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Cover Page Footnote

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REVIEW

Harnessing Nature's Power of *Azolla Pinnata* Superfood With Biofertilizers for a Sustainable Future

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Abstract

Azolla pinnata, a rapidly growing floating aquatic fern, flourishes in freshwater bodies throughout India. It forms clusters in marshes, drains, and rice fields, doubling its biomass every 1.9 days. Azolla, known for its versatility, is used in food, medicinal, water purification, biofertilizers, and livestock feed. Its use in animal diets improves live weight, protein efficiency, and feed conversion rates. Azolla has pharmaceutical benefits in addition to agricultural, including antioxidant and immunological stimulation, as well as hepatoprotective properties. It also plays an important part in phytoremediation and bioremediation. Azolla's potential extends to biofuel production, making it a promising feedstock for sustainable bioenergy.

Keywords: *Azolla pinnata*, Heterosporous, Biofertilizer, Livestock feed

1. Introduction

Azolla pinnata is an aquatic fern that floats on the water's surface. It is also known by many different names in many different languages and countries. These names include Carolina fern, Duck fern, Duckweed fern, Fairy fern, Fairy moss, feathered water fern, Mosquito fern, Pacific mosquito fern, red water fern, and Water Velvet. This species is found throughout much of Africa, Asia, and parts of Australia. [1]. Azolla was named in 1783 by French scientist Jean-Baptiste Lamarck [2]. It's a little aquatic plant that floats on the water's surface, much like Lenticules (Lemna). The name is traditionally derived from a conjugation of two Greek words, azo (to dry) and allyo (to kill), because the fern is killed by drought, but Carrapico (2010) proposes that the name is derived from a local population word in Argentina or Chile, where Commerson collected his azolla specimens [3]. Knowing the local usage, Lamarck used a Greek nomenclatural base to construct a name that sounded comparable. For generations, wetland rice in northern Vietnam and central to southern China has benefited from the high nitrogen content and symbiotic nitrogen fixation of azolla. Early in the 1980s, they had reached South Cotabato in the Philippines [4].

The Indian species *A.pinnata* has a stem that is usually triangular in shape and can grow up to 2.5 cm long and 1−2 cm wide. Numerous 1 or 1 mm-long, overlapping, rounded or angular leaves cover the stem. Tiny leaves, measuring 1−2 mm in length and overlapping in two ranks, have translucent brown lower lobes and green, brown, green, or

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reddish upper lobes [5]. When the plant is fertile, round, 1–1.5 mm wide sporocarps are visible at the bases of the side branches. The upper surface of the leaves is completely water-repellent, with tiny hairs giving them a velvety appearance. This allows the plants to quickly refloat with their right side up if completely submerged. Azolla is special because, although it doesn't need soil to grow, it is among the planet's fastest growing plants [6]. Thus, cyanobacteria called *Anabaena azollae,* which live in cavities on the underside of the upper (dorsal) lobes of leaves, can fix nitrogen from the atmosphere, azolla is beneficial as a fertilizer for rice crops [7].

There are many types of azolla namely *Azolla pinnata*, *Azolla Caroliniana, Azolla rubra, Azolla filiculoides, Azolla nilotica, Azolla Mexicana and Azolla microphylla* as shown in Fig. 1. Out of these all *A.pinnata* is native to India very common and provides good yield in tropical climates, *A.pinnata* chemical composition are present in Table 1. *A.pinnata* classified into distinct categories with different color, common name properties and its uses details are present in Table 2.

2. Application of azolla

Azolla is one of the organic resources with little effort and expense, can be utilized in a variety of ways for economic and environmental advantages in agriculture, livestock, fuels, medical and food as shown in Fig. 2.

Table 1. Chemical composition of the azolla [8].

