasons behind this production gain. Additionally, biofertilizers can somewhat shield plants from specific soil-borne illnesses, although they cannot completely replace pest management techniques [8, 11].

In the quest for sustainable agriculture practices, researchers and farmers alike are turning to nature's solutions to address the challenges facing food production. Among these solutions, Azolla, a humble aquatic fern, has emerged as a promising ally in the cultivation of rice crops. With its unique ability to fix atmospheric nitrogen, suppress weeds, conserve water, and enhance soil fertility, Azolla offers a multitude of benefits that can revolutionize rice farming practices worldwide Adhikari et al. [12] and Tran [13]. Putra et al. [14] documented that Azolla can be used in the field as a companion crop with rice following transplantation, and green manure before transplanting or spreading directly onto the water surface of rice fields. Among these methods, Razavipour et al. [15] noted that dual cropping is more prevalent and is reported to offer benefits to rice cultivation. Azolla adapts to various contexts and environments, providing flexibility and scalability for farmers seeking to incorporate it into their farming systems. Lumpkin and Plucknett [16] found that the inclusion of Azolla in rice paddies led to notable improvements in caryopsis yield, grain yield, overall dry matter output, and straw production.

Azolla is applied in rice fields either as a monocrop or as an intercrop with rice. While intercropping involves growing Azolla alongside rice and incorporating or harvesting as needed, monocropping is carried out before the cultivation of rice [12]. Soon after the rice is moved, it is usually injected into the field. The soil can either mix it with the mud or let it degrade and die naturally [13]. Adding Azolla to the soil, either before or after transplanting, increases soil fertility and reduces the need for synthetic fertilizers, according to a study by Razavipour et al. [15]. Azolla is marketed in various ways due to its numerous benefits and versatility [17]. This covers fish feed, wastewater treatment, livestock feed, and biofertilizers [18]. Additionally, in areas where Azolla is grown, it is frequently traded directly between farmers or at local markets [12, 13].

Furthermore, Kurniawan et al. [19] reported that incorporating Azolla with a lower concentration of nitrogen in cultivated rice fields resulted in increased rice yield, highlighting that a balanced blend of nitrogen and Azolla leads to improved crop output [20]. Several researchers discovered and documented that integrating Azolla into paddy fields resulted in an 8 to 14% increase in rice yield Yao et al. [17]. Thapa and Poudel [21] observed a 34% boost in grain yield with the incorporation of 10 tons of Azolla per hectare. Putra et al. [14], along with Yadav et al. [20], reported a grain yield enhancement of 36 to 38% by using Azolla as a companion crop. Yadav et al. [20] documented a 6 to 29% higher grain yield by cultivating *A. pinnata* alongside rice, while Razavipour et al. [15] observed a 14 to 40% rise in rice production through Azolla dual cropping.

Azolla, also known as water fern, is an aquatic pteridophyte with significant potential in agriculture. The special symbiotic relationship between the nitrogen-fixing cyanobacterium and Azolla is noteworthy. Due to its ability to fix atmospheric nitrogen, Anabaena azollae serves as a useful biofertilizer for soils deficient in nitrogen, particularly in rice paddies. As a green manure, Azolla helps improve soil fertility and reduces weeds in agricultural areas [8, 16]. The dense mat formed by Azolla on the water surface inhibits weed growth and conserves soil moisture, thereby improving overall soil health and crop productivity. Furthermore, Azolla's potential extends beyond its role as a biofertilizer and green manure to its applications in enhancing soil fertility and crop productivity [22]. Emphasizing Azolla's significant potential as an aquatic pteridophyte in agriculture, it highlights its ability to improve soil structure, nutrient availability, and crop yield. Incorporating Azolla into agricultural systems can increase soil organic matter, enhance nutrient cycling, and promote better crop growth, thereby supporting sustainable agricultural practices.

2. Methodology

This study examines the overall information about Azolla as a favorable change in rice cultivation systems, utilizing systematic research and meta-analysis (PRISMA) methodology. It emphasizes prioritized reporting elements used by previous researchers [23, 24].

PRISMA technology provides a checklist of key topics that are addressed simultaneously and ensure proper coverage of the literature. We developed a search method to find relevant materials classified within the scope of our research. Data were collected from three databases: Scopus, PubMed, and Worldwide Science. The search phrases included azolla in rice production with low-income countries, azolla cyanobacterial symbiosis, factors affecting the growth of azolla, nitrogen fixation by azolla, and the potential of azolla as a biofertilizer. The search was limited to articles published up to 2024, including journal articles, review papers, and research reports published in English. To ensure the quality of the reviews, only peer-reviewed articles were considered, and all duplicates were removed. After reviewing the summaries and results, relevant articles were selected for further analysis. This process involved evaluating variables based on article titles, experimental design, and statistical analysis to ensure the reliability and validity of the findings. Figure 1 presents a PRISMA diagram for the systematic review performed for this study [18].

