

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; SP-10(9): 843-846
www.biochemjournal.com
Received: 11-06-2026
Accepted: 14-07-2026

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Combined effect of organic manure and phosphate on growth of *Azolla microphylla*

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i9Sk.9919>

Abstract

The present investigation aimed to assess the effect of varying levels of organic matter on the growth and yield of *Azolla microphylla*. An experiment conducted in the Plant Pathology Section of the College of Agriculture in Nagpur in year 2025-2026 titled "Combined effect of organic manure and phosphate on the growth of *Azolla microphylla*." Ten treatments were used in three replications of the completely randomized design (CRD) experiment. *Azolla microphylla* was subjected to ten micronutrient treatments (K_2HPO_4), $Zn_3(PO_4)_2$, Cowdung, Vermicompost their combinations at 0.6 mg/cm³ and 2kg), with observations made at 15, 30, 45, and 60 DAI. Results revealed that Treatment T5 (Cowdung+ (K_2HPO_4)) consistently gave the highest performance across all parameters fresh weight, dry weight, chlorophyll content, heterocyst percentage, frond area, root length, and N/P uptake followed by T9 (K_2HPO_4)+(Zn₃(PO₄)₂)+Cowdung+Vermicompost), which also delivered significantly improved results. Conversely, Zinc Phosphate treatments (T2 and T6) suppressed biomass, pointing to zinc toxicity effects. Over time, both T5 and T9 showed distinct linear growth trajectories, emphasizing the critical role phosphorus plays in maintaining *Azolla* output. The literature provides good evidence for these results: supplementing *Azolla* with inorganic phosphorus (10-30 mg L⁻¹) is known to increase biomass and nutrient accumulation, but *Azolla's* capacity to absorb heavy metals like zinc depends on concentration, and too much zinc can hinder growth.

Keywords: *Azolla microphylla*, organic manure, phosphorus nutrition, biomass yield, nutrient uptake

Introduction

There are seven well-known species of the fast-growing water fern *Azolla* (family Salviniaceae) include *A. pinnata*, *A. caroliniana*, *A. filiculoides*, *A. mexicana*, *A. microphylla*, *A. nilotica*, and *A. rubra*. The name is typically derived from the Greek words azo, which means "to dry," and ollyo, which means "to kill," emphasizing the plant's vulnerability to drying out. *Azolla* and cyanobacteria (*Anabaena azollae* or *Nostoc azollae*), which are housed in cavities within the dorsal leaf lobes and where heterocysts efficiently fix atmospheric nitrogen, create an obligatory symbiotic relationship. The device can fix roughly 1.1 kg N per hectare each day, supplying up to 20-40 kg N/ha per cropping cycle in rice paddies or more depending on conditions.

In Asian rice fields, *Azolla* produces roughly 8-10 t fresh weight/ha/week and doubles its biomass very quickly often in 2-10 days, depending on species and conditions. It has been used as green manure for rice for generations in China and Vietnam, but its use in India and other countries picked up speed in the late 20th century. Each plant is tiny, delicate, and free-floating, with horizontal rhizomes (0.5-7 cm broad) and densely overlapping branching fronds. Each leaf features a bigger dorsal lobe that houses the symbiotic cyanobacteria and a thinner ventral lobe with few stomata and hairs for buoyancy. *Azolla* reduces reliance on artificial fertilizers by naturally supplying nitrogen to rice and other crops. Cheap, high-yield organic materials that can be used as mulch, compost, or animal feed. It can help with phytoremediation (removing heavy metals) suppress weeds, and lower evaporation. Experiments demonstrate its possible future uses in reducing atmospheric CO₂ as well as its historical significance in carbon sequestration (the so-called *Azolla* Event). Because of its great nutritional value and rapid development, it is often referred to as a "green gold mine" and a "super plant." The purpose of this assessment was to ascertain the impact of zinc, phosphorus, and other micronutrient combinations on *Azolla* growth.

Material and method

Freshly harvested blotted fern *Azolla* fronds from mother culture kept in tap water made up the inoculum. Fresh biomass and the dry weight of the fronds were used to record growth observations. *Azolla* growth was investigated using micronutrients such as potassium hydrogen phosphate (K_2HPO_4), and zinc phosphate ($Zn_3(PO_4)_2$) @ 0.6 mg/cm³ and organic manure such as Vermicompost, cowdung @ 2kg in a 200-liter FRP tank measuring $4 \times 2 \times 1$ ft³. Three-quarters of the tank's capacity, or 150 liters of water each tank, was filled with tap water. Every micronutrient and organic manure was added according to the specifics of the treatment. After growing the *Azolla* inoculum in water for five days, 50 grams of *Azolla* were added to each tank. On the 15th, 30th, 45th, and 60th DAI, observations were made.