Sl: no	Constituents	% DM
1	Acid insoluble ash (AIA)	7.94
2	Total ash (TA)	25.50
3	Nitrogen free extract (NFE)	38.61
4	Ether extract (EE)	2.45
5	Crude fiber (CF)	11.19
6	Crude protein (CP)	22.25
7	Organic matter (OM)	74.50
8	Dry matter (DM)	91.78

2.1. Azolla in agriculture

Modern organic farming is finding new opportunities for the growing demand of organic foods [9]. According to Baroudy et al. (2020), rice (*Oryza sativa L.*), the second most significant cereal crop in the world (behind wheat), is used as a source of nourishment for 50% of the world's population [10]. Azolla is a green nitrogen fertilizer that has been used for thousands of years to increase rice production. It is used as a biofertilizer in agricultural fields, it significantly increases the amount of nitrogen fertilizer used to grow rice. The nitrogen fixation process is carried out by the symbiotic cyanobacterium Anabaena Azolla, which raises the

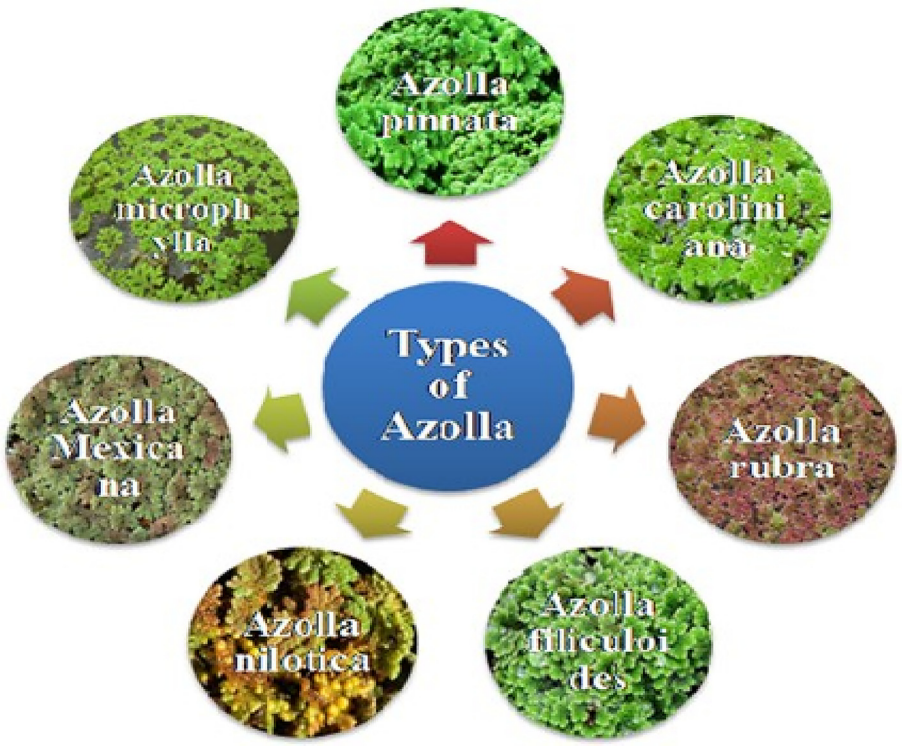

Fig. 1. Types of azolla.

Table 2. Fundamental characteristics of Azolla's properties and its importance.