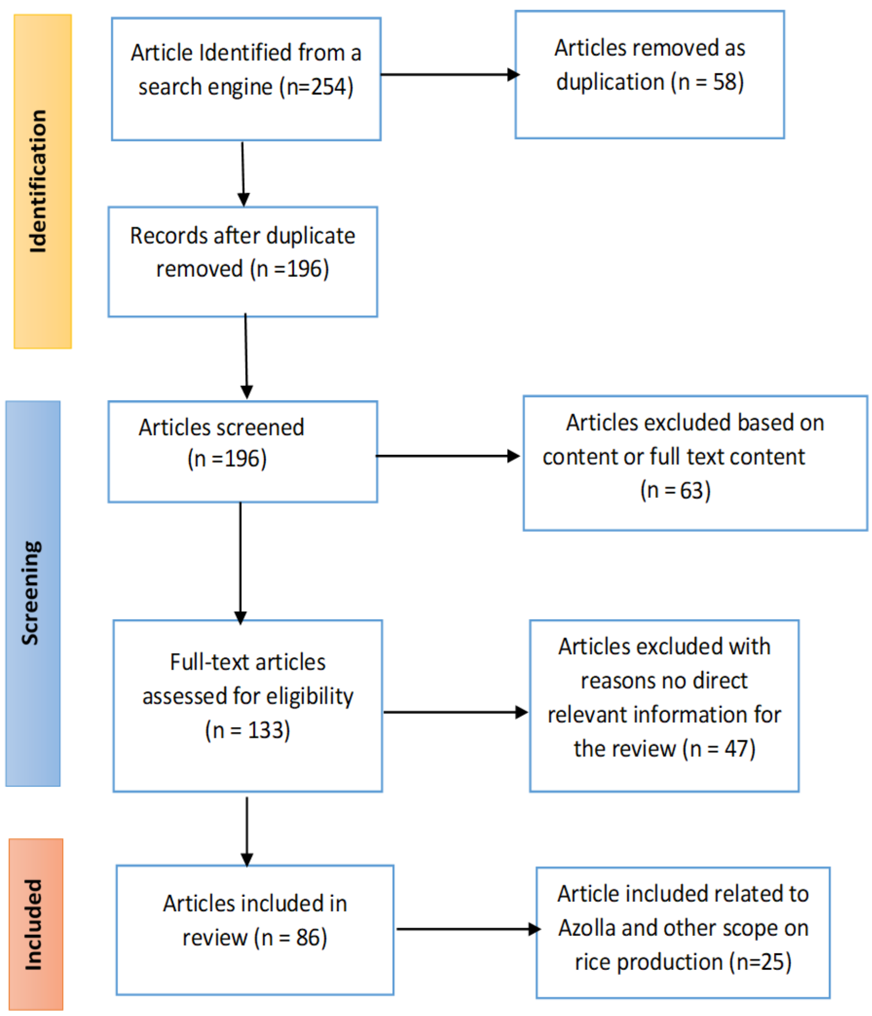

Figure 1. A PRISMA-based systematic review that was altered.
Source: Page, et al. [23].

3. Findings of the Review

3.1. Azolla Cyanobacteria Symbiosis

Azolla is a free-living water fern that can be found throughout warm-temperate, tropical, and subtropical regions [9, 22]. According to Yadav et al. [20], the small, delicate plants have a triangular or polygonal shape and grow luxuriantly in ditches, freshwater areas, and paddy fields. Although some species can grow to a diameter of 15 cm or more, their typical size is between 1 and 2.5 cm [16, 25]. Although Azolla can reproduce asexually as well as sexually, vegetative reproduction is more typical [17]. It can proliferate both vegetatively through fragmentation and reproductively by producing spores. For other species of Azolla, sexual reproduction occurs only occasionally throughout the year; only *A. Mexicana* produces spores year-round [26].

