OPEN ACCESS
Haya: The Saudi Journal of Life Sciences
Abbreviated Key Title: Haya Saudi J Life Sci
ISSN 2415-623X (Print) | ISSN 2415-6221 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article

A Comparative Study of *Azolla* and *Lemna* as Biofertilizer on *Solanum Lycopersicum* L.

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DOI: https://doi.org/10.36348/sjls.2025.v10i11.010 | **Received:** 04.09.2025 | **Accepted:** 18.11.2025 | **Published:** 31.12.2025

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Abstract

The overdependence on chemical fertilizers in conventional agriculture has led to severe ecological degradation, soil fertility loss and health concerns, prompting the urgent need for sustainable alternatives. This study investigates the biofertilizer potential of two aquatic macrophytes *Azolla microphylla* Kaulf. and *Lemna minor* L. on the growth, physiology and yield of *Solanum lycopersicum* L., under field conditions using Randomized Block Design (RBD). For these six treatments were tested such as control, Azolla, *Lemna*, combination of *Azolla* & *Lemna*, cow dung and NPK fertilizer, with three replications each. The growth parameters like plant height, number of branches, number of leaves, leaf area index and reproductive parameters like flowers and fruit count along with physiological parameters, chlorophyll (a, b, total) and carotenoid content were systematically recorded. The combined application of *Azolla* and *Lemna* significantly enhanced all measured parameters compared to both the control and NPK treated plots except for carotenoid content which show high value in *Lemna* only treatment than control and NPK plots. Notably 120.61% increase in plant height, 6463.63% increase in flower production and over 11-fold increase in fruit yield compared to control. Analysis of Variance (ANOVA) confirmed the statistical relevance ($p < 0.001$) of these effects. Physiological parameters such as chlorophyll a, b, total chlorophyll and carotenoids also showed significant enhancement. This study is novel in demonstrating the synergistic effect of *Azolla* and *Lemna* as biofertilizers, surpassing individual and chemical treatments. The dual application offers a low-cost, eco-friendly alternative that enhances crop yield, supports soil health, and promotes sustainable agriculture.

Keywords: Biofertilizer, Azolla, Lemna, Solanum lycopersicum, RBD, Statistical Analysis.

INTRODUCTION

The increasing human population necessitates conventional agricultural to satisfy food requirements, leading farmers to rely on chemical fertilizers and pesticides to boost productivity (Kumar *et al.*, 2018). According to the Food and Agricultural Organization, the world's population will exceed 9 billion people by 2050; therefore, crop yields need increase to meet the demand for food (Chaudhary *et al.*, 2022). The Rapid population growth, industrialization and urbanization have intensified environmental degradation, while the extensive use of chemical fertilizers though ensuring food security has adversely affected ecosystems and living organisms (Mahanty *et al.*, 2017). While fertilizers have ensured food security, they disrupt soil–microbe interactions essential for nutrient cycling and fertility. Nutrient leaching into groundwater causes health risks such as Blue Baby Syndrome and ecological issues like eutrophication. The prolonged dependence on chemicals also reduces soil fertility, increases salinity and weakens overall plant productivity.

Eco-friendly farming practices have increased global interest as biofertilizers, particularly in regions with strict chemical fertilizer regulations. Biofertilizers contain beneficial microorganisms that enhance nutrient availability by fixing nitrogen, solubilizing phosphorus and decomposing organic matter. Since plants absorb only a fraction of applied nutrients, biofertilizers provide a steady nutrient supply through microbial activity, making them essential for Integrated Nutrient Management and sustainable agriculture (Kumar *et al.*, 2018). Nitrogen is essential for crop yield but unavailable to plants in its atmospheric form. The energy-intensive Haber–Bosch process converts nitrogen to ammonia, emitting greenhouse gases. In contrast, biological nitrogen fixation by diazotrophs converts nitrogen to ammonia via nitrogenase enzymes, providing about 65% of agricultural nitrogen globally. Symbiotic associations like *Rhizobium–legume*, *Anabaena azollae–Azolla*, and *Frankia–actinorhizal* plants enhance soil fertility and reduce dependence on chemical fertilizers (Brahmaprakash & Sahu, 2012; Jhu & Oldroyd, 2023).