Sl: no	Types	Common name	Scientific Classification	Color	Properties	Uses
1.	***A.pinnata***	water velvet, mosquito fern, and feathered mosquito fern	Scientific name: Azolla Family: Salviniaceae Kingdom: Plantae Division: Polypodiophyta Clade: Tracheophytes Order: Salviniales	Green, blue-green, or dark red.	➢ A stem that is no longer than 2.5-centimetres ➢ It is coated with tiny hairs that give them a velvety appearance.	➢ It is used as a substrate for growing edible mushrooms. ➢ It is also used as biomass in small-scale biogas production units. ➢ Azolla was used in a variety of dishes for human consumption, including salads, pinangat, mungo, and omelette.
2.	***A. caroliniana***	Water Fern, Carolina Mosquito fern, Eastern mosquito fern	Family: Salviniaceae Species: Azolla caroliniana Growth Habit: Herb Duration: Annual Category: Vascular	Greenish or reddish color.	➢ Leaves are bright or grey green and can turn rusty red, especially if they are grown in direct sunlight. ➢ It quickly forms a mat over surface water, making it difficult for mosquito's to lay their eggs, hence the common name “mosquito fern."	➢ It is used in rice paddies to fertilise the soil and also used as animal feed. ➢ It has been used to reduce pollution and it is currently being researched as a biofuel.
3.	***A. rubra***	Pacific azolla, Red azolla	Kingdom: Plantae Clade: Tracheophytes Division: Polypodiophyta class: polypodiopsida Order: Salviniales Family: Salviniaceae Genus: Azolla Species: A.rubra	Bright red or green.	➢ The branching is erratic, and the roots are peg-like and unbranched. ➢ Triangular leaves with rounded apexes, membranous.	➢ Azolla plants are able to fix atmospheric nitrogen because they contain cyanobacteria. ➢ The Australian frog species Litoria raniformis and Litoria aurea eat Azolla as a food source for their tadpoles
4.	***A. filiculoides***	Red water fern, water fern, mosquito fern	Scientific name: *Azolla filiculoides* Rank: Species Clade: Tracheophytes Division: Polypodiophyta Family: Salviniaceae	Green with a hint of pink orange red around the edges.	➢ Individual plants range in size from 1-2 centimetre across. ➢ Due to its low cold tolerance. It is largely dies back in the winter, in temperate climates only managing to survive through submerged buds.	➢ It is removed from lakes and used as green manure. ➢ It is also used as an ornamental plant in ponds.
5.	***A. nilotica***		Kingdom: Plantae Clade: Tracheophytes Division: Polypodiophyta Class: Polypodiopsida Order: Salviniales Family: Salviniaceae Genus: Azolla Species: Azolla nilotica	Dark green to reddish carpet.	➢ Azolla nilotica is a floating water-bound fern that can reach a length of 32 centimetre long. ➢ The root of Azolla nilotica's roots are also in bundles, whereas the root of all other species is single.	➢ This plant is used as green fertilizer due to its high nitrate content like other Azolla species.

6.	*A. Mexicana*	Mexican Waterfern, Floating Fern	Kingdom: Plantae Clade: Tracheophytes Division: Polypodiophyta Class: Polypodiopsida Order: Salviniales Family: Salviniaceae Genus: Azolla Species: Azolla Mexicana	Blue green to dark red leaves.	➢ Plants are green or frequently blue green to dark red, with some red-fringed leaves. ➢ There are two lobes on the leaf: a translucent cup-shaped lobe at the bottom and an upper green or red photosynthetic lobe with a colourless border.	➢ The name "Mosquito fern" is said to derive from the idea that this plant creates a surface layer of water so densely covered that mosquitoes cannot breed there. ➢ It is widely used in southeast Asia and has been introduced for agricultural and horticultural purposes.
7.	*A. microphylla*	Mexican Mosquito Fern	Kingdom: Plantae Phylum: Tracheophyta Class: Polypodiopsida Order: Salviniales Family: Azollaceae Genus: Azolla Species: Azolla Sub-Species: microphylla		➢ Plants should range from dark red to blue-green or green. ➢ Around 1 to 1.3 centimetre long prostrate stem with 1 centimetre internodes. Leaf the upper leaf lobes have noticeable papillae.	➢ Azolla is used to improve soil fertility and rice yield as a biofertilizer or green manure. ➢ Azolla microphylla is a large, rapidly growing aquatic plant valued for its agronomic, nutritive, and medicinal applications.

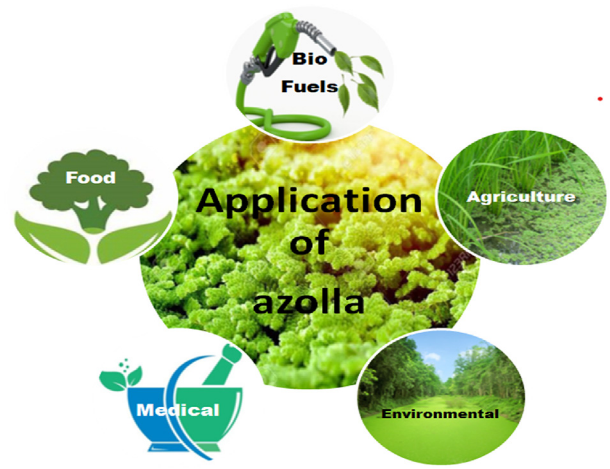

Fig. 2. Application of azolla.

soil's fertility and thus improves yield [11]. Azolla was introduced to agricultural fields. It covers the water surface, reduces light penetration through the soil, and inhibits the germination of weeds. Furthermore, it has been found that using Azolla promotes the growth of micro flora by increasing soil nutrient availability through their biological activity [12]. Importance of azolla in agriculture field shown in Fig. 3.