The symbiosis between Azolla cyanobacteria and the water plant Azolla is characterized by a close relationship between nitrogen-fixing cyanobacteria and the aquatic plant. *Anabaena azollae* represents a remarkable ecological phenomenon with significant implications for nitrogen cycling, agricultural practices, and environmental sustainability. Extensive studies on the symbiotic relationship between Azolla and *Anabaena azollae* have revealed intriguing aspects of its form and function [20]. Azolla, a small aquatic fern, provides a suitable habitat for cyanobacteria within specialized cavities called heterocysts present in its leaves [26]. These heterocysts are the sites of nitrogen fixation, a process that converts atmospheric nitrogen into ammonia, which plants can readily utilize. In return, Azolla provides protection and a consistent supply of carbon dioxide and nutrients to *Anabaena azollae*, supporting its growth and metabolic activities within the symbiotic relationship. The Azolla cyanobacteria symbiosis has historical significance and has been a subject of scientific research for many decades [20]. Researchers have delved into the evolutionary origins of this association and its adaptation to various environmental conditions.

This historical perspective provides valuable insights into the coevolutionary dynamics between Azolla and *Anabaena azollae*, shedding light on the mechanisms underlying their mutualistic interactions. Furthermore, the symbiotic relationship between Azolla and *Anabaena azollae* has drawn interest because of its potential applications in environmental remediation and agriculture [27, 28]. Because of its capacity to fix nitrogen from the atmosphere and improve soil fertility, Azolla has been investigated as a biofertilizer [25]. Farmers can limit nitrogen runoff, reduce their reliance on synthetic fertilizers, and sustainably increase crop yields by incorporating Azolla into their farming practices. Additionally, the symbiosis between Azolla and Cyanobacteria has significant implications for conservation efforts and environmental remediation initiatives [27]. By successfully eliminating contaminants from water bodies and enhancing water quality, Azolla has demonstrated promise in phytoremediation [16]. Its capacity to absorb carbon dioxide from the atmosphere helps reduce greenhouse gas emissions and mitigate climate change.

3.2. Factors Affecting Azolla Growth

The growth of Azolla, a unique aquatic fern genus, is impacted by numerous elements that include ecological and environmental variables. Azolla's adaptation to diverse habitats underscores its resilience and versatility, yet its growth and productivity are intricately linked to various external conditions. Temperature plays a pivotal role in regulating Azolla growth dynamics [29]. While Azolla thrives in warm climates, extreme temperatures can pose challenges. High temperatures can lead to thermal stress, reducing photosynthetic efficiency and inhibiting growth [30]. Similarly, Abd El-AAl [31] reported that Azolla exhibits optimal growth within a temperature range of 18 to 28°C; elevated temperatures, such as 35°C, can impede growth or even cause harm. Conversely, low temperatures of less than -4°C can slow metabolic rates and impede reproductive processes, limiting overall biomass production [26]. However, Putra et al. [14] demonstrated that the temperature sensitivities of various Azolla species, strains, or varieties may differ; for instance, Do Nascimento et al. [9] documented that *A. filiculoides* variety exhibits frost tolerance, unlike other varieties of *A. Mexicana* that cannot withstand such conditions. Additionally, according to Ojha et al. [32], the ideal temperature ranges for *A. filiculoides*, *A. pinnata*, and *A. Carolinian* fall between 25°C and 30°C, although some varieties may not survive within this range.

Light intensity and Light availability are other critical factors shaping Azolla's growth. Azolla relies on adequate light for energy production and biomass accumulation as a photosynthetic organism [25, 30]. Optimal light conditions promote vigorous growth, whereas insufficient light can hinder photosynthesis and stunt development [30]. Managing light exposure is essential, balancing the need for illumination with the risk of photoinhibition under intense sunlight [30]. Nitrogen fixation capacity and the growth rate of Azolla respond to varying light levels. Tangahu et al. [29] and Sadeghi et al. [30] illustrated that as light increases, both the nitrogen fixation activity and growth rate of Azolla change. At high light intensity and under pH 6 and 7, the growth of Azolla is inhibited. Whereas under the same conditions of high light intensity but under the pH of 5, the growth of Azolla is enhanced as noted by Lumpkin and Plucknett [16].

Additionally, the intensity of light beyond 90 Klux suppresses the fixation of nitrogen, while lower light levels impact Azolla proliferation and growth. Suitable air temperature combined with adequate light intensity enables *A. filiculoides* to achieve optimal growth in field conditions, as observed by Sadeghi et al. [33] and Sadeghi et al. [34] in the Anzali wetland. Under natural settings, Azolla thrives in shaded environments, yet its biomass production notably declines when exposed to light intensities below 1.5 Klux [32]. To summarize, the ideal light intensity for the growth of Azolla ranges from 15 to 18 Klux, influencing both photosynthesis and its growth.

