



Water Fern- Azolla: A Review

K. Anokhi Chandrababu, U. Parvathy

10.18805/ag.R-2529

ABSTRACT

The Azolla-Anabaena symbiosis is distinguished by its high productivity and ability to fix nitrogen at high rates. As a result, numerous studies on this association have been conducted over the last few decades, with insufficient synthesis and coordination. As a result, this paper attempts to review and summarise previous and recent findings on the biology and applications of azolla in the hope of facilitating increased future collaborative research on this green gold mine. Azolla is a plant in the Azolla genus. Azolla derived from water can be used as human food, animal feed, green manure, organic fertiliser and to increase soil fertility, as well as for biological wastewater remediation and salt soil reclamation. Because of its high nutritional quality and protein content, azolla is suitable for human consumption as well as as a feed additive for a variety of animals such as fish, ducks, cattle, poultry and others to reduce feed costs. It is also used in the production of biogas and hydrogen, as well as as astronaut food in space. This review provides an overview of Azolla's benefits as well as new developments in its various fields of application.

Key words: Application, Azolla, Nitrogen fixation, Protein food, Water fern.

Nitrogen (N₂) is essential for all living things. Amino acids and proteins, as well as a variety of other chemical molecules, are produced during the nitrogen fixation process. Biological nitrogen fixation is an important aspect of microbial activity (Pereira *et al.*, 2017). Only prokaryotes, which can be symbiotic or free-living in nature, can fix nitrogen biologically. Biological nitrogen fixation, mediated by nitrogenating enzymes, is a well-known and important biological activity mechanism.

Azolla is an aquatic fern in the Salviniaceae family that grows in a symbiotic relationship with the blue-green algae *Anabaena azollae*. The name Azolla is derived from the Greek words azo (to dry) and allyo (to kill), implying that the plant will die if allowed to dry. Azolla contains about seven or eight living species and forty extinct species (Small *et al.*, 2011). The two subgenera of the genus *Euazolla* are *Euazolla* and *Rhizosperma*. *Euazolla* is made up of five species from the New World: *A. caroliniana*, *A. filiculoides*, *A. mexicana*, *A. microphylla* and *A. rubra*. The presence of three megaspore swimmers distinguishes it.

The small, free-swimming, fast-growing fern can be found all over the world. Azolla is a dichotomously branching, free-swimming aquatic fern that grows in moist soil, ditches and swampy ponds. Indian species are triangular in shape, measuring 1.5 to 3.0 cm long and 1 to 2 cm wide. Fronds have small roots that are frequently associated with *microphylla* (Ahmed *et al.*, 2021), as well as a short branched stem called the rhizome that is covered in small, alternately overlapping leaves. The sporophyte is dorsiventrally organised, with dorsal and abdominal lobes on each leaf (Pratte *et al.*, 2021). The ventral lobe is narrow and colourless, with only one cell thick distal half. The aerial dorsal leaflet lobe is composed of layered mesophylladaxial and abaxial epidermal tissue, numerous stomata and unicellular papillae. An elliptical cavity is formed by the folding of the adaxial epidermis in the dorsal leaf lobe. The cavity, which is mostly gas-filled, is lined with mucus and contains the cyanobiont

ICAR-Central Institute of Fisheries Technology, Kerala University of Fisheries and Ocean Studies, Kochi-682 506, Kerala, India.

Corresponding Author: K. Anokhi Chandrababu, ICAR-Central Institute of Fisheries Technology, Kerala University of Fisheries and Ocean Studies, Kochi-682 506, Kerala, India.
Email: anokhikc@gmail.com

How to cite this article: Chandrababu, K.A. and Parvathy, U. (2022). Water Fern- Azolla: A Review. *Agricultural Reviews*. DOI: 10.18805/ag.R-2529.

Submitted: 22-03-2022 **Accepted:** 24-11-2022 **Online:** 02-12-2022

Anabaena azollae as well as a gram-positive, non-nitrogen-fixing bacterium identified as an *Arthrobacter* species (Sebastian *et al.*, 2021). These green plants are considered high in proteins, fats, pharmacologically active secondary metabolites and antioxidants. As a result, their extracts were used in biocomposite films (Eltabakh *et al.*, 2021). It also contains macronutrients like calcium, magnesium and potassium, as well as vitamins like A (precursor beta-carotene) and B12. It can be used as a food supplement due to its ease of cultivation, high productivity and high nutritional value (Sreenath *et al.*, 2016).