2.2. Environmental application

Environmental pollution is increasing as cities and industries grow toxic wastes generated by industrial units, automobile engines, and domestic operators are primarily disposed of in components of our environment such as soil, air, and water. The accumulation of such toxic water essentially into the soil and water eventually enters the food chain and causes health hazards [13]. When the accumulation of these toxic substances in water and soil exceeds allowable levels, applications or remediation strategies become necessary. Waste treatment methods include bioremediation and phytoremediation. Azolla grows efficiently and cost effectively in simple wetlands and wastewater. Azolla macroalgae have the capability and potential to be used in bioremediation due to their high cellulosic content. These methods are less expensive than other conventional wastewater treatment methods. Azolla may be useful in phytoremediation methods to remove heavy metals from wastewater and improve environmental conditions [14]. Fig. 4 depicts various azolla environmental applications.

2.2.1. Azolla in bioremediation

The term "bioremediation" refers to the process of reducing or removing toxic pollutants from

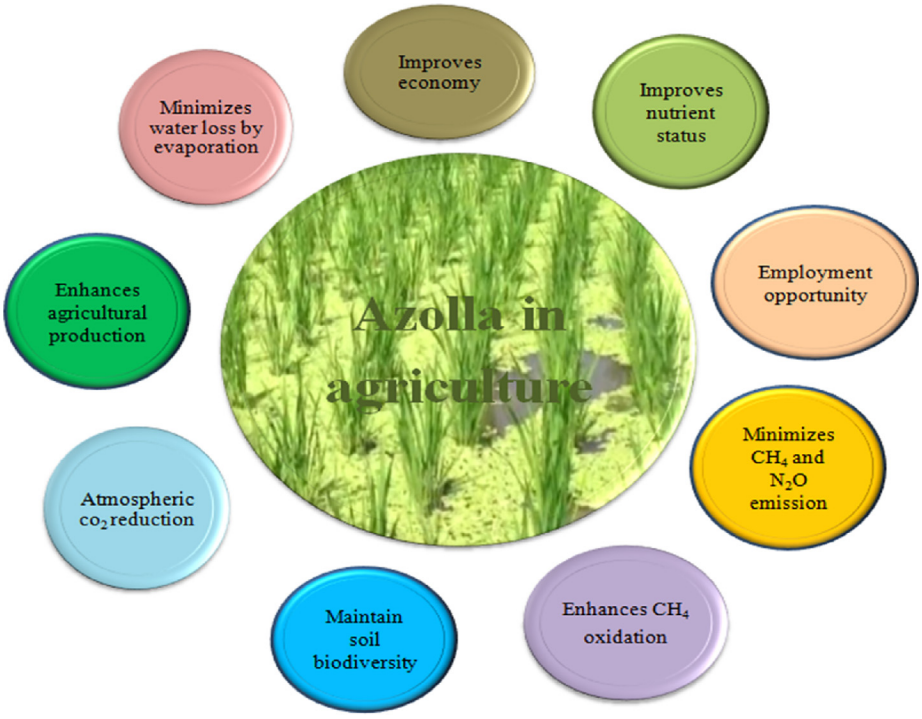

Fig. 3. Importance of azolla in agriculture.

Fig. 4. Environmental application of azolla.

contaminated sites using lower organisms like bacteria, fungi, and algae. It is the technology used to clean up polluted areas, stop further pollution, and restore contaminated areas. Organic contaminant bioremediation primarily relies on either microorganism already present at the site or on microbial inoculants created in a lab and introduced at the site. Azolla is an efficient and eco-friendly bioremediation device that can be used to treat wastewater, including industrial effluents and sewage water [15]. *A. pinnata* and *Lamna minor* were found to remove the heavy metals copper and iron from

polluted water. When pollutants are present in low concentrations, they can be cleaned up by being processed through ponds and then used again for agricultural purposes [16]. Analysis of Azolla's capacity for bioremediation of wastewater produced positive results for the removal of total organic carbon, phosphorus, and nitrogen, as well as demonstrating the plant's effectiveness in removing ammonia [17].