Water depth and water quality profoundly impact Azolla's growth and health [14, 25]. It has been observed that Azolla is present on the surface of some slow-moving river systems, lakes, ponds, and canals. Clean, nutrient-rich water promotes robust Azolla populations, facilitating nitrogen fixation and nutrient assimilation [30]. Conversely, poor water quality, characterized by high salinity, pollution, or nutrient deficiencies, can suppress Azolla growth and compromise its ecological function [9]. Maintaining water quality standards is imperative for sustaining thriving Azolla communities. In their study, Lumpkin and Plucknett [16], Sadeghi et al. [33], and Sadeghi et al. [34] noted that a potential correlation exists between Azolla and water depth, as excessively shallow water levels could hinder growth and consequently reduce biomass production. Water depth plays a crucial role in shaping Azolla habitat suitability [26]. Azolla typically thrives in shallow water bodies, where it forms dense floating mats. Optimal water depths facilitate light penetration, gas exchange, and nutrient uptake, promoting vigorous Azolla growth. Adjusting water depth is essential to create favorable conditions for Azolla proliferation and ecosystem functioning [29, 32].

One of the main factors influencing Azolla growth is the availability of nutrients, especially nitrogen. Effective nitrogen fixation is facilitated by the symbiotic interaction between Azolla and nitrogen-fixing cyanobacteria, which promotes Azolla growth in nitrogen-deficient environments [31]. Azolla's production is also influenced by the availability of other essential minerals such as potassium, phosphate, and micronutrients [32]. This highlights the importance of balanced nutrient management. Interactions with other organisms, including symbiotic relationships, competition, and predation, can significantly affect Azolla growth dynamics [25, 30]. Symbiotic associations with nitrogen-fixing bacteria enhance Azolla's nitrogen-fixation capacity, augmenting its growth potential. However, competition from other aquatic plants, algae, or weeds can impede Azolla expansion, necessitating effective weed management strategies to maintain Azolla dominance. Pests and diseases also pose potential threats to Azolla's health and productivity [29]. Although relatively resilient, Azolla can be susceptible to certain pathogens, insects, and herbivores, particularly under stress conditions.

3.3. Potential of Azolla-Cyanobacteria Symbiosis in Rice Production

3.3.1. Biofertilizer

Azolla biofertilizer is considered a promising biofertilizer for most semi-aquatic crops, including lowland paddy, and is regarded globally as a valuable source of nitrogen due to its high potential for biological nitrogen fixation (BNF) [26, 35]. Azolla has an excellent capacity to fix nitrogen, and it can provide rice farms with up to 600 kg of N per hectare annually. Owing to its ability, Asians have been utilizing it as their main supply of green manure for rice fields [26]. Even though Azolla's leaves are home to over 8000 different cyanobacteria, only nitrogen fixation is performed by *Anabaena azollae*, also termed blue-green algae [13]. When *A. azollae* are present on leaves, it has the remarkable capacity to fix atmospheric nitrogen, making it usable for crops. Lumpkin and Plucknett [16] and Yadav et al. [20] revealed that Azolla is expected to be able to repair 1.1 kg of nitrogen per hectare per day. This amount is sufficient to meet the entire nitrogen requirements of rice. A study conducted by various scientists demonstrated Azolla's ability to fix nitrogen ranging from 40 to 120 kg of N per hectare when inoculated with different amounts. According to Marzouk et al. [24], azolla inoculated with 20 t ha^{-1} with a maximum of 120 kg N ha^{-1}. Additionally, a study by Kimani et al. [36] and Talley and Rains [37] demonstrates a similar capacity of Azolla to fix atmospheric nitrogen when used in Yoneda, with values ranging from 33.80 to 55.1 kg Nha^{-1} (Table 1).

Table 1. Nitrogen fixation of Azolla and different inoculation amounts.

Azolla species	Amount of Azolla inoculated	Inorganic N	N-fixed	Source
Azolla filiculoides	20 t ha^{-1}	165 kg N ha^{-1}, reduced to 50% + Azolla	120 kg N ha^{-1}	Marzouk, et al. [24]
Azolla filiculoides	12.2 t dry matter ha^{-1}	Applied N with biochar 330 kg N ha^{-1} reduced to 15% + *Azolla*	33.80 kg N/ha	Kimani, et al. [36]
Azolla filiculoides Azolla pinnata	Applied at 1.5, 3.5, and	Half recommended	40 kg N ha^{-1}	Setiawati, et al. [35] and Seleiman, et al. [38]
A. filiculoides	50 g fresh wt. ha^{-1} equivalents to 22.8 kg dry wt. ha^{-1}	Half and full recommended rate (90 and 180 kg N ha^{-1})	40 kg N ha^{-1}	Talley and Rains [37]
Azolla pinnata	Fresh Azolla at 10 and 20 t ha^{-1}, while powder was applied at 2.5 and 5 t ha^{-1}	80 kg N t ha^{-1}, 40 kg N t ha^{-1}	50 kg N ha^{-1}	Ahmad, et al. [39]
Azolla filiculoides	5 t DM ha^{-1}	100 kg ha^{-1}	52.5–55.1 kg N ha^{-1}	Marzouk, et al. [24]