Azolla is a free-floating aquatic fern that fixes atmospheric nitrogen in collaboration with the nitrogen-fixing cyanobacterium *Anabaena azollae*. This is found endophytically in Azolla leaves' dorsal lobes (Ghadimi *et al.*, 2021). Nitrogen is obtained for the rice crop from this source. The only existing cyanobacterial plant symbiosis is that between Azolla and *Anabaena*. It is used as organic fertiliser in agriculture. The primary aquatic symbiotic system is Azolla. The environment contains seven different species. Azolla is a nitrogen-rich plant that was once used as a green. Wetland rice obtains organic matter from manure and organic matter. In North Vietnam and China, After 20 days of inoculation, approximately 70%

of the Azolla nitrogen content was mineralized to NH_4 . The rice plant readily absorbs NH_4 (Maham *et al.* 2020). Excess Azolla is used as compost and is fed to dryland crops and vegetables to increase crop yield. Azolla has a high protein content of about 23-27% on a dry weight basis, followed by 3-6% nitrogen, 0.5-0.9% phosphorus and 2-4.5% potassium, among other majors and micronutrients present (Sonowal *et al.*, 2021). Azolla can reclaim soil and reduce weed infestation in rice crops and it has also been used in wastewater treatment and heavy metal degradation. Aside from these applications, it can also be used directly as aquaculture feed, which is known as azobiofer (Kumar *et al.*, 2021).

Azolla is grown as a cover crop in flood plains for two to three weeks. The water is then drained and the Azolla fern is planted in the field before the rice is planted. Otherwise, 4-5 quintal of fresh Azolla are added to stagnant water a week after planting rice. Green Azolla makes excellent fish feed, while dry Azolla flakes make excellent chicken feed. It is a bio-fertilizer, insect repellent, salad dressing and most importantly, a bio-scavenger because it removes all heavy metals (Mlhm *et al.*, 2021).

Azolla's possible benefits

The overall Azolla biosystem was depicted in Fig 1, with the following potential benefits:

1. It grows easily in the wild and under controlled conditions.
2. It is simple to produce in large quantities as green manure.
3. It can fix atmospheric CO_2 and nitrogen to produce carbohydrates and ammonia and after decomposition, it provides soil accessible nitrogen for plant uptake as well as organic carbon content.
4. The oxygen produced by oxygenated photosynthesis helps plant root systems and other soil microbes respire.

5. It dissolves Zn, Fe and Mn in water, making them available to rice.
6. Azolla can be used to supplement chemical nitrogen fertilisers (20 kg/ha) and improve crop yield and quality. It improves the efficiency with which chemical fertilizers are used.
7. It reduces the rate of evaporation in irrigated paddy fields.
8. Lower disease prevalence.
9. Increase the number of flowers and fruits.
10. Improve seedling or transplanted plant establishment and survival.
11. Improve drought tolerance by watering less frequently.

Advantages of Azolla

Azolla as a biofertilizer for rice production

Azolla is a bio-fertilizer that produces approximately 300 tonnes of green bio-hectares per year in a typical subtropical environment, equivalent to 800 kg of nitrogen (1800 kg of urea). The rapid decomposition of Azolla in soil and the efficient availability of its nutrients are critical factors in its use as a nitrogen-fixing biofertilizer for rice crops (Adhikari *et al.*, 2021). The rapid reproduction rate of Azolla and its ability to decompose have become critical factors in its use as a green manure and organic fertiliser in paddy fields.

The Azolla benefits listed below are suitable for use as organic rice fertiliser:

1. Azolla is a nitrogen-fixing plant with a high nitrogen fixation rate.
2. Azolla is rapidly expanding.
3. Because azolla floats on the water's surface, it cannot compete with rice for light and space.
4. Azolla thrives in partial shade, which a rice canopy can easily provide in its early and mid-growing stages in most regions.
5. Azolla begins to die and decompose when the rice reaches maturity due to low light intensity under the canopy and a lack of nutrients, releasing nutrients into the medium.