2.2.2. Phytoremediation of Azolla

Azolla is the primary species studied in phytoremediation because of its high tolerance to environmental pollutants and capacity to hyperaccumulate heavy metals [18]. Phytoremediation involves suspending live *A.pinnata* on the wastewater's surface. It has been reported that heavy metals (such as zinc, lead, chromium [19], mercury, cadmium [20], copper, and arsenic) and organic dyes [21] can be removed from industrial wastewater by phytoremediation. Using plants to detoxify their substrate, a novel approach known as phytoremediation is both economically and environmentally advantageous [22].

Numerous studies have shown that it is capable of absorbing metal ions from wastewater, single- and multi-metal solutions, and media [23]. Living things suffer from the harmful effects of heavy metals that enter the environment through different channels [24]. One of the aquatic ferns, *Azolla filiculoids*, is a hyperaccumulator; it can bind certain heavy metals and store a considerable amount of them [25]. Using Waste Metal Cutting Fluid (WMCF) and temperature and humidity stress. *Azolla imbricata* was employed to test the phytoremediation of Metals from Water media (WM) and Nutrient Media (NM). When it came to phytoremediation in multi-metal contaminated WMCF, *Azolla imbricata* performed exceptionally well. As such, it is a financially feasible approach to use WM to extract metals from WMCF [26].

2.2.3. Azolla as Mosquito repellent

Azolla has earned the title of "mosquito fern" due to studies showing that it can significantly lower mosquito breeding populations by up to 95%. By coating the surface of slow-moving, still, or stagnant freshwater bodies, it reduces the emergence and development of mosquito larvae and stops adult mosquitoes from laying eggs [27]. Infectious diseases like malaria, chikungunya, dog heartworm, dengue fever, and yellow fever are less likely to spread because of this [28].

The Zika outbreak has the potential to become pandemic [29], the risk is increased because pregnant women can transmit the Zika virus to their unborn child, potentially leading to microcephaly in newborns as we can see in Fig. 5. There is no known cure or vaccine for the Zika virus, the only way to reduce the threat currently is to reduce mosquito populations that transmit the virus. Ability of Azolla to lessen danger one of the planet's fastest-growing plants Azolla-covered pools, wells, and ponds in the nearby Indian city of Ghaziabad nearly completely suppressed the breeding of malaria-carrying mosquitoes. Like this, azolla mats covering the water in rice paddies in southern India significantly reduced the number of immature mosquito populations [30,31]. Freshwater fish, like carp or tilapia, which eat azolla plants and mosquito larvae and provide a nearby, renewable source of high-protein food, can help reduce mosquito populations.

A. pinnata studies were only done to determine the species' ability to kill *Aedes larvae* and to analyze its chemical makeup using gas chromatographic methods. Plant extracts from A. pinnata demonstrated encouraging results. The oviposition

Fig. 5. Zika virus affects the babies.

deterrence test revealed that the *A. pinnata* extract contains repellent qualities [32].

2.2.4. Azolla in weed control

In agricultural systems, weeds are an inevitable element that poses the biggest obstacle to high-yield production in rice crops. 10%–15% of losses worldwide are thought to be caused by weeds [33]. In many parts of the world, azolla is a helpful bio-herbicide that lets Japan's rice paddies use less herbicide; azolla are weeds that cover some bodies of water entirely [34]. Three fields with different weed flora were used for the study. Among the weed species that azolla suppressed were Monochoria vaginalis Presl, Echinochloa glabrescens Munro ex Hook.f, Cyperus difformis L and Paspalum species. Azolla could inhibit the growth of some weed species by up to 100% during rice flowering and 86%–95% during harvest, depending on the type of weed. However, other weed species were not affected by this phenomenon [35].