According to some studies, 12.2 tons of dry matter per hectare adds roughly 33.80 kg of nitrogen per hectare when incorporated, but 16.5–17.5 tons per hectare fix between 52.5 and 55.1 kg of nitrogen per hectare when fresh Azolla is inoculated [9, 36]. Cyanobacteria's main goal is to manufacture nitrogen for both itself and its host (Azolla). Azolla is projected to generate between 30 and 120 kg N ha^{-1}, which is comparable to the yield that rice produces when it generates between 30 and 60 kg N ha^{-1} [36]. Furthermore, it has been noted that under ideal circumstances, Azolla may double its biomass every three to five days and collect between 70 and 110 kg N ha^{-1} [29]. According to Kandel et al. [26], 2 to 3 million hectares of rice fields worldwide employ Azolla as a biofertilizer. Furthermore, some studies examine the increase in yield as Azolla is used as a biofertilizer, revealing an increase in yield between 56% and 80% [26] increase in dry weight of more than 50% [9]. Increase in nitrogen use efficiency by 69% [17], increase in yield of fresh weight by applying 2.78 tons of Azolla to 37.8 tons ha^{-1} [16] (Table 2).

Table 2. Studies and their results on the use of Azolla as a biofertilizer.

Studies	Results	Reference
Biofertilizer on Rice Production	Increase yield between 56–80%	Kandel, et al. [26]
Production of organic fertilizer and soil conditioner	Increase > 50% of the dry weight	Do Nascimento, et al. [9]
Biofertilizer	The recovery efficiency of fertilizer N by 69% and 59%	Yao, et al. [17]
Effects of soil salinity and water content on soil microbes	Increase yield between 13–20%	Yan, et al. [25]
Properties of *Azolla*	Yield of 37.8 tons ha^{-1} fresh weight containing 2.78 tons dry weight of *A. pinnata*	Lumpkin and Plucknett [16]

3.3.2. Increase Soil Fertility

Azolla improves soil fertility in the long term and increases crop yields in rice production systems, claims [40]. Azolla releases some ammonium into the water during its growth, but most of the fixed nitrogen is available in rice only when Azolla breaks down [12]. Azolla biomass has a higher nitrogen and potassium content than other green fertilizers, according to Lumpkin and Plucknett [16] which is 3-5% and 3-6% by dry weight, respectively. Furthermore, Azolla, like other biological features in soil, has long-term effects on increasing the total levels of nitrogen (N), phosphorus (P), potassium (K+), calcium (Ca2+), magnesium (Mg2+), and phosphatases, contributing to soil fertility and plant growth enhancement [32, 41]. Azolla disintegrates quickly, resulting in more humus being released into the soil, increasing its porosity (3.7 4.2%), its specific gravity decreases, increasing soil capacity and retaining water.

In addition, studies have shown that the inclusion of Azolla in the soil improves microbial activity, which enhances soil fertility and nutrient cycling Razavipour et al. [15] and Xie et al. [42]. Goala et al. [28] conducted a study to assess the effect of Azolla on soil physicochemical properties. For example, Azolla compost was mixed with different rates of 0, 20, 40, 60, and 80 kg per hectare. This information was obtained from a study comparing synthetic fertilizers with Azolla compost. Azolla was found to grow significantly with increasing soil pH, organic matter (OM), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg), with pH values increasing from 2.42 to 3.67 g kg^{-1}, OM from 2.42 to 3.67%, P from 0.15 to 0.47 ppm, K from 0.5 to 3.8%, and Ca and Mg levels also showing notable increases. The correlation coefficients ranged from 0.93 to 0.97, indicating a strong positive relationship between Azolla growth and soil nutrient levels. Conversely, as Azolla decomposes, phosphorus becomes free and more accessible in the soil, enhancing nutrient availability for plants [43]. The amount of available soil P added with azolla increased by 89% at the start of the rice panicle [44]. Azolla was also found to have higher P and Ca contents, with average values of 345 mg $100g^{-1}$ and 125 ppm, respectively [45]. The same team of researchers found that after two more years of study, the soil treated with Azolla had a 29% increase in K and a 43% increase in P over the initial value when treated with Azolla and cow dung [46]. Table 3 details the effect of azolla on soil chemical properties.