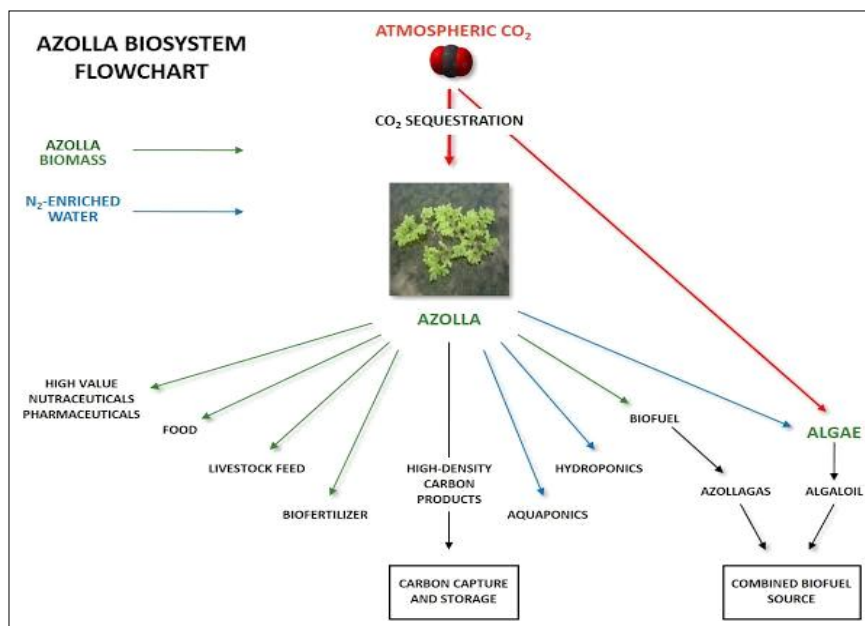


Fig 1: Azolla biosystem flow chart.

6. Because Azolla decomposes quickly, the nitrogen it has absorbed, as well as phosphorus and other nutrients it may have noticed in the water, possibly in competition with the rice, are quickly released back into the medium and made available to the rice to grow during crop development (Thapa *et al.*, 2021).
7. Azolla accumulates more potassium in its tissues than rice in low-potassium environments, making this nutrient available to rice after decomposition (Bin *et al.*, 2021).
8. A thick Azolla mat provides additional weed suppression in a paddy field.

Azolla as a green fertilizer

Azolla as a cover crop can benefit water bamboo, arrowhead, taro, wheat and rice (Kimani *et al.*, 2021). Incubating Azolla in moist soil as a green manure resulted in rapid mineralization, with 60-80% of the nitrogen released after two weeks (Ahmad *et al.*, 2021).

Azolla: Beneficial effects on crops

Azolla is beneficial to wheat when grown in a rice-wheat rotary cultivation system (Lubis *et al.*, 2021). Azolla increases mung bean production by increasing water holding capacity, organic carbon, ammoniacal nitrogen, nitrate nitrogen and accessible phosphorus, potassium, calcium and magnesium while decreasing pH and bulk density (Vat *et al.*, 2021).

Azolla: Beneficial effects on soil physiological and chemical properties

Azolla is a herb that increases soil fertility. The application of Azolla, which improves soil fertility by increasing total soil nitrogen, organic carbon and accessible phosphorus, confirmed these findings (Youssef *et al.*, 2021).

Azolla in reclamation of saline soils

Although Azolla is salt sensitive, growing it in a saline environment for two years reduced salinity from 0.35 to 0.15 and the desalination rate (71.4%) was 1.8 times faster than water leaching and 2.1 times faster than *Sesbania*, as well as decreased electrical conductivity, pH of acidic soil and increased soil calcium content (Shariffi *et al.*, 2021).

Azolla for bioremediation

The heavy metals iron and copper can be removed from contaminated water by *A. pinnata* and *Lamna minor*. Water. Pollutants can be harmful even in low concentrations. It is processed through ponds and can be reused for a variety of purposes (Annisa *et al.*, 2021). This is done for agricultural purposes. Tolerance and acceptance have proven to be advantageous. Three Azolla species have chromium accumulation in their leaves (Goala *et al.*, 2021). Azolla biomass also acts as a pollutant biofilter. Azolla has a remarkable ability to directly concentrate metals from impurities such as Cu, Cd, Cr, Ni, Pb and nutrient effluents (Prabhakaran *et al.*, 2022).