2.3. Medical application

Dental caries is a major health issue that can cause severe pain in the mouth. *A.pinnata* extracts in ethanol, chloroform, and aqueous form have a significant inhibitory effect on dental pathogens. Azolla has antifungal, antiviral, antibacterial, antioxidant, anti-inflammatory, and anticancer properties [36]. Azolla is used to make cough medicine in *Tanzania* [37]. Oxidative stress, an uptick in pro-inflammatory cytokines, and pro-apoptotic proteins are signs of lead acetate-induced hepatotoxicity in rats. *A.pinnata* ethanolic extract, on the other hand, mitigated lead acetate-induced hepatotoxicity [38]. Antibiotic therapy may be hampered by bacterial resistance to antibiotics [39]. *A. pinnata* extract administered and the ability of that extract to prevent the growth of *Salmonella typhi* [40].

2.4. Food application

Azolla is one of the best alternative feed ingredients for cows, chickens, pigs, and fish, as shown in Fig. 6 [41]. Azolla is used as a feed because it contains more protein and amino acids than soybean [42]. Azolla is a simple to digest supplement that increases feed efficiency, average daily gain of animals, and milk production by 15–20% [43]. The most common species of azolla used as animal feed is *A. pinnata*. It is recommended to incorporate azolla at a level of less than 15% for poultry, 5% for grill chicken, and less than 25% for fish [44].

2.4.1. Azolla as space food

The potential of azolla as a food source for humans is less well known. This is now beginning to change, in part due to research into possible diets for space stations, space travel, and human habitation on the Moon and Mars. Because azolla habitats have limited space and little to no gravity, they are ideal for use as food in controlled ecological life support systems in space travel. Azolla was found to satisfy human nutritional

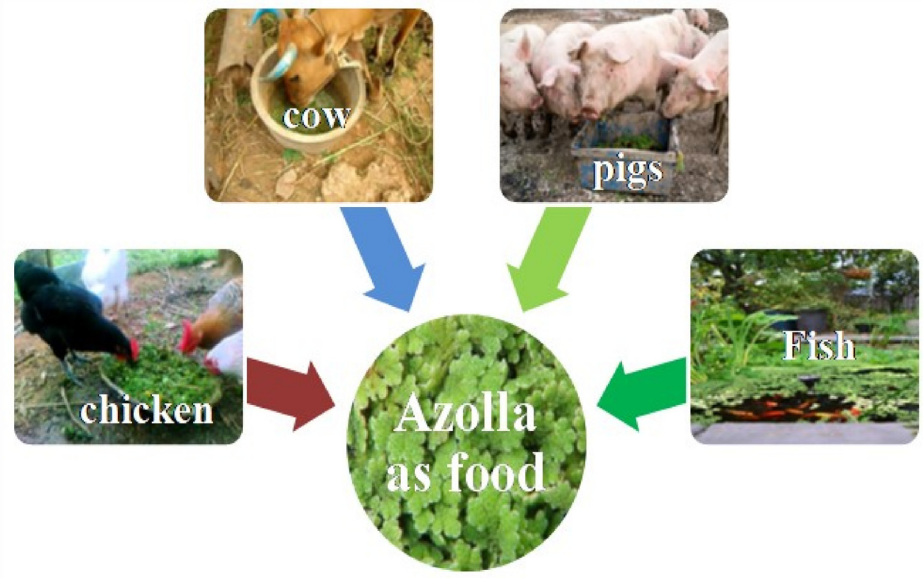

Fig. 6. Azolla as animal food.

Fig. 7. Azolla as a food for humans.

needs on Mars, according to recent research by Katyama in collaboration with the Space Agriculture Task Force (SATF) [45]. To protect the crew's health while they are exposed to high levels of ionising radiation and other harmful environmental factors during space travel, the food must be balanced with the necessary nutrients [46]. The recipes that are included include those by Erik Sjödin for azolla soup, burgers, pancakes, hardtack, balls, and bread. Fig. 7 illustrates this. There are researchers who have tried cooking Azolla in soups or making "Azolla meatballs as human food." It's in Tanzania. In Western nations, azolla is also used as a salad due to its high protein content [47].

2.5. Azolla as a fuel

The demand for energy and the scarcity of fossil fuels are increasing all the time. As a result, there has been a search for sustainable, renewable, and low-cost biofuel, which has prompted a search for potential bioenergy crops [48]. Fig. 8 illustrates that bio-oil, which is produced through pyrolysis and is carbon neutral, is among the most appealing liquid biofuels (bioethanol, biodiesel, and biooil). When biooil is used, it emits less sulphur dioxide and nitrogen dioxide when compared to conventional fossil fuels. With the intention of producing ethanol, a sample of the Azolla plant was collected [49].

