International Journal of Ecology and Environmental Sciences 50: 661-672, 2024
ISSN: 2320-5199 (Online); https://doi.org/10.55863/ijees.2024.0264

Review article

Exploring the Multifaceted Benefits of Azolla: A Comprehensive Review of an Aquatic Fern's Biological and Practical Contributions

SARANYA DEVI, K.[1*], VIJAYAN, K.T.V.[1], DEEPTHI, K.S.[1], RESHMA, C.V.[1] AND SANGEETHA MENON[2]

[1]*Department of Microbiology, Government Arts and Science College Kozhinjampara, Palakkad, 678554, Kerala, India*

[2]*Department of Life Science, Kristu Jayanti College Autonomous, Bengaluru, 560077, India*

E-mail: saranyadevimicro@gmail.com, drvijayanktv@gmail.com, nivedithasreekumar@gmail.com, reshmavalsalan@gmail.com, sangeethamenon@kristujayanti.com

[*]**Corresponding author**

ABSTRACT

Azolla, also known as "green gold" or "super plant," is a nitrogen-fixing pteridophyte found in temperate and tropical freshwater ecosystems. This free-floating aquatic fern, native to Asia, Africa, and the Americas, thrives in diverse aquatic habitats, including swamps, ditches, lakes, and shallow rivers. It generates full biomass in a relatively short period of time. It acts as a nitrogen biofertilizer and boosts rice productivity. Beyond its role in agriculture, *Azolla* serves as a versatile resource, finding applications in livestock feed, human nutrition, hydrogen fuel production, biogas generation, pest control, and water purification. Notably, *Azolla* exhibits hyperaccumulation capabilities for various heavy metal contaminants and can effectively absorb ammonium and phosphorous from wastewater. Moreover, it possesses antimicrobial properties and a rich array of phytochemical compounds, promising multifaceted benefits in diverse fields. This review highlights the vast potential of *Azolla*, emphasizing its suitability for extensive research and development in food, feed, and fodder applications, as well as household cultivation and utilization. To fully harness the myriad advantages offered by *Azolla* and promote sustainability across various sectors, further exploration and investigation into its untapped applications remain imperative. *Azolla* holds the key to addressing numerous environmental, agricultural, and industrial challenges, paving the way for a more sustainable and resource-efficient future.

Key words: *Azolla,* Cyanobacterium, Salviniaceae, Pteridophyte, Sustainability

INTRODUCTION

Azolla, belonging to the Salviniaceae family, encompasses seven distinct aquatic fern species, including *Azolla caroliniana, A. filiculoides, A. mexicana, A. microphylla, A. nilotica,* and *A. pinnata* (Raja et al. 2012). Recognized by various names such as mosquito fern, duckweed fern, fairy moss, Carolina fern, and water fern (Soman and Arora 2018), this plant can double its biomass within a week under favourable conditions, exhibiting reported yields ranging from 8 to 10 tonnes of fresh matter per ha in Asian rice fields (Brouwer et al. 2018). *Azolla* is recognized for its role as a symbiotic nitrogen fixer, engaging in a mutually beneficial association with the cyanobacterium *Anabaena azollae*, which effectively converts atmospheric nitrogen into a form usable by plants (Ran et al. 2010). Its capacity for nitrogen fixation has resulted in its global acceptance as a widely adopted biofertilizer.

Extensive research has unveiled numerous potential applications of *Azolla*, including its use as livestock feed (Shukla et al. 2018), a source of human nutrition (Liu et al. 2008), a resource for biofuel production (Miranda et al. 2016), a contributor to biogas generation (Sathammaipriya et al. 2018), a promising biolarvicide (Ravi et al. 2010), a tool for water purification (Liu et al. 2017, Arora and Kaur 2019), an agent with antimicrobial properties (Ekawati and Pradana 2019), and a means to enhance soil microbial diversity (Lu and Lu 2018). In addition, with this, it plays a significant role in mitigating atmospheric CO_2 levels (Speelman et al. 2009, Verma et al. 2022, Korsa et al. 2024). The beneficial properties of *Azolla* are outlined in Figure 1.