Treatments details

Treatments
T1 - Potassium hydrogen phosphate (K_2HPO_4)
T2 - Zinc phosphate ($Zn_3(PO_4)_2$)
T3 - Cowdung
T4 - Vermicompost
T5 - Cowdung+Potassium hydrogen phosphate (K_2HPO_4)
T6 - Cowdung+ Zinc phosphate ($Zn_3(PO_4)_2$)
T7 - Vermicompost+Potassium hydrogen phosphate (K_2HPO_4)
T8 - Vermicompost+ Zinc phosphate ($Zn_3(PO_4)_2$)
T9 - Potassium hydrogen phosphate (K_2HPO_4) +Zinc phosphate ($Zn_3(PO_4)_2$) + Cowdung+Vermicompost
T10 - Uninoculated Control

The colorimetric method recommended by Subudhi and Watanabe (1981) [13] was used to measure the total chlorophyll content (mg g⁻¹) of the dried leaves. Jackson (1967) provided the vanadomolybdate yellow color method, which was used to assess the phosphorus content of leaves. The Micro Kjeldhal technique described by Ali and Watanabe (1986) [6] was used to analyze the nitrogen content of *Azolla*. The following formula was used to compute the heterocyst percentage in accordance with.

$$\text{Heterocyst percentage} = \frac{\text{No. of heterocyst cell}}{\text{Total no. of vegetative cell}} \times 100$$

Result and Discussion

Statistical analysis revealed significant differences among the treatments in terms of both fresh and dry biomass of *Azolla microphylla* when compared with the uninoculated control. Among the treatments, T5 (Cowdung + K_2HPO_4) recorded the maximum fresh biomass throughout the experimental period. The fresh weight under T5 was 830.66 g/pot at 15 DAI, which increased to 1330.66 g/pot at 30 DAI, 1930.89 g/pot at 45 DAI, and 2034.22 g/pot at 60 DAI. Thus, T5 maintained its superiority over the other treatments at all observation intervals. This result are in accordance with earlier findings of Hamid *et al.* (2007) [6], Ali and Watanabe (1986) [6], Dehane (2022) [3]. A comparable pattern was observed for dry biomass. Treatment T5 exhibited the highest dry weight, with values of 52.20, 83.62, 115.85 and 126.24 g/pot at 15, 30, 45 and 60 DAI, respectively. The results indicate the better performance of the Cowdung + K_2HPO_4 combination in promoting biomass accumulation in *Azolla microphylla*.

The chlorophyll concentration also differed considerably among the treatments. Treatment T5 showed the highest

chlorophyll content, recording 1.39 and 1.62 mg/g at 15 and 30 DAI, respectively, followed by 1.74 and 1.66 mg/g at 45 and 60 DAI. The next superior treatment was T9 (K_2HPO_4 + $Zn_3(PO_4)_2$ + Cowdung + Vermicompost), which recorded chlorophyll values of 1.35 and 1.57 mg/g at 15 and 30 DAI, followed by 1.67 and 1.56 mg/g at 45 and 60 DAI respectively. Treatment T7 (Vermicompost + K_2HPO_4) ranked next, with chlorophyll concentrations of 1.32 and 1.53 mg/g at 15 and 30 DAI and 1.63 and 1.56 mg/g at 45 and 60 DAI, respectively. Overall, all treatments exhibited higher chlorophyll content than the uninoculated control, whereas T10 recorded the lowest chlorophyll concentration. A declining trend in chlorophyll content was observed from 45 to 60 DAI, although most treatments remained statistically comparable during this period. Same findings were recorded by Ghatge (2013) [5], Andreeilee *et al.* (2015) [2], and Ismail *et al.* (2010) [7].

Heterocyst percentage, an important indicator of nitrogen-fixing activity in *Azolla*, was evaluated at each observation interval. Treatment T5 (Cowdung + K_2HPO_4) recorded the highest heterocyst percentage, with values of 12.05% at 15 DAI, 29.27% at 30 DAI, 33.01% at 45 DAI and 35.01% at 60 DAI. This was followed by T9 (K_2HPO_4 + $Zn_3(PO_4)_2$ + Cowdung + Vermicompost) and T7 (Vermicompost + K_2HPO_4). The treatments differed significantly from the uninoculated control. The heterocyst percentage generally showed an increasing tendency up to 60 DAI, while a decline was observed from 45 to 60 DAI according to the overall trend described for the treatments. Despite these variations, several treatments were statistically at par with one another. The reported by Potdukhe *et al* (2020) [10] documented the same.