3. Conclusion

Azolla is a natural wonder that is high in protein, lowers environmental risks, and can be developed at a reasonable cost as wetlands. Animals and birds fed Azolla show significant increases in body weight, milk production, and egg production. Azolla significantly increases rice production by more than 50%. The Azolla reduces the cost of nitrogen fertilisers, herbicides, and insecticides by fixing atmospheric nitrogen, controlling weed emergence, and preventing insects like mosquito's from laying eggs.

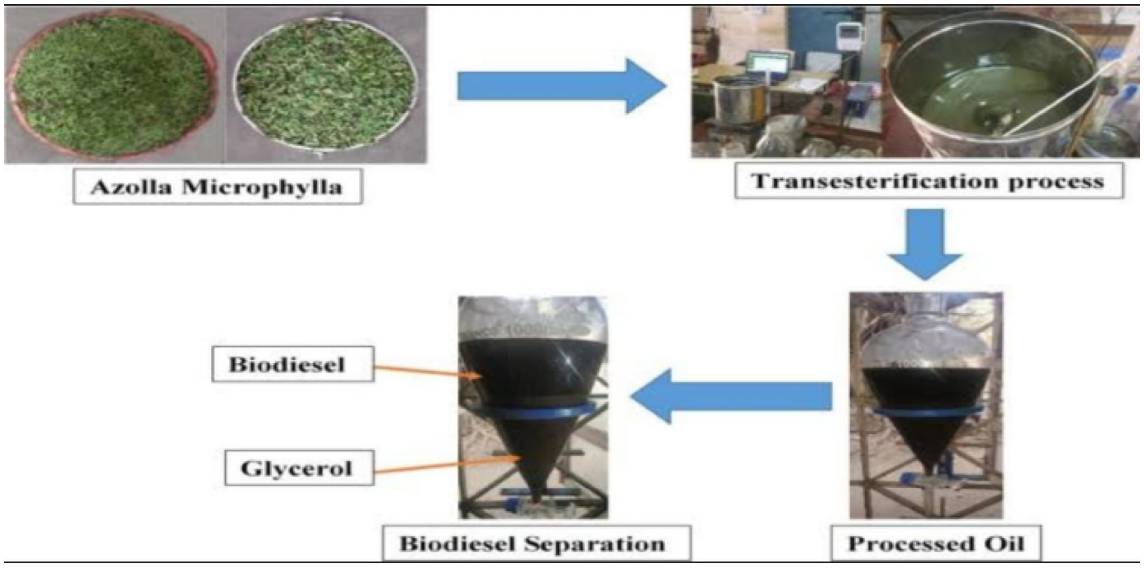

Fig. 8. Azolla used as biofuel (S.Thiruvenkatachari et al., 2021) [47].

Azolla produces a large amount of biomass in a short period of time, compost for organic farming and kitchen gardening, or converted into a biofuel such as bioethanol. Azolla is used in the bioremediation and phytoremediation of heavy metal-contaminated sewage and industrial water. Azolla is a powerful herbal remedy used in conventional medicine. As a result, by growing it in a clean and controlled environment, we can use it as human food. It has the potential to improve global food security and reduce global hunger.

4. Future prospective

- ➢ Azolla has been proposed as a food item for humans to eat, but there are currently no human studies on the safety of azolla consumption.
- ➢ Soil microbiologists can also work on Azolla roots to identify sulphur (S) and zinc (Zn) solubilizing bacteria.
- ➢ Specifically in the livestock, poultry, and aquaculture sectors, more research on the life cycle assessment of azolla-based animal feed production is needed to improve its environmental impact.
- ➢ Azolla in medical field there is growing hope in the field of medicine and dentistry will result in improvement in the diagnosis, treatment and prevention of disease.

Ethics information

This review paper does not require ethical clearance as it synthesizes and analyzes existing research rather than involving new data collection from human or animal subjects.

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Conflicting interest

None.

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