Azolla as a mosquito repellent

By laying a thick mat of Azolla on the water's surface, you can prevent mosquito hatching and adult emergence (Hossain *et al.*, 2021). According to a study of ponds, ponds, wells, paddy fields and sewers, *Anopheles* spp. brood was almost completely suppressed in waters completely covered with Azolla. *A. pinnata* significantly reduced oviposition and adult emergence in *Culex quinquefasciatus* and *Anopheles culicifacies* giles, but larval survival was unaffected (Sumitha *et al.*, 2021). The hatchability of the eggs was reduced in part.

Azolla for weed control

Weeds have also been found to be inhibited by Azolla. Our findings were supported by the fact that an Azolla cover significantly reduced the total amount of weeds, particularly the most common weed, *Monochoria vaginalis*, which grasses and hedges could not always control (Singh *et al.*, 2021).

Azolla in biogas production

The anaerobic fermentation of Azolla (or a combination of Azolla and rice straw) produces methane gas, which can be used as fuel and the remaining effluent, which contains all of the nutrients originally incorporated into plant tissues with the exception of a small percentage of lost nitrogen *B. ammonia*, can be used as fertiliser (Thiruvengkatachari *et al.*, 2021).

Azolla and bioenergy

Nitrogenization in the symbionts evolves hydrogen rather than nitrogen fixation when *Azolla anabaena* is grown in a nitrogen-free environment and/or a nitrate-rich aquatic medium (Hamdan *et al.*, 2021).

Controlling the spread of aquatic weeds

Weed growth is stifled when Azolla forms a thick, almost opaque mat. There are most likely two mechanisms at work here, the most efficient of which is light deprivation for immature weed seedlings due to sunlight obstruction (Ouedraogo *et al.*, 2021). The other is a dense, interlocking Azolla mat that provides physical resistance to the emergence of weed seedlings (Marzvan *et al.*, 2021). Rice seedlings are not harmed by Azolla's weed suppression function because they are planted above the Azolla mat.

Azolla for human consumption

A few researchers have experimented with preparing Azolla in soups or "Azolla meatballs as human food." Sjodin and colleagues (2012) Traditional cough treatment has been reported to be effective in Tanzania when Azolla is used (Rahal *et al.*, 2019). Azolla is widely used as a food fortifier due to its high protein content (Divya *et al.*, 2020).

Azolla as a space diet ingredient

Azolla has been proposed as part of the space diet while on Mars in collaboration with the Space Agriculture Task Force and it has been discovered that Azolla meets human nutritional needs on Mars (Katayama *et al.*, 2008). They used combination of Azolla, spirulina and carbohydrate.

Azolla as a livestock nutritional supplement

Azolla is consumed as a dietary supplement by pigs, rabbits, chickens, ducks and fish (Verma *et al.*, 2021). Azolla is harvested in large quantities and fed to cattle and pigs as feed (Tarrif *et al.*, 2021). It was also demonstrated that feeding Azolla to broilers produced growth and body weight values comparable to corn-soybean meal (Ouedraogo *et al.*, 2021). Digested Azolla manure left over from biogas production was suitable as fish pond fertiliser and in a study of lactating cows (Abdelatty *et al.*, 2021), it was discovered that Azolla could be used as a feed ingredient, with milk yield and fat percentage remaining at the same levels as when fed conventional feed.

Present status of Azolla

Azolla is already grown commercially in India and China, where its utility has long been recognised. The fern's use was once limited due to propagation issues, but it is now used in larger growing areas. The Chinese have been using azolla for hundreds of years. It was first used in India in the 11th century. These are the only two countries that have long been cultivating Azolla. The practise most likely began when it was discovered that growing wild Azolla in paddy fields was beneficial for harvesting. However, until reliable methods of overwintering and oversummering the fern were developed, organised use of the fern would be impossible. Because Azolla can only be grown from vegetative material, it requires protection during seasons when the weather is too harsh to grow.