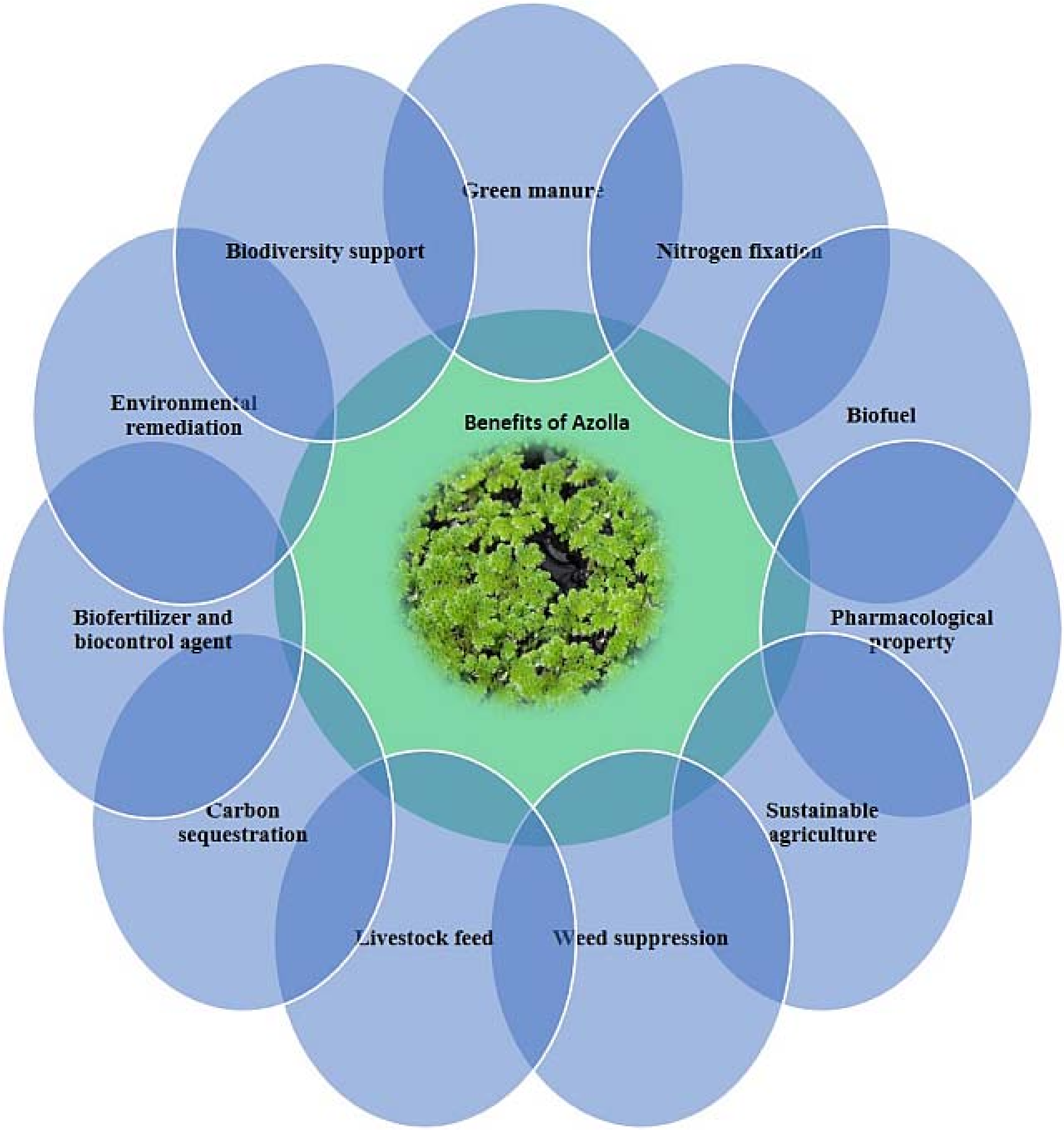

Figure 1. Beneficial role of *Azolla* in various sectors

Overall, the multifaceted advantages of *Azolla* make it a valuable asset with wide-ranging implications across various sectors. In recent decades, numerous studies have highlighted the profits of *Azolla,* making it a versatile and highly valuable resource. Despite its small size, measuring only a few millimetres, this aquatic fern possesses remarkable qualities that make it indispensable across various fields. This comprehensive review explores the varied benefits of *Azolla*, shedding light on its pivotal contributions in industrial, medical, agricultural, and across various fields.

Historical significance and reproduction of *Azolla*

The genus *Azolla* has a significant presence in the fossil record, with origins dating back to the Late Cretaceous, approximately 70 million years ago. Notably, *Azolla arctica*, was deposited fifty million years ago during the Eocene Arctic *Azolla* event. The fossil record has revealed a minimum of six extinct species, such as *A. intertrappea, A. berryi, A. prisca, A. tertiaria, A. primaeva,* and *A. boliviensis* (Evrard and van Hove 2004). It is hypothesized that during the Eocene, *Azolla* proliferated at an extraordinary rate, leading to substantial carbon absorption and, intriguingly, setting a global cooling effect that persists to the present day (Speelman et al. 2009). This historical context sheds light on the diverse nature of *Azolla* species and their substantial environmental impact. *Azolla* exhibits both sexual and asexual reproduction methods. Unlike typical ferns, *Azolla* is heterosporous, producing two types of spores (separate female and male spores in sac known as sporangia) (Nagalingum et al. 2006).

Symbiotic partners of *Azolla*

Azolla stands out as a botanical marvel, achieving unparalleled growth rates on a global scale, all while thriving without the need for soil. It shows unique symbiotic partnership with the cyanobacterium *Anabaena azollae*, an extracellular endosymbiont with the remarkable capacity to fix atmospheric nitrogen. The leaves of *Azolla* create a specialized microenvironment, fostering the growth of *Anabaena* within their confines. This mutualistic association between *Azolla*, the fern, and the cyanobacterium *A. azollae* is evidence to nature's intricacies, wherein the algae contribute nitrogen to the fern, while the fern, in return, provides a suitable habitat for the cyanobacteria. This exceptional nitrogen-fixing ability has positioned *Azolla* as an economically vital biofertilizer, particularly in the context of rice cultivation across Asia (Herath et al. 2023).

Initially, *Cyanobacterium* was categorized under the name *Nostoc*, however, its nomenclature was later revised to *A. azollae* (Carrapico 2010). Yet, the precise identification of the cyanobionts, which encompass genera such as *Anabaena, Nostoc*, and *Trichormus*, remains a subject of ongoing debate (Brouwer et al. 2014). Cyanobacteria, particularly those proficient in nitrogen fixation, hold pivotal roles in numerous cyanobacterial symbioses by providing essential combined nitrogen to their host organisms (Adams et al. 2012). Notably, *Azolla*, despite its classification as a lower vascular plant, exhibits more advanced and mature symbiotic properties compared to other vascular plant-cyanobacterial symbiotic relationships (Carrapico 2010). This strange mutuality between *Azolla* and *A. azollae* offers profound insights into sustainable agriculture.

Symbiotic association in the plant kingdom have increasingly revealed remarkable complexities, and *Azolla*, a water fern, stands as an exemplary model. In addition to its well-known partnership with cyanobacterium *A. azollae*, several reports have unveiled the presence of a third symbiotic partner - bacteria, residing within the leaf cavity of *Azolla* (Pratte and Thiel 2021). These include *Pseudomonas* (Bottomley 1920, Peters et al. 1978), *Caulobacter fusiformis, Alcaligenes faecalis* (Newton and Herman 1979), Coryneform bacteria (Gates et al. 1980), *Arthrobacter, Corynebacterium* and *Agrobacterium* (Petro and Gates 1987), *Bacillus cereus* (Si-Ping et al. 2008), *Microbacterium, Hypomicrobium, Shinella, Ralstonia, Rhizobium* and *Hydrocarboniphaga* (Dijkhuizen et al. 2021), as well as Proteobacteria, Actinobacteria, Chlorobillobacteria, and Firmicutes (Yang et al. 2022). This dynamic microcosm within the leaves of *Azolla* cavities enhances its ecological significance by facilitating the coexistence of diverse microbial communities. Furthermore, exploring the potential of endosymbiotic bacteria and its beneficial role in different sectors is essential in current scenario.

GREEN GOLD: *AZOLLA'S* ECONOMIC ADVANTAGE

Azolla as a nitrogen-fixing biofertilizer in rice cultivation

The use of *Azolla* as a biofertilizer in Southeast Asia has gained prominence due to its nitrogen-fixing capabilities, offering an eco-friendly alternative to nitrogenous chemical fertilizers. The sustained application of chemical fertilizers has detrimentally affected soil organic matter and worsened nitrogen deficits (Hossain et al. 2001). Furthermore, these fertilizers have contributed to soil acidification, leading to a long-term reduction in soil microbial activity.

Azolla, an aquatic fern's role as a biofertilizer dates back over 1,500 years, as documented in "The Art of Feeding the People", a book authored by Jia Si Xue in 540 A.D (*Azolla* Foundation 2014). The historical use of *Azolla* as green compost is evident from records at the end of the Ming dynasty (Lumpkin and Plucknett 1982). For centuries, *Azolla* has been involved in enhancing agricultural productivity in China. When rice paddies are flooded in the spring, *Azolla* can be introduced, rapidly covering the water surface and suppressing weed growth. As *Azolla* decomposes, it releases nitrogen into the water, providing up to 9 kg of protein per hectare annually (Tung and Shen 1985). With dry matter production reaching 80 MT/ha and annual biomass production at 1000 MT/ha, *A. anabaena* is capable of fixing nearly three times the atmospheric nitrogen compared to legumes. Typical nitrogen fixation rates for legumes are 400 kg of nitrogen per ha per yr, whereas *A. anabaena* can fix 1100 kg of nitrogen per ha per yr. This translates to *Azolla* producing approximately 8-10 tonnes of green biomass, equivalent to 25-30 kg of nitrogen, or 55-60 kg of urea.