Phosphorus and nitrogen concentrations of *Azolla microphylla*. Treatment T5 consistently recorded the highest nitrogen concentration, whereas T9 exhibited the highest phosphorus concentration throughout the observation period. Phosphorus concentration among the treatments ranged from 0.36 to 0.80% at 15 DAI, 0.42 to 0.83% at 30 DAI, 0.48 to 0.88% at 45 DAI and 0.45 to 0.95% at 60 DAI. Similarly, nitrogen concentration varied from 3.58 to 4.58% at 15 DAI, 3.78 to 4.75% at 30 DAI, 3.89 to 4.83% at 45 DAI and 3.72 to 4.93% at 60 DAI. The findings indicated that the important role of phosphorus in the growth and productivity of *Azolla microphylla*. Phosphate deficiency was associated with reduced biomass production, lower chlorophyll concentration, decreased heterocyst percentage and reduced nitrogen and phosphorus accumulation. Earlier studies conducted by Subudhi *et al.* (1981) [13], Hamid *et al* (2007) [6], Potdukhe *et al* (2020) [10], Gatole (2019) [4].

Frond size of *Azolla microphylla* was recorded at 15, 30, 45 and 60 DAI. Treatment T5 (Cowdung + K_2HPO_4) produced the largest fronds throughout the experimental period. The corresponding values were 1.67, 1.73, 1.81 and 1.89 cm² at 15, 30, 45 and 60 DAI, respectively, indicating its superior performance over the remaining treatments. In contrast, T2 ($Zn_3(PO_4)_2$) resulted in the smallest frond size during the observation period. The values recorded under this treatment were 1.12, 1.20, 1.25 and 1.66 cm² at 15, 30, 45 and 60 DAI, respectively. The results of the present investigation are similar to those of Dehane (2022) [3].

Root length was also assessed at 15, 30, 45 and 60 DAI. The longest roots were observed in T5 (Cowdung + K_2HPO_4), with recorded values of 7.56, 7.66, 7.76 and 7.86 cm at 15, 30, 45 and 60 DAI, respectively. Treatment T9 (K_2HPO_4 +

$Zn_3(PO_4)_2$ + Cowdung + Vermicompost] ranked next, recording root lengths of 7.51, 7.61, 7.21 and 7.81 cm at the respective observation intervals. The shortest roots were recorded in T2 ($Zn_3(PO_4)_2$), with values of 5.72, 5.84, 5.94

and 6.04 cm at 15, 30, 45 and 60 DAI, respectively. Overall, the results demonstrated better root development under T5 compared with the other treatments.

Table 1: Effect of different treatments on growth parameters on Azolla microphylla at various stages

Treatments	Fresh weight (g/tank)				Dry weight (g/tank)				Chlorophyll content (mg/g)				Heterocyst Percent			
	15 DAI	30 DAI	45 DAI	60 DAI	15 DAI	30 DAI	45 DAI	60 DAI	15 DAI	30 DAI	45 DAI	60 DAI	15 DAI	30 DAI	45 DAI	60 DAI
T1 - K_2HPO_4	322.16	822.16	1045.15	1122.15	20.10	51.30	62.71	68.84	1.20	1.26	1.36	1.33	17.18	19.21	22.41	24.41
T2 - $Zn_3(PO_4)_2$	94.66	147.99	334.5	320.85	6.35	11.27	20.09	21.86	0.90	1.15	1.26	1.17	10.64	12.65	10.68	14.68
T3 - Cowdung	534.33	1034.33	1504.27	1610.27	32.85	63.59	90.26	98.72	1.30	1.40	1.43	1.38	19.37	21.77	23.67	25.67
T4 - Vermicompost	485.16	985.16	1385.13	1595.13	30.25	61.67	83.11	91.18	1.26	1.36	1.42	1.37	25.93	21.33	22.50	23.17
T5 - Cowdung+ (K_2HPO_4)	830.66	1330.66	1930.89	2034.22	52.20	83.62	115.85	126.24	1.39	1.62	1.74	1.66	12.05	29.27	33.01	35.01
T6 - Cowdung+ ($Zn_3(PO_4)_2$)	96.66	196.66	516.86	636.86	6.80	13.77	31.01	33.76	1.11	1.18	1.30	1.20	20.70	14.06	18.06	20.06
T7 - Vermicompost+ (K_2HPO_4)	645.16	1145.16	1545.33	1646.33	39.85	70.38	92.72	101.16	1.32	1.53	1.63	1.56	13.04	22.72	24.92	26.92
T8 - Vermicompost+ ($Zn_3(PO_4)_2$)	102.16	602.16	802.19	905.52	7.05	41.34	48.13	52.46	1.14	1.21	1.35	1.24	22.66	15.14	19.14	22.14
T9 - (K_2HPO_4) + ($Zn_3(PO_4)_2$) + Cowdung+ Vermicompost	792.50	1292.50	1692.47	1761.14	50.15	81.71	101.55	110.72	1.35	1.57	1.67	1.56	16.53	24.70	27.70	31.57
T10 - Control	79.36	80.67	89.57	83.36	4.30	4.93	4.47	4.70	0.94	1.02	1.70	0.93	16.53	24.70	27.70	31.57
F test	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig
SE $\pm$ (m)	10.30	13.41	12.24	12.44	0.27	0.44	0.47	0.43	0.02	0.03	0.02	0.01	0.17	0.19	0.17	0.19
C.D (P=0.01)	41.46	53.95	49.24	50.07	1.08	1.75	1.87	1.72	0.07	0.11	0.07	0.6	0.67	0.78	0.70	0.77