CONCLUSION

The utilization of Azolla as food, as well as any remaining significant purposes, contribute fundamentally to the conservation or improvement of the world's biological system. There is a reasonable need to boost oceanic potential. Biotechnological medicines could utilize Azolla all the more productively later on. The utilization of maintainable and earth sound farming practices can make a significant commitment to the biological steadiness of the planet. The utilization of Azolla as a bio-manure for rural harvests, to decrease or supplant substance composts, as a human dietary enhancement, as a feed for poultry and creatures can assume a huge part in keeping up with or further developing the utilization status of Azolla in the worldwide climate. A few different purposes of it can likewise be extremely helpful for people. More cooperative exploration endeavors are expected to capitalize on this significant normal asset, this little superplant.

Conflict of interest: None.

REFERENCES

Abdelatty, A.M., Mandouh, M.I., Mousa, M.R., Mansour, H.A., Ford, H., Shaheed, I.B. and Bionaz, M. (2021). Sun-dried Azolla leaf meal at 10% dietary inclusion improved growth, meat quality and increased skeletal muscle Ribosomal protein S6 kinase β 1 abundance in growing rabbit. *Animal*. 15(10): 100348.

- Abomohra, A.E.F., El-Hefnawy, M.E., Wang, Q., Huang, J., Li, L., Tang, J. and Mohammed, S. (2021). Sequential bioethanol and biogas production coupled with heavy metal removal using dry seaweeds: Towards enhanced economic feasibility. *Journal of Cleaner Production*. 316: 128341.
- Adhikari, K., Bhandari, S. and Acharya, S. (2021). An overview of Azolla in rice production: A review. *Reviews in Food and Agriculture*. 2(1): 4-08.
- Ahmad, N. and Tariq, H. (2021). Azolla as Waste Decomposer and Bio-fertilizer: A review. *Journal of Applied Research in Plant Sciences (JOARPS)*. 2(1): 108-116.
- Ahmed, I.M., Saad, S.M., Abd, A.E.Y., Suliman, M.A. and Mustafa, T.M. (2021). A new record for the invasive aquatic fern *Azolla filiculoides* Lam. in the White Nile, Sudan. *Dysona-Life Science*. 2(2): 18-24.
- Annisa, K., Sutarno, S. and Santosa, S. (2021). Azolla microphylla and Pseudomonas aeruginosa for bioremediation of bioethanol wastewater. *Biodiversitas Journal of Biological Diversity*. 22(4). <https://doi.org/10.13057/biodiv/d220425>.
- Bin, M., Khair, M.I., Azman, E.A., Ismail, R. and Rani, M.N.F.A. (2021). Biofertilizer: *Azolla pinnata* in-combination with Inorganic Fertilizer on Growth and Yield of Rice. DOI: 10.21203/rs.3.rs-871462/v1.
- Divya, P., Kanimozhi, K., Poornima, S., and Tamilarasu, S. (2020). Shelf life and physicochemical evaluation of Azolla pinnata incorporated yogurt. *Journal of Critical Reviews*. 7(7): 770-773.
- Eltabakh, M., Kassab, H., Badawy, W., Abdin, M. and Abdelhady, S. (2021). Active bio-composite sodium alginate/maltodextrin packaging films for food containing *Azolla pinnata* leaves extract as natural antioxidant. *Journal of Polymers and the Environment*. 1-11.
- Ghadimi, M., Sirousmehr, A., Ansari, M.H. and Ghanbari, A. (2021). Organic soil amendments using vermicomposts under inoculation of N₂-fixing bacteria for sustainable rice production. *Peer J*. 9: e10833.
- Goala, M., Yadav, K.K., Alam, J., Adelodun, B., Choi, K.S., Cabral-Pinto, M.M. and Shukla, A.K. (2021). Phytoremediation of dairy wastewater using *Azolla pinnata*: Application of image processing technique for leaflet growth simulation. *Journal of Water Process Engineering*. 42: 102152.
- Hamdan, H.Z. and Huri, A.F. (2021). CO₂ sequestration by propagation of the fast-growing Azolla spp. *Environmental Science and Pollution Research*. 1-13.
- Hossain, M.A., Shimu, S.A., Sarker, M.S.A., Ahsan, M.E. and Banu, M.R. (2021). Biomass growth and composition of Azolla (*Azolla pinnata* R. BR.) supplemented with inorganic phosphorus in outdoor culture. *SAARC Journal of Agriculture*. 19(1): 177-184.
- Katayama, N., Yamashita, M., Kishida, Y., Liu, C.C., Watanabe, I., Wada, H. and Force, S.A.T. (2008). Azolla as a component of the space diet during habitation on Mars. *Acta Astronautica*. 63(7-10): 1093-1099.
- Kimani, S.M., Bimantara, P.O., Kautsar, V., Tawaraya, K. and Cheng, W. (2021). Poultry litter biochar application in combination with chemical fertilizer and Azolla green manure improves rice grain yield and nitrogen use efficiency in paddy soil. *Biochar*. 1-12.