Table 3. Effect of Azolla on soil chemical properties.

Treatment Level g kg⁻¹	OM g kg⁻¹	N %	P ppm	K Cmol kg⁻¹	Ca %	Mg %
0	2.42	2.42	0.15	0.50	0.18	0.33
20	3.38	3.38	0.25	0.56	0.92	0.45
40	3.55	3.55	0.31	0.58	0.99	0.55
60	3.60	3.60	0.44	0.63	1.10	0.75
80	3.67	3.67	0.47	0.63	1.38	0.93
LSD (0.05)	0.22	0.22	0.09	0.01	0.76	0.04

Putra et al. [14] found that adding azolla significantly increases the amount of organic matter in the soil. Azolla at varying rates of 0, 10, 30, 60, and 90 kg^{-1} was incubated for different durations of 10, 20, 30, 40, and 50 days to assess the impact of Azolla on soil organic matter content. This study compared the amount of Azolla compost with different inoculation times to determine its effectiveness in enhancing soil fertility and organic matter levels [47]. It was discovered that the rate of Azolla significantly improved the amount of organic matter in the soil (Table 4)

Table 4. Effects of Azolla on soil organic matter (SOM).

Treatment	Incubation time (days)				
Azolla kg⁻¹	10	20	30	40	50
0	2.29	2.39	2.39	2.39	2.30
10	3.01	3.17	3.49	3.43	3.91
30	3.21	3.36	3.61	3.53	3.41
60	3.18	3.41	3.71	3.71	3.61
90	3.32	3.52	3.81	3.87	3.81

Source: Bhuvaneshwari and Kumar [47]

Azolla compost improves plant development and yield and increases the amount of organic matter in the soil. Putra et al. [14] and Goala et al. [28] state that soil organic carbon and humic compounds would be produced throughout the mineralization process. Azolla inoculation was found to considerably raise the soil's organic carbon content [35]. Equal amounts of cow dung and azolla increased the soil's organic carbon content, which varied between 1.3 and 1.7%, according to a group of researchers' findings [35]. According to reports, oxidizable organic C in soil treated with Azolla increased by 25.51% [45]. Other investigators observed increased cellulolytic and urea-hydrolyzing activities, along with a notable increase in the bacterial population [31].

3.3.3. Nitrogen Mineralization

According to Kandel et al. [26], organic nitrogen mineralizes more gradually after the first two weeks of decomposition. The main form in which N is ammonium. According to Abd El-AAl [31], the amount of ammonium-nitrogen released stabilized at about 1 mg of fresh Azolla, or 26-28% of all nitrogen content of the plant. The rates of mineralization under the incorporation of fresh and air-dried Azolla were compared by Putra et al. [14]. They found that, in the first four weeks of incubation, fresh-incorporated Azolla showed a higher rate of mineralization than air-dried Azolla. But from the fourth to the eighth week, air-dried Azolla's rate of mineralization accelerated. However, after incubation for six weeks, 75% of the dried Azolla had undergone ammonification, in contrast to 62% of the fresh Azolla [14]. Additionally, when Azolla is applied at a basal rate of 10 to 12 tons per hectare, the amount of nitrogenous fertilizer required for rice crops is reduced to 30-35 kg, and the soil's nitrogen content increases by 50 to 60 kg per hectare. By injecting 500 kg per hectare of green Azolla, the soil's nitrogen content is increased by 50 kg per hectare, resulting in a reduction of 20–30 kg per hectare in the requirement for nitrogen fertilizer [48].

3.3.4. Improves the Soil Microbial Activity

Growth-promoting chemicals such as gibberellic acid (GA) and indole acetic acid (IAA) are found in Azolla, along with specific bioactive compounds like polysaccharides, fatty acids, and phenolic compounds. These were identified through the application of *A. azollae* and examined for microbicidal activities by Abd El-Aal [49]. It was discovered that these compounds possess both in vitro and in vivo microbicidal properties. Furthermore, it is now recognized that *A. azollae*'s high nitrogenase activity is an early indicator of its capacity for biofertilization. Notably, it has been demonstrated that soil microbial populations increase as a result of enhanced dehydrogenase activity and the excretion of polysaccharides, thereby improving soil fertility. Consequently, *A. azollae* may support the agricultural industry in various ways and is considered a safe and promising bio-inoculant, especially in light of current organic farming trends [31].