This study evaluates the efficiency of two aquatic macrophytes, *Azolla microphylla* Kaulf. and *Lemna minor* L., as sustainable biofertilizers for *Solanum lycopersicum* L. *Azolla microphylla* is a free-floating aquatic fern that forms a symbiotic relationship with the cyanobacterium *Anabaena azollae*, enabling atmospheric nitrogen fixation in nitrogen-deficient environments. Azolla enhances soil fertility, crop yield and weed suppression while serving as livestock feed, wastewater purifier and biofuel feedstock (Wijeysingha & Amarasinghe, 2023). *Lemna minor* (duckweed), a small, fast-growing aquatic plant, reproduces rapidly and thrives in stagnant freshwater. With 20–35% protein and high nutrient recycling ability, it serves as green manure, animal feed and a biomass source for bioethanol and biochar. Its ecological adaptability and high productivity make it valuable for sustainable agriculture and bioenergy production (Vulpe *et al*., 2025).

MATERIALS AND METHODS

The healthy, vibrant and disease-free fronds of *Azolla microphylla* and *Lemna minor* were manually collected from naturally thriving freshwater ponds in Ithikkara, Kollam (8.8606°N, 76.7533°E) and Muhamma, Alappuzha (9.6069°N, 76.3930°E) respectively. *Azolla* was drained on clean mesh, packed in moist newspaper and *Lemna* in polythene bags to prevent desiccation during transport. Both were rinsed in freshwater to remove debris, mud and insects and maintained under suitable sunlight and aeration until transferred to cultivation bed. Fresh cowdung were collected from organically fed, healthy cows under hygienic conditions and were free from contamination. Commercial grade NPK fertilizer (10:10:10) were procured from a certified store and was stored properly in a dry and ventilated place. The yielding, uniform seeds of *Solanum lycopersicum* were bought from a seed store.

Temporary nursery ponds for *Azolla* and *Lemna* were established at Adichanalloor, Kollam, Kerala (8.8878°N, 76.7381°E) by manually excavating two identical rectangular pits (6 ft × 3 ft × 1 ft). After levelling and clearing sharp objects, UV-stabilized HDPE tarpaulin sheets were used to line the pits, with edges anchored using bricks and compacted soil to ensure water retention. Each pond was filled to three-fourths with clean ground water, maintaining a depth of 20–25 cm. To promote initial growth, a cow dung slurry (1:10 ratio of fresh cow dung to water) was prepared, settled briefly, and evenly distributed into both ponds.

Following the preparation of two ponds, *A. microphylla* and *L. minor* were cultivated separately under controlled natural conditions. After allowing the slurry enriched water to stabilize for two days, 500 grams of each plant were inoculated into their respective ponds. *A. microphylla* in Pond A with a water depth of 10–25 cm, and *L. minor* in Pond B with 5–20 cm, both maintained at 20–30°C and pH 5.9 under open sunlight. Daily monitoring ensured optimal plant health, with manual removal of weeds, debris, and pests like snails. No chemical inputs were used, to keep the process entirely organic and sustainable.

The experimental plot was prepared on a well-drained, sunlight site with uniform topography and irrigation access. The land was ploughed, harrowed, and cleared of debris for a fine tilth. A Randomized Block Design (RBD) with three replications (R1, R2, R3) was used, each measuring 2.5 m × 1.5 m with 0.5 m buffer zones between replications to prevent edge effect and nutrient crossover. Each block was divided into six 60 cm × 60 cm sub-plots for treatments:

- T1 – Control,
- T2 – *Azolla*
- T3 – *Lemna*
- T4 – *Azolla & Lemna*
- T5 – Cow dung
- T6 – NPK fertilizer.

Sub-plots were spaced 30 cm apart and raised 5–10 cm to improve drainage. A field layout map was also drawn and recorded to track plant growth and measure accurate data collection. Treatments were applied uniformly included: T1 (Control) with no nutrient input, T2 with 1 kg fresh *Azolla,* T3 with 1 kg fresh *Lemna*, T4 with a combination of 1 kg *Azolla* and 1 kg *Lemna,* T5 with 1 kg of cowdung and T6 with 7.2 g of NPK fertilizer (10:10:10). The organic inputs were evenly applied and integrated into the topsoil, while all treatments were followed by immediate irrigation to enhance nutrient uptake and minimize volatilization. Organic treatments were reapplied at 15–30 days intervals to sustain nutrient availability throughout the growth period.