Azolla was used as a cover on the floodwater surface of rice fields, effectively reducing ammonia volatilization. The gaseous ammonia emissions into the atmosphere are major contributors to the low efficiency of nitrogen fertilizer use. These losses not only incur financial costs for farmers but also have adverse environmental consequences. The presences of an *Azolla* on the water surface during the initial urea application, successfully inhibited the rapid increase in floodwater pH resulting from urea hydrolysis. *Azolla*, with its low C:N ratio, mineralizes faster than other species, supplying nitrogen to agricultural plants (Macale and Vlek 2004). Continuous *Azolla* application significantly enhances the soil organic nitrogen content (Yadav et al. 2014). Its cultivation in paddy fields, either as a monoculture or intercrop, serves to increase soil humus and nutrient content, resulting in a remarkable 20-30% boost in rice yields (Raja et al. 2012).

Marzouk et al. (2023) described various positive impacts of *Azolla* as a biofertilizer in lowland rice production. These include the improvement of soil fertility, reduction of weed growth, increased soil organic carbon levels, and the enhancement of microbial biomass, leading to improved nutrient cycling and increased rice growth and yield. Furthermore, *Azolla* has been found to enhance soil nutrient availability through biological activity and foster the development of microflora.

Azolla as a nutrient-rich feed

The application of *Azolla* as a feed for animals and birds has gained substantial attention due to its nutritional richness. The utilization of *Azolla* as an animal feed also presents an opportunity to reduce dependence on conventional feed sources, aligning with sustainable livestock farming practices. The Food and Agriculture Organization (FAO) recognized *Azolla*'s role in tropical biomass agricultural systems as early as 1993, emphasizing its capacity to reduce the need for supplementary food. Researchers have demonstrated the application of *Azolla* as fodder for various animal species, including cows, goats, pigs, rabbits, chickens, ducks, and fish (van Hove 1989). Its high protein content, coupled with low lignin levels, renders it highly digestible by animals, contributing to its role as a growth promoter (Liu et al. 2017).

Azolla is well known for its protein content, essential amino acids, vitamins, and minerals. These attributes make *Azolla* an attractive dietary supplement for livestock and poultry, with the potential to enhance growth, health, and overall productivity (Shukla et al. 2018). Extensive research has affirmed the advantages of incorporating *Azolla* into animal diets. Buffaloes exhibited accelerated growth rates when *Azolla* was added to their diet along with rice straw, in comparison to diets composed solely of rice straw (Anonymous 1985). Improved growth performance in 'Black Bengal' goats was reported when *Azolla* replaced up to 20% of their feed concentrate (Tamang and Samanta 1995, Varun et al. 2021). Furthermore, the addition of *Azolla* meal to the diets of Nellore sheep and buffalo led to enhanced dry matter digestibility, daily weight gain, and feed efficiency (Indira et al. 2009, Ahmed et al. 2016, Sihag et al. 2018). Increased milk production in cattle has been reported; when *Azolla* was included in diet (Mathur et al. 2013, Kumar et al. 2020).

Although, *Azolla* has primarily been explored in the context of livestock, particularly cattle and goats, its potential in poultry diets has also gathered attention. The benefits of incorporating *Azolla* into broiler rations; results in cost reductions and improved returns (Parthasarathy et al. 2002). Higher body weight and increased egg production were observed in layer birds fed with fresh *Azolla*. The immune-enhancing effects of *Azolla* protein in commercial broilers have also proved (Chichilichi et al. 2015). *Azolla*'s nutritional profile, (Asim et al. 2022), includes essential vitamins (B_{12}, beta-carotene, vitamin A), biopolymers, minerals, and amino acids. The potential of *Azolla* extends to hepatoprotection and its anti-inflammatory, antioxidant, and anti-apoptotic properties, suggesting its utility in preventing super-hepatotoxicity. Recent investigations, have confirmed the positive influence of *Azolla*-based diets on various aspects of poultry performance, including the feed conversion ratio, egg production, and yolk color, further expanding the scope of *Azolla*'s application in poultry nutrition (Swain et al. 2022). *Azolla*'s journey from its nutritional discovery to its multifaceted application in animal feed reflects its versatility as a sustainable and nutrient-rich resource. Its potential to enhance animal growth, productivity, and health makes it a valuable asset in promoting sustainable and efficient animal farming practices.

Azolla, with its abundant production and cost-effectiveness, has emerged as a promising ingredient in fish feed formulations. A notable innovation is the utilization of *Azolla* slurry, a byproduct of biogas generation, as a potent fishpond fertilizer, as exemplified by Mosha (Mosha 2018). A comprehensive investigation of the feeding of Pangasius catfish with *Azolla* was undertaken (Rahmah et al. 2022). The results of this study demonstrated notable improvements in weight gain, specific growth rate, protein efficiency ratio, and feed conversion ratio in fish that were supplemented with *Azolla*. The cultivation of *Azolla* in Red Tilapia aquaculture wastewater, could effectively serve as a substitute for fishmeal protein in the diets of Pangasius catfish juveniles up to 10 g per kg. This substitution not only contributed to enhanced growth and feed efficiency but also optimized the utilization of nutrients, while having no adverse effects on fish survival, body parameters, or body composition. Furthermore, recent research (El-Daim et al. 2021) has emphasized the benefits of supplements containing *A. nilotica* (5%) and *Spirulina platensis* (1%) in enhancing the growth performance of *Nile tilapia*. This positive trend extends to various other fish species, including common carp, fringed-lipped peninsula carp, and Thai silver barb, where *Azolla* successfully replaced portions of traditional soybean meal in their diets without compromising flesh quality or incurring additional feed costs (Das et al. 2018, Ammar et al. 2020). *Azolla caroliniana*, a water fern, was recently validated (Lumsangkul et al. 2022) for its positive effects on the growth rate and feed efficiency of Nile tilapia. Additionally, in *Penaeus monodon* (black tiger shrimp), *A. pinnata* proved to be a valuable dietary component, leading to substantial weight gain compared to conventional soybean meals (Sudaryono 2006). A previous study suggested that dietary replacement of up to 20% fresh *Azolla* holds the promise of enhancing fish growth, quality, profitability, and sustainability in mono-sex Nile Tilapia (Refaey et al. 2023). Consistent findings across these studies underline the growing significance of *Azolla* as an economical and nutrient-rich component within fish feeds, ultimately contributing to the enhancement of growth, feed efficiency, and overall sustainability in aquaculture practices.

Azolla as affordable biofuel producer

The increasing demand for cost-effective and sustainable biofuel production has led to the exploration of next-generation bioenergy crops. Aquatic plants, known for their rapid colonization of wetlands, have received significant attention because of their ability to thrive in wastewater and yield substantial biomass. Among these, *Azolla* species stand out as exceptionally fast-growing plants, capable of thriving in contaminated water and natural habitats, making them appealing candidates for bioenergy production. *Azolla,* as a novel bioenergy feedstock, holds great promise because of its unique chemical composition (Miranda et al. 2018). Its biomass possesses a distinctive chemical makeup, encompassing three major categories of bioenergy molecules: cellulose/hemicellulose, starch, and lipids. This chemical profile resembles a hybrid of conventional terrestrial bioenergy crops and microalgae, enhancing its suitability as a biofuel source (Miranda et al. 2016). A few studies have been conducted on *Azolla* for producing hydrogen, a non-polluting, high-energy fuel. When *A. anabaena* is grown in a nitrogen-free atmosphere or in a nitrate-containing water medium, the nitrogenase of the symbiont produces hydrogen rather than nitrogen

(Peters and Meeks 1989).