Azolla is an organic matter substrate that decomposes quickly; therefore, incorporating it into the soil will accelerate microbial activity. In the profile of wet paddy soil, there is a thin aerobic layer on top of a reduced lower layer [19]. Greater than the oxygen that diffuses through the profile, aerobic bacteria utilize the oxygen found in this upper layer [29]. Due to oxygen depletion caused by the active flora's microbial activity on the easily decomposable organic materials, the aerobic layer will be reduced [50]. After flooding the profile, it will take the bacteria a few hours to deplete the oxygen that is still present in the lower soil layer and either die or become dormant [19, 48]. Next in line will be the facultative anaerobes, followed by the obligate soil anaerobes. These anaerobes cause the reduction of various elements in the soil as they consume soil constituents and dissimilation products. According to Lumpkin and Plucknett [16], there is a chance that the same genera of facultative anaerobes reduce one element. Nonetheless, research has shown that multi-element-reducing bacteria first reduce the more oxidized element, followed by the less oxidized element [19, 51].

The combination of reducible substrate availability and microbial activity rate determines how much of the soil component is reduced upon submergence [25]. Consequently, this will accelerate the rate at which soil component degradation occurs [19]. The ferric iron to ferrous iron ratio is always the most significant in lowland rice fields because iron is more prevalent than other reducible components [51]. The pH of paddy soil decreases after flooding due to the buildup of CO_2 from soil microbial activity. This decrease is subsequently followed by an increase in pH, which stabilizes at a range of 6.7 to 7.2 within a few weeks [29].

3.3.5. Reduction of NH_3 Volatilization

Rajendran et al. [52] reveal that accelerated amounts of nitrogen in the parterre Azolla soil of standing rice contribute to maximizing efficiency of use in the early stages of rice growth and are released later. Research findings show that Azolla can effectively reduce the loss of volatility as a cover for rice flood surfaces by affecting flood pH values. The rapid increase in floodwater pH values caused by urea hydrolysis indicates the importance of managing nitrogen application to optimize rice cultivation and minimize environmental impacts [53]. Algae photosynthetic activity was effectively inhibited by complete azolla covering of the flood surface. Ammonia coverage is assumed as a major cause of fertilizer efficiency with low N fertilizers, and gaseous NH3 emissions to the atmosphere are an important method for low-lying n -loss. These gas losses also affect water and air quality, causing significant economic losses for farmers. As a result, there are concerns that the use of Azolla will increase the efficiency of N-fertilizers [19, 47]. Additionally, the Azola cover significantly reduces flood temperatures compared to the Azolla-free diagram.

The relative amount of NH3 volatilization present at a particular pH value is affected by flood temperature [54]. At the beginning of the vegetation period, rice is not a good competitor for the available nitrogen. Therefore, Azola uses N to efficiently compete with young rice plants and absorb almost twice as much urea as rice plants. This leads to the system's urine material, leading to a complete N-reproducing at the beginning of the vegetation period [55]. Many studies have shown that the addition of azolla to rice increases. When using Azolla, the NUSE efficiency increased by 32%.

3.3.6. Controlling the Growth of Aquatic Weeds

According to Goala et al. [28] and Roy et al. (2016), weeds can reduce the mobility yield by 15-20%, and in extreme cases by 50%. Weed control is an added benefit of thick azolla in rice fields. Azola, like other seaweeds, covers the surface of the water and reduces the penetration of light. This means that 70% of weeds cannot germinate [56]. Therefore, *Echinochloa Crus-galli*, *Cyperus spp*., *Paspalum spp*., and other aquatic biological agents are reduced by the growth of azolla in flooded rice fields, which improves harvest growth and productivity [19, 51]. According to Roy et al. (2016), the degree of suppression increases with increasing azolla cover percentage and water depth. The use of 10 t ha-1 azolla produced the lowest weed density and the highest weed control index in travel harvest, as leafy growth formed a very thick mat on the water surface, creating a light-limited amount, allowing weed seeds and seedlings to be intercepted [57]. Depending on the nature of the weeds, the use of Azolla was shown to be reduced by 69% during harvest and during the harvesting of rice flowers [54].