Healthy and uniform seeds of *Solanum lycopersicum* were directly sown into the soil, with each subplot containing six seeds spaced 30 cm apart. The seeds were planted at a depth of 1–1.5 cm, lightly covered with topsoil, and gently pressed. Regular irrigation was provided at appropriate intervals to support their growth. Growth parameters like plant length, number of branches, number of leaves of *Solanum lycopersicum* were manually recorded at two-week intervals starting from 30 days after sowing. Leaf Area Index (LAI) was estimated using a graph-based manual method using the formula:

LAI = Total Leaf Area / Ground Area, with the ground area standardized to 1 m² (Pandey & Sinha, 2009). Reproductive parameters like number of flowers and fruits upto 90 days of growth were recorded.

Physiological parameters including chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids were estimated from *S. lycopersicum* leaves collected from different treatments. For each sample, 100 mg of fresh leaf tissue was ground with 100 ml of 80% acetone, centrifuged at 5000 rpm for 5 minutes, and the supernatant was collected. The residue was re-extracted with 10 ml of acetone, centrifuged again, and the final volume adjusted to 25 ml. Absorbance readings at 663, 645, and 480 nm were taken against an 80% acetone blank. The Concentrations were calculated using formulas:

mg of Chlorophyll a / Gram tissue = $[12.7(A663) - 2.69(A645)] \times V / (1000 \times W)$

mg of Chlorophyll b / Gram tissue = $[22.9(A645) - 4.68(A663)] \times V / (1000 \times W)$

mg of Total chlorophyll / Gram tissue = $[20.2(A645) + 8.02(A663)] \times V / (1000 \times W)$

mg of Carotenoid / Gram tissue = $[A480 - (0.17 \times A663 - 0.638 \times A645)] \times V / (1000 \times W)$

Where, V is volume and W is tissue weight (Arnon, 1949). The collected data was tabulated along with average and percentage increase with control. Then it was statistically analyzed by using Analysis of Variance (ANOVA).

RESULTS AND DISCUSSION

The effect of different types of organic fertilizers and chemical fertilizers on the vegetative growth, physiological and reproductive traits indicate the importance of *Azolla* and *Lemna* as a promising organic fertilizer. The vegetative traits like plant height, branch number and leaf count were assessed on 30th, 45th and 60th day after sowing (DAS). Leaf area index (LAI) also measured at 60 DAS.

- **30 DAS:** The *Azolla* and *Lemna* treatment significantly outperformed other treatments. Plant height increased by 68.65%, branch number by 84.53%, and leaf count by 42.90% over the control. In contrast, NPK treatment yielded only modest increases (33.19%, 31.08% and 8.70% respectively).
- **45 DAS:** Combination treatment of *Azolla* and *Lemna* continued to show superior results with 88.17% height gain, 67.36% increase in branch number and 40.43% rise in leaf count. NPK again showed lower increases (28.17%, 20.73% and 6.65% respectively).
- **60 DAS:** The *Azolla* and *Lemna* treatment showed the highest enhancement plant height (120.61%), branch number (27.09%), and leaf count (28.80%) compared to NPK-treated plots (2.98%, 10.38% and 12.08% respectively).
- **Leaf Area Index:** The combination of *Azolla* and *Lemna* recorded a 171.80% increase in LAI compared to control, surpassing the NPK treatment (113.90%) (Table 1).

Table 1: Effect of different types of organic fertilizers and chemical fertilizers on the vegetative growth

Treatment	Plant Height						Number of Branches					
	30 DAS		45 DAS		60 DAS		30 DAS		45 DAS		60 DAS	
	A	% increase	A	% increase	A	% increase	A	% increase	A	% increase	A	% increase
Control	6.61	0%	8.92	0%	20.71	0%	2.13	0%	3.86	0	6.36	0%
Azolla	7.14	12.05%	11.64	30.53%	33.13	59.98%	3.18	49.24%	5.43	40.67	7.57	19.12%
Lemna	8.29	25.39%	13.72	53.83%	39.65	91.45%	3.27	53.30%	5.9	52.85	7.8	22.64%
Azolla&Lemna	11.15	68.65%	16.78	88.17%	45.69	120.61%	3.93	84.53%	6.46	67.36	8.08	27.09%
Cow dung	7.99	20.85%	14.81	66%	27.95	34.95%	2.98	39.86%	5.43	40.67	7.33	15.25%
NPK	8.08	33.19%	11.43	28.17%	21.33	2.98%	2.79	31.08%	4.66	20.73	7.02	10.38%