- Kodape, A. and Sharma, S. Anabaena Azollae Culture Water, A. Source For Bioremediation.
- Kumar, U., Rout, S., Kaviraj, M., Swain, P., and Nayak, A.K. (2021). Uncovering morphological and physiological markers to distinguish Azolla strains. *Brazilian Journal of Botany*. 44(3): 697-713.
- Lubis, K.S., Pratiwi, N. and Rauf, A. (2021, June). The Effect of the Combination of Urea, Manure of Cow and Azolla on the Growth and N Uptake of the Black Rice of Ominio Variety. In IOP Conference Series: Earth and Environmental Science (Vol. 782, No. 4, p. 042042). IOP Publishing.
- Maham, S.G., Rahimi, A., Subramanian, S. and Smith, D.L. (2020). The environmental impacts of organic greenhouse tomato production based on the nitrogen-fixing plant (Azolla). *Journal of Cleaner Production*. 245: 118679.
- Marzvan, S., Moravej, K., Felegari, S., Sharifi, A. and Askari, M.S. (2021). Risk assessment of alien Azolla filiculoides lam in anzali lagoon using remotesensing imagery. *Journal of the Indian Society of Remote Sensing*. 1-9.
- Maswada, H.F., El-Razek, U.A.A., El-Sheshtawy, A.N.A., and Mazrou, Y.S. (2021). Effect of Azolla filiculoides on growth, physiological and yield attributes of maize grown under water and nitrogen deficiencies. *Journal of Plant Growth Regulation*. 40(2): 558-573.
- Mibm, K., Azman, E.A., Ismail, R. and Rani, M.N.F.A. (2021). Biofertilizer: *Azolla pinnata* in-combination with Inorganic Fertilizer on Growth and Yield of Rice. DOI: 10.21203/rs.3.rs-871462/v1.
- Ouedraogo, S.W. N., Komoe, K., Lompo, D.J.P., Da, S.S., Isimemen, O. and Masse, D. (2021). Effect of invasive aquatic plants (*Azolla a.*, *Myriophyllum a.* and *Cyperus a.*) biochar amendment on maize growth: An assessment. *Journal of Agricultural Studies*. 9(3): 336-351.
- Pereira, A.L. (2017). The Unique Symbiotic System Between a Fern and a Cyanobacterium, Azolla-Anabaena azollae: Their potential as biofertilizer, feed and remediation. In *Symbiosis*. IntechOpen.
- Pourkarimi, S., Hallajisani, A., Alizadehdakhl, A. and Nouralishahi, A. (2021). Bio-oil production by pyrolysis of *Azolla filiculoides* and *Ulva fasciata* macroalgae. *Global Journal of Environmental Science and Management*. 7(3): 331-346.
- Prabakaran, S. and Mohanraj, T. (2021). Optimization of biodiesel production from *Azolla pinnata*: Investigation of CI engine characteristics using nano dosed biodiesel. *Journal of Mechanical Science and Technology*. 35(10): 4765-4773.
- Prabakaran, S., Mohanraj, T., Arumugam, A. and Sudalai, S. (2022). A state-of-the-art review on the environmental benefits and prospects of Azolla in biofuel, bioremediation and biofertilizer applications. *Industrial Crops and Products*. 183: 114942.
- Pratte, B.S. and Thiel, T. (2021). Comparative genomic insights into culturable symbiotic cyanobacteria from the water fern Azolla. *Microbial Genomics*. 7(6). <https://doi.org/10.1099/mgen.0.000595>.
- Rahal, A. (2019). Azolla-emerging animal feed. *International Research Journal of Natural and Applied Sciences*.
- Sebastian, A., Deepa, P. and Prasad, M.N.V. (2021). Azolla farming for sustainable environmental remediation. *Handbook of Assisted and Amendment: Enhanced Sustainable Remediation Technology*. 517-533.