Azolla is the most attractive, sustainable, and versatile feedstock for the development of cost-effective, low-energy demanding, and nearly maintenance-free systems for producing a wide array of renewable biofuels (Miranda et al. 2016). Recently, researchers implemented *Azolla* to develop a self-sustaining biorefinery model (Sathish et al. 2022). Tannery effluents have been subjected to phytoremediation using *Azolla*, taking advantage of its rapid growth to effectively treat and mitigate pollutants. Additionally, algal oil extracted from cultivated *Azolla* has been harnessed as a raw material for biodiesel production. *Azolla* species, with their rapid growth, unique chemical composition, and suitability for low-cost and low-energy biofuel production systems, offer a promising and sustainable solution to the increasing demand for cost-effective renewable biofuels.

Medicinal potential of *Azolla*

Increased concerns about the efficacy of antibiotic treatments due to bacterial resistance against antibiotics have prompted researchers to search for plant-based remedies. Several bioactive compounds discovered in medicinal plants have the ability to cure numerous diseases in humans. *Azolla,* an aquatic fern, is regarded as the most promising antibacterial choice because of its simple growth process, low water requirements, high yield, and significant phytochemical content. This plant is also a source of probiotics and biopolymers, and offers pharmacological benefits, functioning as an antioxidant, immune enhancer, and hepatoprotective agent. (Pillai et al. 2002). Historical and cultural references have highlighted the diverse uses of *Azolla* in traditional medicine. The use of *Azolla* for treating sore throat and cough in New Zealand has been reported (Pereira et al. 2015), while other researchers have noted its role as a traditional cough medicine in Tanzania (Wagner 1996). Shi and Hall, (1988) pointed out the medicinal properties of *Azolla*, although they did not specify the target diseases. Additionally, scientific studies have demonstrated the antimicrobial potential of different *Azolla* species; such as *A. filiculoides, A. caroliniana,* and *A. microphylla*, against various bacteria and fungi.

Azolla demonstrates efficacy in protecting the liver from hepatotoxic substances and possesses anti-inflammatory, antioxidant, and anti-apoptotic properties in its decoction, presenting a compelling solution for the prevention and treatment of severe hepatotoxicity (Asim et al. 2022). The anti-caries properties of *A. pinnata* and *A. rubra* against cariogenic bacteria were studied previously (Prashith 2014). Utilizing the agar well diffusion technique, *A. pinnata* and *A. rubra* were tested against oral isolates, particularly *Streptococcus mutans*. This study revealed that *A. pinnata* exhibited superior anti-caries activity compared to *A. rubra*. In recent years, *A. microphylla* has emerged as a key player in antimicrobial properties, demonstrating its effectiveness against a range of bacterial species and fungi. The antibacterial and antifungal potential of *A. microphylla* extracts has been reported (Sathammaipriya et al. 2018), particularly methanolic and ethanolic extracts, which showed activity against various bacteria and fungi (especially *Aspergillus* sp.). Additionally, the antimicrobial properties of *A. pinnata* extracts against *Salmonella typhi* have been highlighted (Arora and Kaur 2011), and its potential as a traditional remedy for inhibiting the growth of this bacterium has been demonstrated.

For instance, *A. filiculoides* exhibited lipophilic activity against *Penicillium expansum* and hydrophilic activity against *Agrobacterium vitis* and the crustacean *Artemia salina* (Piccardi et al. 2000). The methanolic extract of *A. microphylla* exhibits antimicrobial activity against *Xanthomonas* sp., a plant pathogen (Abraham 2013). These investigations have revealed the promising role of *Azolla* as a natural biocontrol agent. *Azolla* is emerging as a promising medicinal plant with both antibacterial and antifungal properties. Its rich bioactive compounds, ease of cultivation, and traditional uses make it an interesting candidate for further research and potential for applications in medicine and pharmacology. Nevertheless, the bioactivity of *Azolla* and its potential applications in medicine and pharmacology; as well as its prospective pathogenic targets; remain largely unexplored.

Environmental benefits of *Azolla* in water purification and phytoremediation

Azolla offers a promising path for environmental conservation along with its agricultural utility. Its rapid growth forms a dense water surface cover that; effectively shades the water beneath. This shading delays the growth of unwanted aquatic plants and algae causes, eutrophication and preserves the ecological balance of aquatic ecosystem. *Azolla* also acts as a natural water purifier, absorbing excess nutrients, heavy metals, and pollutants from the water, thereby enhancing water quality. Phytoextraction, a simple and cost-effective method that utilizes living plants, has been successfully applied to remediate wastewater containing heavy metals, hydrocarbons, and dyes. Furthermore, the water purification capabilities of *Azolla* were noteworthy. *Azolla* finds application in wastewater treatment by reducing nitrogen, phosphorus, heavy metals, Chemical Oxygen Demand (COD), and Biochemical Oxygen Demand (BOD). Notably, it generates a substantial amount of biomass during wastewater treatment. Additionally, *Azolla* has been used to combat eutrophication in lakes and streams (Jayasundara 2022). Its rapid growth, with a doubling time of 2-4 days, is attributed to pectin, a component of its cell wall that; functions as a biofilter in wastewater treatment. *Azolla* has shown its potential for phytoremediation and metal removal, as demonstrated by (Golzary et al. 2018, Soman and Arora 2018). *Azolla* is efficient in purifying ammonium and; phosphorus, and aggregating a wide range of toxic metals. It also contributes to water quality by reducing nitrate and phosphorus levels (Liu et al. 2017).

Phytoextraction has been used to remediate wastewater containing heavy metals (Ali et al. 2013), hydrocarbons (Agamuthu et al. 2010) and dyes (Torbati et al. 2014). Various plants have been studied for their phytoextraction potential, including water lettuce, *Tagetes patula*, sunflowers etc. *Azolla* is a candidate for the biological remediation of polluted water because of its ability to accumulate a range of heavy metals, such as arsenic, mercury, zinc, lead, and more (Valderrama et al. 2013). Its advantages over chemical methods in wastewater treatment include eco-friendliness and effectiveness. Arora et al. (2006) highlighted *Azolla*'s tolerance and phytoaccumulation of heavy metals such as Cu, Cd, Cr, Ni, and Pb from sewage water. Moreover, *Azolla* is a promising candidate for the phytoremediation of polluted waterways; because it effectively absorbs pollutants, heavy metals, and dyes (Sood et al. 2011). The role of *Azolla* as a metal hyperaccumulator was explored, and next generation sequencing was used to identify metal-tolerant strains isolated from *Azolla* (Banach et al. 2020). In general, *Azolla* demonstrates an excellent capacity to absorb heavy metals from aqueous solutions, particularly *A. filiculoides*, which absorbs high metal contents from nickel-rich solutions.

Azolla's phytoextraction potential for removing hazardous dyes, such as methylene blue, achieves high removal efficiency (Imron et al. 2019). *Azolla*'s dye absorbent properties, demonstrated its ability to significantly reduce dye concentrations (Israa 2018). The textile industry discharge consists of a large number of dyes or effluents, which contaminate rivers and other waterways. Various studies have demonstrated the ability of *Azolla* to remove water-soluble dyes such as Acid Red 88, Acid Green 3, Acid Orange 7, and Basic Orange, further emphasizing its potential as a sustainable solution (Tan et al. 2010).