Treatment	Plant Height						Number of Branches					
	30 DAS		45 DAS		60 DAS		30 DAS		45 DAS		60 DAS	
	A	% increase	A	% increase	A	% increase	A	% increase	A	% increase	A	% increase
Control	6.61	0%	8.92	0%	20.71	0%	2.13	0%	3.86	0	6.36	0%
Azolla	7.14	12.05%	11.64	30.53%	33.13	59.98%	3.18	49.24%	5.43	40.67	7.57	19.12%
Lemna	8.29	25.39%	13.72	53.83%	39.65	91.45%	3.27	53.30%	5.9	52.85	7.8	22.64%
Azolla&Lemna	11.15	68.65%	16.78	88.17%	45.69	120.61%	3.93	84.53%	6.46	67.36	8.08	27.09%
Cow dung	7.99	20.85%	14.81	66%	27.95	34.95%	2.98	39.86%	5.43	40.67	7.33	15.25%
NPK	8.08	33.19%	11.43	28.17%	21.33	2.98%	2.79	31.08%	4.66	20.73	7.02	10.38%

(Where, A=Average & % increase= Percentage increase with control)

The physiological parameters, including chlorophyll a, chlorophyll b, total chlorophyll and carotenoids, were significantly influenced by organic treatment. Table 2 depicts the effect of different fertilizers on physiological parameters

- **Chlorophyll a and b:** The treatment of *Azolla* and *Lemna* combination increased chlorophyll a by 81.50% and chlorophyll b by 90.50% compared to the control. NPK yielded only 33.18% and 18.61% increase respectively.
- **Total Chlorophyll:** The organic combination treatment led to an 89.05% increase, whereas NPK resulted in a 21% rise.
- **Carotenoids:** *Lemna* alone produced the highest carotenoid increase (89.05%), while the *Azolla* and *Lemna* combination showed a 39.07% rise. NPK-treated plants showed only a 21% increase (Table 2).

Table 2: Effect of different fertilizers on physiological parameters

Treatment	Chlorophyll a		Chlorophyll b		Total chlorophyll		Carotenoid	
	A	% ^	A	%^	A	%^	A	%^
Control	2.54	0%	3.62	0%	3.83	0%	3.8	0%
Azolla	2.90	14.46%	4.93	36.23%	5.08	32.80%	5.0	32.77%
Lemna	3.40	34.25%	5.07	40.00%	5.33	39.07%	7.24	89.05%
Azolla& Lemna	4.61	81.50%	6.90	90.50%	7.24	89.05%	5.33	39.07%
Cow dung	3.48	37.32%	6.30	73.81%	6.43	68%	6.43	68%
NPK	3.38	33.18%	4.30	18.61%	4.63	21%	4.63	21%

(Where, A=Average & % increase= Percentage increase with control)

- **Flower Production:** The *Azolla* and *Lemna* treatment resulted in a remarkable 6463.63% increase in flower number compared to control, significantly outperforming NPK (100% increase).
- **Fruit Yield:** The same organic combination produced 11.66 times more fruits than control, while NPK- treated (Table 3).

Table 3: Effect on flower and fruit production

Treatment	No. of Flowers		No. of Fruits
	A	% increase	A
Control	0.33	0%	0
Azolla	6	1718.18%	4
Lemna	15.67	4645.45%	9.33
Azolla & Lemna	21.67	6463.63%	11.67
Cow dung	2.33	606.06%	1.33
NPK	0.67	100%	0.33

ANOVA revealed that both treatment type and time (DAS) significantly influenced all measured parameters.

- **Plant Height:** Treatment ($F = 6.61$, $p < 0.001$) and days ($F = 95.67$, $p < 2e-16$) had significant effects.
- **Branch Number:** Highly significant effects were observed for both treatment ($F = 19.88$, $p = 1.67e-10$) and days ($F = 374.74$, $p < 2e-16$).
- **Leaf Count:** Treatment ($F = 23.95$, $p = 8.86e-12$) and days ($F = 1337.01$, $p < 2e-16$) were both significant.
- **Flower and Fruit Count:** Treatment effects were highly significant for flower number ($F = 75.97$, $p = 1.16e-08$) and fruit yield ($F = 73.56$, $p = 1.75e-07$), indicating clear advantages of organic treatments.