3.3.7. Carbon Sequestration

Carbon sequestration is a critical process that occurs primarily in terrestrial ecosystems, particularly in forests. These ecosystems act as CO2 sinks in the atmosphere, helping to reduce the substantial amount of carbon released by anthropogenic activities. Proper understanding and enhancement of this process are essential for mitigating climate change and maintaining ecological balance [20, 58]. It is used to increase the organic content of beds, thus increasing plant quality and harvesting at a low cost [59]. This makes production and use cheaper. 50% intake reduced CO2 emissions by 35%, N2O, and CH4 by 5% [19, 60]. Reduced traditional feeding has a positive net effect on 28% of climate change in GWP KG CO2. Azolla's ability to quickly isolate CO2 is always remembered, considering the Azolla Bloom episode. This played an important role in the atmospheric CO2 recordings and subsequent Earth cooling [60]. Because of its ability to grow very quickly, Azolla can be used to absorb a significant amount of CO2 from the atmosphere as biomass. This biomass can be stored to effectively remove carbon from the ongoing carbon cycle. It has been reported that each hectare pond can save approximately 21,266 kg of CO2 annually, highlighting its potential for carbon sequestration and climate change mitigation [19, 20].

3.3.8. Increase in Rice Yield

Yields of grain, straw, kalionis, and dry matter increased in the rice fields with the addition of Azolla [61]. This symbiosis is suitable for use as a biofertilizer due to the ability of Azollae to fix nitrogen and the nitrogen content of ferns [20]. According to a Peters study, the use of Azolla as a biofertilizer increased yields by 112% compared to control plots. When applied as a spread with rice, yields increased by 23%. It was then applied as a monoculture and intercropped land, resulting in a yield increase of 216% [60]. The combination of Azolla and nitrogen is more effective because it increases rice yield by 8% when used together with a low nitrogen dose in planted ODA Yao et al. [17] and Yang et al. [57]. Lumpkin and Plucknett [16] reported that the additional 10 tons per ha of Azolla increased revenues at Rice Corner by 34%.

Yadav et al. [20] and Yang et al. [57] show that if Azora is installed in rice fields, the increase in income from corner sales ranges from 36.6% to 38%. Elmachliy et al. [62] reported that using Azolla can increase rice yields by 14% to 40%. However, Tangahu et al. [29] noted that the use of Azolla in rice fields can increase grain yield by up to 29% [29]. Researchers recognize a significant increase in rice corner operations by using azolla in conjunction with urea coated with neem cake [57]. The combination of the relationship between Azola infection and its relationship was due to Seleiman et al. [38] comparing different inorganic derivatives and finding that the use of azolla in all variants affects rice growth and grain yield [17, 23]. Table 5 presents a summary of studies on the impact of Azolla biofertilizer on rice grain yield.

Table 5. Summary of studies on the impact of Azolla biofertilizer on rice grain yield.

Studies	Grain yield (t/ha)				Reference
	Control Plot	Inorganic N	1/2 inorganic N + Azolla	Azolla	

Comparing the time of Azolla incorporation and synthetic fertilizer on the growth and yield of rice	0.51	6.3	Not specified	6.21	Page, et al. [23]
Use of Azolla in conserving urea-N in rice production	1.1	6	Not specified	5.1	Page, et al. [23]
Effect of P on Azolla growth	1.9	3.3	2.9	2.4	Yao, et al. [17]
Azolla in rice production	2.64	3.5	Not specified	3.95	Page, et al. [23]
Impact of Azolla on CH4 Emission	3.58	4.58	4.38	4.38	Kondo, et al. [63]
Use of Azolla for improving low N-use efficiency	4.58	8.99	9.84	9.64	Kimani, et al. [36]
Application of biochar and Azolla in Rice production	8	Not specified	Not specified	11	Page, et al. [23]
Use of Azolla in moderating the dose of N fertilizer and mitigating GHG	426 g m^{-1}	518 g m^{-1}	515 g m^{-1}	528 g m^{-1}	Setiawati, et al. [35]
Azolla in fresh and powder form improves soil and rice yield	49.76 g pot^{-1}	Not specified	Not specified	64.9 g pot^{-1} 3.95	Liu, et al. [64]
Azolla cover reduces NH3 volatilization	56.81 g pot^{-1}	81.6 g pot^{-1}	85.17 g pot^{-1}	83.63 g pot^{-1}	Kimani, et al. [36]

4. Conclusion

Azolla was identified as a potential organic matter source in lowland rice agriculture. The use of Azolla offers a cost-effective alternative with significant long-term environmental benefits and a high potential for supplying solid nitrogen and other nutrients in lowland rice systems. When Azolla is properly employed and utilized, it enhances nitrogen use efficiency, reduces nitrogen loss, and decreases the dependence on synthetic fertilizers. Additionally, Azolla exhibits high growth rates, leading to increased organic carbon content, microbial biomass, and improved nutrient cycling within the soil. Consequently, farmers and other stakeholders are more likely to adopt Azolla as a biofertilizer. Considering these factors, the application of Azolla as a biofertilizer presents a practical option for rice farmers, as it promotes sustainable soil health and simultaneously boosts rice productivity.

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