- Sharifi, P., Shorafa, M. and Mohammadi, M.H. (2021). Investigating the effects of cow manure, vermicompost and *Azolla fertilizers* on hydraulic properties of saline-sodic soils. *International Journal of Recycling Organic Waste in Agriculture*. 10(1): 43-51.
- Singh, S., Elamathi, S., Ghosh, G. and Abeysingha, N.S. (2021). Sesbania, Azolla and herbicide use for weed management and optimizing yield in direct-seeded rice. *Indian Journal of Weed Science*. 53(1): 30-35.
- Sjödén, E. (2012). The Azolla cooking and cultivation project. Erik Sjödén.
- Small, E. and Darbyshire, S.J. (2011). 35. Mosquito Ferns (*Azolla* species)-tiny 'superplants'. *Biodiversity*. 12(2): 119-128.
- Sonowal, M., Bhattacharya, H.K., Kalita, M.C., Sarma, M., Bharali, D. and Boro, P. (2021). A comparative study on growth and nutritive value of *Azolla pinnata* and common duck weed (*Lemna minor*) under agro climatic condition of Assam. *Journal of Entomology and Zoology Studies*. 2021: 9(1): 1781-1783.
- Sreenath, K.B., Sundaram, S.O.W.M.Y.A., Gopalakrishnan, V.K. and Poornima, K.A.N.N.A.P.P.A.N. (2016). Quantitative phytochemical analysis, *in vitro* antioxidant potential and gas chromatography-mass spectrometry studies in ethanolic extract of *Azolla Microphylla*. *Asian J. Pharm Clin Res*. 9(2): 318-23.
- Sumitha, V., Mini, I. and Lajja, S.N. (2021). Larvicidal efficacy of *Hyptis capitata* Jacq. Against *Culex quinquefasciatus* mosquito (Culicidae). *International Journal of Mosquito Research*. 8(4): 42-46.
- Taghilou, S., Peyda, M. and Mehrasbi, M.R. (2021). Modeling of waste water treatment by *Azolla filiculoides* using response surface methodology. *Journal of Environmental Health Science and Engineering*. 1-11.
- Tarif, S.T. (2021). Use of azolla as a substitute poultry feed and its effect on growth and production: A review. *Plant Archives*. 21(1): 947-950.
- Thapa, P. and Poudel, K. (2021). Azolla: Potential biofertilizer for increasing rice productivity and government policy for implementation. *Journal of Wastes and Biomass Management (JWBM)*. 3(2): 62-68.
- Thiruvenkatachari, S., Saravanan, C.G., Geo, V.E., Vikneswaran, M., Udayakumar, R. and Aloui, F. (2021). Experimental investigations on the production and testing of *Azolla* methyl esters from *Azolla microphylla* in a compression ignition engine. *Fuel*. 287: 119448.
- Vats, S., Srivastava, P., Saxena, S., Mudgil, B. and Kumar, N. (2021). Beneficial Effects of Nitrogen-Fixing Bacteria for Agriculture of the Future. In *Soil Nitrogen Ecology* Springer, Cham. (pp. 305-325).
- Verma, D. and Dey, K.P.S.D. (2021). Effect of supplementation of *Azolla (Azolla pinnata)* on productive performance in cattle and economics of farmers: A field study. *The Pharma Innovation Journal*. 2021: 10(4): 336-339.
- Youssef, M.A., AL-Huqail, A.A., Ali, E.F. and Majrashi, A. (2021). Organic amendment and mulching enhanced the growth and fruit quality of squash plants (*Cucurbita pepo* L.) grown on silty loam soils. *Horticulturae*. 7(9): 269. <https://doi.org/10.3390/horticulturae7090269>.