The study was the first to compare the combined use of *Azolla* and *Lemna* as biofertilizers on *S. lycopersicum,* revealing that the combination treatment outperformed both individual applications and chemical fertilizers. Previous studies showed that *Lemna minor* improved lettuce yield (Baldi *et al.,* 2024) and *Azolla* extracts enhanced tomato growth (Hanafy & El-Emary, 2018), but none examined their combined effect or biomass incorporation. The present findings introduced a novel and sustainable fertilization approach, demonstrating superior plant growth, efficient nutrient recycling, and reduced input costs. This highlights the potential of these aquatic macrophytes as eco-friendly fertilizer alternatives. The future research refine has to application rates, evaluate long-term soil effects and test other crops to advance climate-resilient and low-input agriculture.

Ahmad and Tariq (2021) reiterated the use of Azolla as waste decomposer and bio-fertilizer. In another study, Sonowal *et al.* (2021) observed that both *Azolla pinneta* and *Lemna minor* possess good nutritive values and suitable production behaviour in agro climatic conditions of Assam. But, *Azolla pinneta* showed a better results than L. minor in the study. In addition, the use of Azolla as a source of biofertilizer for sustainable crop production was also well reviewed (Santhiya and Jeeva, 2022). These findings encourage the use of Azolla-based biofertilization techniques in environmentally responsible and sustainable agricultural systems.

To conclude, the dual application of *Azolla* and *Lemna* offers a pragmatic, low-cost, and climate-resilient pathway to reduce global dependence on energy-intensive synthetic fertilizers. By recycling nutrient-rich aquatic biomass, this approach not only elevates crop productivity but also fosters ecological stewardship, mitigates agricultural runoff, prevents eutrophication and enhances long-term soil sustainability. Therefore, promoting the decentralized cultivation of these macrophytes among rural farming communities emerges as a transformative strategy for advancing sustainable, organic and resilient agricultural systems in the face of escalating environmental degradation and global food-security challenges.

CONCLUSION

The present investigation conclusively establishes that the combined application of *Azolla microphylla* and *Lemna minor* represents a highly efficacious, sustainable and economically viable biofertilization strategy for enhancing the productivity of *Solanum lycopersicum* L. The synergistic integration of these two aquatic macrophytes consistently outperformed all other treatments, including conventional NPK (10:10:10) and traditional cow dung across the vast majority of assessed vegetative, physiological and reproductive parameters. Notably, the combination treatment yielded a 120.61% increase in plant height at 60 DAS, an extraordinary 6463.63% surge in flower production, and a remarkable 11.66-fold enhancement in total fruit yield relative to the unfertilized control. Furthermore, the substantial improvements in chlorophyll a (81.50%), chlorophyll b (90.50%) and total chlorophyll (89.05%) underscore the profound physiological priming effect induced by this organic amendment, which significantly bolstered photosynthetic efficiency and overall plant vigour. The Analysis of Variance (ANOVA) corroborated these findings with high statistical significance ($p < 0.001$) across all critical parameters, validating the reproducibility and reliability of the observed effects.

The superior performance of the combined treatment can be mechanistically attributed to the complementary nutrient profiles and decomposition dynamics of these macrophytes. This complementary synergy mitigates the limitations of individual applications, ensuring synchronized nutrient release that aligns seamlessly with the phenological demands of the tomato crop. These findings resonate strongly with the previous observations. This study is novel in providing comprehensive empirical evidence for the synergistic biofertilizer potential of *Azolla* and *Lemna* biomass incorporation under field conditions using a Randomized Block Design. In future, the applicability of this combined biofertilizer strategy must also be extended to other economically vital crops, including cereals, legumes and leafy vegetables, to validate its broader agricultural relevance.

Acknowledgement

The authors are thankful to the Principal, faculties and librarian of Sree Narayana College, Cherthala and Dr. Sevichan P.J., Rtd Associate professor, St. Joseph's College Devagiri, Kozhikode.

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