Although much research has been conducted on *Azolla*'s effectiveness in treating wastewater, purifying water, and phytoextracting heavy metals, there is still a clear knowledge gap regarding the long-term environmental effects and scalability of *Azolla*-based remediation techniques in practical settings. Furthermore, additional research is required to evaluate any potential trade-offs or synergies between *Azolla*'s function in environmental conservation and its agricultural value, as well as to create comprehensive methods for widespread use in a variety of environments.

***Azolla* as a natural larvicide for mosquito control**

Throughout the twentieth century, *Azolla*, often referred to as the "mosquito fern" held the promise of preventing mosquito breeding and, consequently, the transmission of diseases like malaria. Researchers conducted controlled experiments, revealing that *Azolla* covering up to two-thirds of the water surface could effectively limit *Culex* mosquito oviposition (Mwingira et al. 2009). Although it did not completely prevent *Anopheles sinensis* from ovipositing, it restrained the emergence of adult mosquitoes. Subsequent field tests further supported these findings, demonstrating that when *Azolla* covered 75% of the water surface, larval density decreased significantly (Baolin 1987). Some researchers have investigated *Azolla*'s larvicidal potential against mosquito larvae and established its effectiveness in reducing their population (Ravi et al. 2018). This study underscores *Azolla*'s role as a natural and eco-friendly method for mosquito control. Furthermore, additional research is required to evaluate the scalability of *Azolla*-based mosquito prevention strategies in various ecological and geographical contexts as well as to optimize application techniques.

Azolla: A promising biogas source for sustainable energy

Azolla, has proven to be a valuable resource for both biogas and biofertilizer production due to its high nutrient content. When *A. pinnata* is combined with cow dung, biogas production increases significantly, reaching 1.4 times the output compared to using cow dung alone (Sarkar et al. 2023). This synergy takes advantage of the organic matter of *Azolla* and its compatibility with anaerobic digestion. Various researchers have explored the potential of *Azolla* as a feedstock for biogas production (Sathammaipriya et al. 2018). The organic matter can be efficiently converted into biogas through anaerobic digestion, which is a renewable and sustainable energy source. This is not only an eco-friendly alternative to fossil fuels, but also contributes to the reduction of greenhouse gas emissions. Further exploration is necessary to boost bioenergy production as there is a research gap in the understanding of the precise aspects impacting the optimisation of this synergistic strategy.

CONCLUSIONS

Azolla emerges as a multifaceted and invaluable resource with a myriad of benefits across industrial, medical, and agricultural domains. Its diverse applications, ranging from serving as a biofertilizer in Southeast Asian rice fields to potential uses as livestock feed, biofuel production, biogas generation, and water purification, underscore its versatility and significance. The remarkable productivity of *Azolla*, coupled with its symbiotic nitrogen-fixing abilities, contributes not only to agricultural sustainability but also addresses environmental concerns by mitigating atmospheric CO_2 levels. Furthermore, its role as a bio-larvicide, antimicrobial agent, and enhancer of soil microbial diversity adds depth to its potential contributions in medical and ecological contexts. Its significance extends beyond its size, with promising implications for addressing global challenges. Further exploration and utilization of *Azolla* in various fields can unleash its full potential, and contribute to innovative solutions and sustainable practices that benefit both human society and the environment.

ACKNOWLEDGEMENTS

The authors express their gratitude to the Head of the institution and the entire staffs of Government Arts and Science College, Kozhinjampara, Palakkad, Kerala, for their unwavering assistance and support during the entire preparation of this review.

Authors' contributions: Corresponding author contributed significantly to the conception, design, as well as drafting the manuscript. Second author provided final approval for publication and drafting manuscript. Third author contributed to drafting the manuscript and provided critical revisions for important intellectual content. Fourth author assisted in drafting the manuscript and organizing its content. Fifth author contributed to drafting and grammar checking. All authors have read and approved the final version of the manuscript.

Conflict of interest: The authors declare no conflict of interest.

REFERENCES

Abraham, G. 2013. Evaluation of antimicrobial activity of methanolic extracts of *Azolla microphylla*. Vegetos, 26(1), 200-204. https://doi.org/10.5958/J.2229-4473.26.1.029

Adams, D.G., Duggan, P.S. and Jackson, O. 2012. Cyanobacterial symbioses. Pp. 593–564. In: Whitton, B.A. (Ed.). Ecology of Cyanobacteria II: Their diversity in space and time. Springer science business media, BV.

Agamuthu, P., Abioye, O.P. and Aziz, A.A. 2010. Phytoremediation of soil contaminated with used lubricating oil using *Jatropha curcas*. Journal of Hazardous Materials, 179(1-3), 891-894. https://doi.org/10.1016/j.jhazmat.2010.03.088

Ahmed, H.A., Ganai, A.M. and Beigh, Y.A. 2016. Performance of growing sheep on *Azolla* based diets. Indian Journal of Animal Research, 50, 721-724. https://doi.org/10.18805/ijar.9642.

Ali, H., Khan, E. and Sajad, M.A. 2013. Phytoremediation of heavy metals - Concepts and applications. Chemosphere, 91(7), 869-881. https://doi.org/10.1016/j.chemosphere.2013.01.075.

Ammar, A.A.A. and Taha, N.T. 2020. Effect of use of the *Azolla* plant instead partial for soybean meal in the ration of common carp fish *Cyprinus carpio* L. in some production standards. Plant Archives, 20(2), 5177-5185. https://doi.org/10.13140/RG.2.2.32337.35686

Anonymous. 1985. Propagation and agricultural use of *Azolla* in Vietnam. FAO-AGPIRC/85/7(bl). FAO, Rome, Italy. 16 pages.

Arora, A., Sexana, S. and Sharma, D.K. 2006. Tolerance of phyto-accumulation of chromium by three *Azolla* species. World Journal of Microbiology and Biotechnology, 22, 97-100. https://doi.org/10.1007/s11274-005-9000-9.

Arora, M. and Kaur, A. 2019. *Azolla pinnata, Aspergillus terreus* and *Eisenia fetida* for enhancing agronomic value of paddy straw. Scientific Reports, 9(1), 1341. https://doi.org/10.1038/s41598-018-37880-1.

Asim, R., Khan, M.S., Saeed, M., Kamboh, A.A., Khan, R.U., Farooq, Z., Imran, S., Farid, M.U. and Farid, M.U. 2022. Importance of *Azolla* plant in poultry production. World's Poultry Science Journal, 78(3), 789-802. https://doi.org/10.1080/00439339.2022.2054752.

Azolla Foundation. 2014. The *Azolla* Foundation. Retrieved from http://theazollafoundation.org/azolla/azollas-use-in-the-east/.

Banach, A.M., Kuzniar, A., Grz¹dziel, J. and Wolinska, A. 2020. *Azolla filiculoides* L. as a source of metal-tolerant microorganisms. PLoS ONE, 15(5), e0232699. https://doi.org/10.1371/journal.pone.0232699.

Baolin, L. 1987. Environmental management for the control of rice field-breeding mosquitoes in China. Pp. 111–121. In: Vector-borne disease control in humans through rice agroecosystem management. Proceedings of the research and training needs in the field of integrated vector-borne disease control in rice land agroecosystems of developing countries. March 9–14, (ed. IRR Institute). International Rice Research Institute in collaboration with the WHO/FAO/UNEP Panel of Experts on Environmental Management for Vector Control, Manila, Philippines.

Bottomley, B. 1920. The effect of organic matter on the growth of various plants in culture solutions. Annals of Botany, 39, 353-365.

Brouwer, P., Brautigam, A., Kulahoglu, C., Tazelaar, A.O.E., Kurz, S., Nierop, K.G.J., van der Werf, A., Weber, A.P.M. and Schluepmann, H. 2014. *Azolla* domestication towards a biobased economy. New Phytologist, 202(3), 1069-1082. https://doi.org/10.1111/nph.12708.

Brouwer, P., Schluepmann, H., Nierop, K.G., Elderson, J., Bijl, P.K., van der Meer, I., de Visser., W., Reichart, G.J., Smeekens, S. and van der Werf, A. 2018. Growing *Azolla* to produce sustainable protein feed: the effect of differing species and CO_2 concentrations on biomass productivity and chemical composition. Journal of the Science of Food and Agriculture, 98(12), 4759-4768. https://doi.org/10.1002/jsfa.9016.

Carrapico, F. 2010. *Azolla* as a superorganism. Its implication in symbiotic studies. pp. 225-242. In: Symbioses and Stress. Seckbach, J. and Grube, M. (Eds) Symbioses and Stress: Cellular Origin, Life in Extreme Habitats and Astrobiology, vol 17. Springer, Dordrecht.https://doi.org/10.1007/978-90-481-9449-0_11.

Chichilichi, B., Mohanty, G.P., Mishra, S.K., Pradhan, C.R., Behura, N.C., Das, A. and Behera, K. 2015. Effect of partial supplementation of sun-dried *Azolla* as a protein source on the immunity and antioxidant status of commercial broilers. Veterinary World, 8(9), 1126-1130. https://doi.org/10.14202/vetworld.2015.1126-1130.

Das, M., Rahim, F.I. and Hossain, M.A. 2018. Evaluation of fresh *Azolla pinnata* as a low-cost supplemental feed for Thai silver Barb *Barbonymus gonionotus*. Fishes, 3(1), 15. https://doi.org/10.3390/fishes3010015

Dijkhuizen, L.W., Brouwer, P., Bolhuis, H., Reichart, G.J., Koppers, N., Huettel, B., Bolger, A.M., Li, F.W., Cheng, S., Liu, X. and Wong, G.K.S. 2021. Red light-induced *Azolla filiculoides* symbiosis sexual reproduction: Responsive transcripts of symbiont *Nostoc azollae* encode transporters whilst those of the fern relate to the angiosperm floral transition. Frontiers in Plant Science, 12, 693039. https://doi.org/10.3389/fpls.2021.693039.

Ekawati, E.R. and Pradana, M.S. 2019. The effectiveness of *Azolla pinnata* in inhibiting the growth of *Salmonella typhi*. Jurnal Biota, (1), 1-5. https://doi.org/10.19109/Biota.v5i1.2696.

El-Daim, A., Asely, M., Kandiel, A., El-Gawad, H., Elabd, A.S. and Abbass, A. 2021. Effect of *Spirulina platensis* and *Azolla nilotica* as feed additives on growth performance, antioxidant enzymes and fecundity of *Oreochromis niloticus*. Iranian Journal of Fisheries Sciences, 20(3), 846-862. http://www.agrijournals.ir/article_124136.html

Evrard, C. and van Hove, C. 2004. Taxonomy of the American *Azolla* species (Azollaceae): A critical review. Systematics and Geography of Plants, 74, 301-318. https://doi.org/10.2307/3668500.

Gates, J.E., Fisher, E.W. and Candler, R.A. 1980. The occurrence of the *Coryneform* bacteria in the leaf cavity of *Azolla*. Archives of Microbiology, 127, 163-165. https://doi.org/10.1007/BF00428020.

Golzary, A., Tavakoli, O., Rezaei, Y. and Karbassi, A. 2018. Wastewater treatment by *Azolla filiculoides*: A study on color, odor, COD, nitrate, and phosphate removal. Pollution, 4 (1), 69-79. https://doi.org/ 10.22059/POLL.2017.236692.290.

Herath, B.M., Yapa, N. and Karunarathna, S. 2023. *Azolla* as the multifunctional fern in organic agriculture: Prospects and challenges. International Journal of Agricultural

Technology, 19, 63-82.

Hossain, M.B., Mian, M.H., Hashem, M.A., Islam, M.Z. and Shamsuddoha, A.T.M. 2001. Use of *Azolla* as biofertilizer for cultivation of BR26 Rice in Aus season. Journal of Biological Sciences, 1(12), 1120-1123. https://doi.org/10.3923/jbs.2001.1120.1123

Imron, M.F., Kurniawan, S.B., Soegianto, A. and Wahyudianto, F.E. 2019. Phytoremediation of methylene blue using duckweed (*Lemna minor*). Heliyon, 5(8), e02206. https://doi.org/10.1016/j.heliyon.2019.e02206.

Indira, D., Rao, K.S. and Suresh, J. 2009. *Azolla* (*Azolla pinnata*) as feed supplement in buffalo calves on growth performance. Indian Journal of Animal Nutrition, 26, 345-348.

Israa, A.A.B. 2018. Removal of 1,2-Dichloroethane from real industrial wastewater using a sub-surface batch system with *Typha angustifolia* L. Ecotoxicology and Environmental Safety, 147, 260-265. https://doi.org/10.1016/j.ecoenv.2017.08.022

Jayasundara, P. 2022. Wastewater treatment by *Azolla*: A review. Diyala Agricultural Sciences Journal, 14(1), 40-46. https://doi.org/10.52951/dasj.22140105.

Korsa, G., Alemu, D. and Ayele, A. 2024. *Azolla* plant production and their potential applications. International Journal of Agronomy, 12. https://doi.org/10.1155/2024/1716440.

Kumar, A.S., Murugesan, S.V.M. and Balamurugan, P. 2020. Feeding of *Azolla* as a green fodder feed supplement on productive performance and milk composition of crossbred dairy cows in Theni District of Tamil Nadu, India. International Journal of Current Microbiology and Applied Science, 9(6), 1388-1392. https://doi.org/10.20546/ijcmas.2020.906.171

Liu, J., Xu, H., Jiang, Y., Zhang, K., Hu, Y. and Zeng, Z. 2017. Methane emissions and microbial communities as influenced by dual cropping of *Azolla* along with early rice. Scientific Reports, 7, 40635. https://doi.org/10.1038/srep40635.

Liu, X., Min, C., Xia-Shi, L. and Chungchu, L. 2008. Research on some functions of *Azolla* in CELSS system. Acta Astronautica, 63(7), 1061-1066. https://doi.org/10.1016/j.actaastro.2008.03.001.

Lu, X.M. and Lu, P.Z. 2018. Response of microbial communities to pesticide residues in soil restored with *Azolla imbricata*. Applied Microbiology and Biotechnology, 102(1), 475-484. https://doi.org/10.1007/s00253-017-8596-7.

Lumpkin, T.A. and Plucknett, D.L. 1982. *Azolla* as a green manure: Use and Management. Westview Press, USA. 230 pages.

Lumsangkul, C., Vu Linh, N.V., Chaiwan, F., Abdel-Tawwab, M., Dawood, M.A.O., Faggio, C., Jaturasitha, S. and van Doan, H. 2022. Dietary treatment of Nile tilapia (*Oreochromis niloticus*) with aquatic fern (*Azolla caroliniana*) improves growth performance, immunological response, and disease resistance against *Streptococcus agalactiae* cultured in bio-floc system. Aquaculture Reports, 24, 101114. https://doi.org/10.1016/j.aqrep.2022.101114.

Macale, M.A.R. and Vlek, P.L.G.2004. The role of *Azolla* cover in improving the nitrogen use efficiency of lowland rice. Plant and Soil, 263(1), 311-321. https://doi.org/10.1023/B:PLSO.0000047742.67467.50.

Marzouk, S.H., Tindwa, H.J., Amuri, N.A. and Semoka, J.M. 2023. An overview of underutilized benefits derived from *Azolla* as a promising biofertilizer in lowland rice production. Heliyon, 9(1), e13040. https://doi.org/10.1016/j.heliyon.2023.e13040.

Mathur, G.N., Ramakant, S. and Choudhary, P.C. 2013. Use of *Azolla* (*Azolla pinnata*) as cattle feed supplement. Journal of Krishi Vigyan, 2(1),7375. https://www.indianjournals.com/ijor.aspx?target=ijor:jkv&volume=2&issue=1&article=018&type=pdf

Miranda, A.F., Liu, Z.Q., Rochfort, S. and Mouradov, A. 2018. Lipid production in aquatic plant *Azolla* at vegetative and reproductive stages and in response to abiotic stress. Plant Physiology and Biochemistry. 124. https://doi.org/10.1016/j.plaphy.2018.01.012.

Miranda, A.F., Biswas, B., Ramkumar, N., Singh, R., Kumar, J., James, A., Roddick, F., Lal, B., Subudhi, S., Bhaskar, T. and Mouradov, A. 2016. Aquatic plant *Azolla* as the universal feedstock for biofuel production. Biotechnology for Biofuels, 9(9), 221. https://doi.org/10.1186/s13068-016-0628-5.

Mosha, S.S. 2018. A review on significance of *Azolla* meal as a protein plant source in finfish culture. Journal of Aquaculture Research and Development, 9(7), 544. https://doi.org/10.4172/2155-9546.1000544.

Mwingira, V.S., Mayala, B., Senkoro, K., Rumisha, S., Susan, F.R., Shayo, H.E., Mlozi, M.R.S. and Mboera, L. 2009. Mosquito larval productivity in rice-fields infested with *Azolla* in Mvomero District, Tanzania. Tanzania Journal of Health Research, 11, 17-22. https://doi.org/10.4314/thrb.v11i1.43246.

Nagalingum, N.S., Schneider, H. and Pryer, K.M. 2006. Comparative morphology of reproductive structures in heterosporous water ferns and a re-evaluation of the sporocarp. International Journal of Plant Science, 167, 805-815. https://doi.org/10.1086/503848.

Newton, J.W. and Herman, A.I. 1979. Isolation of cyanobacteria from the aquatic fern, *Azolla*. Archives of Microbiology, 120 (2), 161-165. https://doi.org/10.1007/BF00409103.

Parthasarathy, R., Kadirvel, R. and Kathaperumal, V. 2002. *Azolla* as a partial replacement for fish meal in broiler rations. Indian Veterinary Journal, 79(2), 144-146.

Pereira, A. L., Lucinda, J. B., Pedro, L., Vasconcelos, V. and Martins da Costa P. 2015. Bioactivity of *Azolla* aqueous and organic extracts against bacteria and fungi. Symbiosis, 65, 10. https://doi.org/10.1007/s13199-015-0316-4.

Peters, G. and Meeks, J. 1989. The *Azolla-anabaena* symbiosis: basic biology. Annual Review of Plant Physiology and Plant Molecular Biology, 40(1), 193-210. https://doi.org/10.1146/annurev.pp.40.060189.001205.

Peters, G.A., Toia, R.E., Jr Raveed, D. and Levine, N.J. 1978. The *Azolla-Anabaena azollae* relationship. VI.

Morphological aspects of the association. New Phytologist, 80(3), 583-593. https://doi.org/10.1111/j.1469-8137.1978.tb01591.x.

Petro, M.J. and Gates, J.E. 1987. Distribution of *Arthrobacter* sp. in the leaf cavities of four species of 29 the N-fixing *Azolla* fern. Symbiosis, 3, 41-48.

Piccardi, R., Frosini, A., Tredici, M.R. and Margheri, M.C. 2000. Bioactivity in free-living and symbiotic cyanobacteria of the genus *Nostoc*. Journal of Applied Phycology, 12(3/5), 543-547. https://doi.org/10.1023/A:1008106715148

Pillai, K.P., Premalatha, S. and Rajamony, S. 2002. *Azolla* – A sustainable feed substitute for livestock. Leisa India, 4, 15-17.

Prashith, K.T.R. 2014. Anticaries activity of *Azolla pinnata* and *Azolla rubra*. Science. Technology and Arts Research Journal, 3(3), 119121. https://doi.org/10.4314/star.v3i3.21.

Pratte, B.S. and Thiel, T. 2021. Comparative genomic insights into culturable symbiotic cyanobacteria from the water fern *Azolla*. Microbial Genomes, 7(6), 000595. https://doi.org/10.1099/mgen.0.000595.

Rahmah, S., Nasrah, U., Lim, L.S., Ishak, S.D., Rozaini, M.Z.H. and Liew, H.J. 2022. Aquaculture wastewater-raised *Azolla* as partial alternative dietary protein for Pangasius catfish. Environmental Research, 208, 112718. https://doi.org/10.1016/j.envres.2022.112718.

Raja, W., Rathaur, P., John, S.A. and Ramteke, P.W. 2012. *Azolla-Anabaena* association and its significance in supportable agriculture. Journal Biology and Chemistry, 40(1), 1-6. https://dergipark.org.tr/en/pub/hjbc/issue/61880/926016

Ran, L., Larsson, J., Vigil-Stenman, T., Nylander, J.A., Ininbergs, K., Zheng, W.W., Lapidus, A., Lowry, S., Haselkorn, R. and Bergman, B. 2010. Genome erosion in a nitrogen-fixing vertically transmitted endosymbiotic multicellular cyanobacterium. PLoS ONE, 5(7), e11486. https://doi.org/10.1371/journal.pone.0011486

Ravi, R., Zulkrnin, N.S.H., Rozhan, N.N., Nik Yusoff, N.R., Mat Rasat, M.S. and Ahmad, M.I. 2018. Chemical composition and larvicidal activities of *Azolla pinnata* extracts against *Aedes* (Diptera:Culicidae). PLoS ONE, 13(11), e0206982. https://doi.org/10.1371/journal.pone.0206982.

Refaey, M.M., Mehrim, A.I., Zenhom, O.A., Areda, H.A., Ragaza, J.A. and Hassaan, M.S. 2023. Fresh Azolla, *Azolla pinnata* as a Complementary Feed for *Oreochromis niloticus*: Growth, digestive Enzymes, intestinal Morphology, Physiological Responses, and Flesh Quality. Aquaculture Nutrition, 14(03), 704. https://doi.org/10.1155/2023/1403704.

Sarkar, A., Bhakta, J.N. and Bhakta, B.O.K. 2023. Evaluating growth-dependent enhanced carbon dioxide sequestration potential of *Azolla pinnata* using cattle wastes (cow dung and cow urine). Heliyon, 9(3), e14610. https://doi.org/10.1016/j.heliyon.2023.e14610.

Sathammaipriya, N., Thamilmaraiselvi, B., Steffi, P.F. and Sangeetha, K. 2018. Investigation of phytochemical constituents in *Azolla microphylla* for antibacterial activity. National Journal of Physiology, Pharmacy and Pharmacology, 8(9), 1500-1504. https://doi.org/10.5455/njppp.2018.8.0310430072018.

Sathish, S.S., Supriya, P., Andal, D., Prabu, J., Aravind Kumar, M., Rajasimman, Sabah, A. and Shahabaldin, R. 2022. Effective utilization of *Azolla filiculoides* for biodiesel generation using graphene oxide nano catalyst derived from agro-waste. Fuel, 329. 125412.https://doi.org/10.1016/j.fuel.2022.125412.

Shi, D.J. and Hall, D.O. 1988. The *Azolla-Anabaena* association: Historical perspective, symbiosis and energy metabolism. Botanical Review, 54(4), 353-386. https://doi.org/10.1007/BF02858416.

Shukla, M., Bhattacharyya, A., Shukla, P.K., Roy, D., Yadav, B. and Sirohi, R.2018. Effect of *Azolla* feeding on the growth, feed conversion ratio, blood biochemical attributes and immune competence traits of growing turkeys. Veterinary World, 11(4), 459-463. https://doi.org/10.14202/vetworld.2018.459-463.

Sihag, S., Sihag, S.Z., Kumar, S. and Singh, N. 2018. Eect of feeding *Azolla (Azolla pinnata*) based total mixed ration on growth performance and nutrients utilization in goats. Forage Research, 43, 314-318.

Si-Ping, Z., Bin, C., Xiong, G. and Wei-Wen, Z. 2008. Diversity analysis of endophytic bacteria within *Azolla microphylla* using PCR-DGGE and electron microscopy. Chinese Journal of Agricultural Biotechnology, 5(3), 269-276. https://doi.org/10.1017/S1479236208002441.

Soman, D.V.A. and Arora, A. 2018. Bioremediation of municipal sewage water with *Azolla microphylla*. International Journal of Advanced Research, 6(5), 101-108. https://doi.org/10.21474/IJAR01/7012.

Sood, A., Uniyal, P.L., Prasanna, R. and Ahluwalia, A. S. 2011. Phytoremediation potential of Aquatic Macrophyte, Azolla. *Ambio*, 41, 122-137. https://doi.org/10.1007/s13280-011-0159-z.

Speelman, E.N., Van Kempen, M.M.L., Barke, J., Brinkhuis, H., Reichart, G.J., Smolders, A.J.P., Roelofs, J.G.M., Sangiorgi, De Leeuw, J.W., Lotter, A.F. and Sinninghe Damste, J.S. 2009. The Eocene Arctic *Azolla* bloom: Environmental conditions, productivity and carbon draw down. Geobiology, 7(2), 155170. https://doi.org/10.1111/j.14724669.2009.00195.x.

Sudaryono, A. 2006. Use of *Azolla* (*Azolla pinnata*) meal as a substitute for defatted soybean meal in diets of juvenile black tiger shrimp (*Penaeus monodon*). Journal of Coastal Development, 9, 145-154. http://eprints.undip.ac.id/473/1/use_of_azolla_meal.pdf

Swain, B.K., Naik, P.K. and Beura, C.K. 2022. Nutritive value of *Azolla* as poultry feed - A review. Indian Journal of Animal Nutrition, 39, 1-11. https://doi.org/10.5958/2231-6744.2022.00001.9.

Tamang, Y. and Samanta, G. 1995. Feeding value of *Azolla* (*Azolla pinnata*) in goats. Annales de Zootechnie, 44(sup 1), 62. https://doi.org/10.1051/animres:19950532

Tan, C.Y., Li, G., Lu, X.Q. and Chen, Z.L. 2010. Biosorption of basic orange using dried *A. filiculoides*. Ecological Engineering, 36(10), 1333-1340. https://doi.org/10.1016/

j.ecoleng.2010.06.009.

Torbati, S., Khataee, A.R. and Movafeghi, A. 2014. Application of watercress (*Nasturtium officinale* R. Br.) for biotreatment of a textile dye: Investigation of some physiological responses and effects of operational parameters. Chemical Engineering Research and Design, 92(10), 1934-1941. https://doi.org/10.1016/j.cherd.2014.04.022.

Tung, H.F.T.C. and Shen, T.C. 1985. Studies of the *Azolla pinnata* - *Anabaena azollae* symbiosis: Concurrent growth of *Azolla* with rice. Aquatic Botany, 22(2), 145-152. https://doi.org/10.1016/0304-3770(85)90043-9.

Valderrama, A., Tapia, J., Penailillo, P. and Carvajal, D.E. 2013. Water phytoremediation of cadmium and copper using *Azolla filiculoides* Lam. in a hydroponic system. Water and Environment Journal, 27(3), 293-300. https://doi.org/10.1111/wej.12015.

van Hove, C. 1989. *Azolla* and its multiple uses with emphasis on Africa. Rome, Italy: Food and Agriculture Organization, Rome, Italy. 59 pages.

Varun S.T., Prasad, R.M.V., Bindu Madhuri, S., Jayalaxmi, P. and Shashi Kumar, M. 2021. Effect of *Azolla* supplementation on the carcass characteristics of Deccani Ram Lambs reared under grazing based production system. International Journal of Livestock Research, 11(2), 78-84.

Verma, G., Prakriti, K.A. and Babu, S. 2022. Implications and future prospects of *Azolla* as a low-cost organic input in agriculture. Agriculture, 1(6), 1-7.

Wagner, G.M. 1996. The *Utricularia-Cyanophyta* and *Azolla* - *Anabaena* associations: Their ecology, N fixation rates, and effects as biofertilizers on rice. Ph.D. thesis, University of Dar es Salaam, Dar et Salaam, Tanzania.

Yadav, R.K., Abraham, G., Singh, Y.V. and Singh, P.K. 2014. Advancement in the utilization of *Azolla-Anabaena* system in relation to sustainable agricultural practices. Proceedings of the Indian National Science Academy, 2(2), 301-316. https://doi.org/10.16943/ptinsa/2014/v80i2/55108.

Yang, Y.Q., Deng, S.F., Yang, Y.Q. and Ying, Z.Y. 2022. Comparative analysis of the endophytic bacteria inhabiting the phyllosphere of aquatic fern *Azolla* species by high-throughput sequencing. BMC Microbiology, 22(1), 246. https://doi.org/10.1186/s12866-022-02639-2.

Received: 20th April 2024
Accepted: 27th May 2024