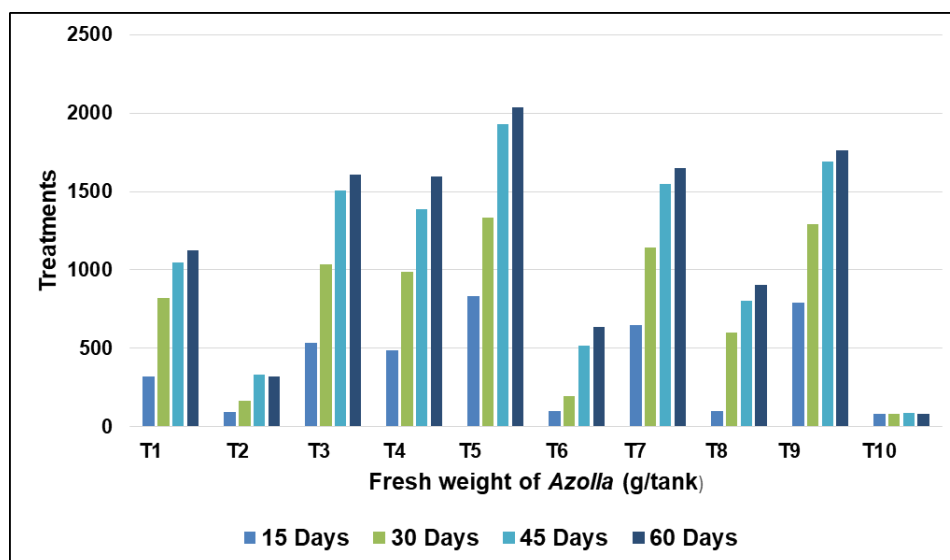


Fig 1: Fresh weight (gm/tank) of Azolla as influenced by different treatments at various intervals

Table 1: Effect of different treatments on growth parameters on Azolla microphylla at various stages

Treatments	Nitrogen content (per cent)				Phosphorous content (per cent)				Frond size (cm ²)				Root length (cm)			
	15 DAI	30 DAI	45 DAI	60 DAI	15 DAI	30 DAI	45 DAI	60 DAI	15 DAI	30 DAI	45 DAI	60 DAI	15 DAI	30 DAI	45 DAI	60 DAI
T1 - K_2HPO_4	4.26	4.47	4.65	4.77	0.62	0.68	0.72	0.82	1.21	1.32	1.38	1.50	7.04	7.11	7.21	7.31
T2 - $Zn_3(PO_4)_2$	4.14	4.28	4.33	4.20	0.48	0.57	0.61	0.68	1.12	1.20	1.25	1.33	5.72	5.84	5.94	6.04
T3 - Cowdung	4.40	4.66	4.72	4.82	0.68	0.74	0.80	0.93	1.39	1.48	1.55	1.66	7.20	7.28	7.38	7.48
T4 - Vermicompost	4.30	4.61	4.69	4.79	0.60	0.70	0.75	0.85	1.25	1.35	1.42	1.54	7.10	7.17	7.27	7.37
T5 - Cowdung+ (K_2HPO_4)	4.58	4.75	4.83	4.93	0.80	0.83	0.88	0.95	1.67	1.73	1.81	1.89	7.56	7.66	7.76	7.86
T6 - Cowdung+ ($Zn_3(PO_4)_2$)	4.17	4.37	4.47	4.57	0.52	0.60	0.64	0.74	1.15	1.25	1.29	1.37	6.18	6.28	6.45	6.55
T7 - Vermicompost+ (K_2HPO_4)	4.29	4.69	4.78	4.88	0.76	0.78	0.81	0.91	1.43	1.57	1.61	1.76	7.43	7.53	7.63	7.73
T8 - Vermicompost+ ($Zn_3(PO_4)_2$)	4.25	4.44	4.57	4.67	0.57	0.66	0.71	0.81	1.13	1.29	1.32	1.43	6.99	6.83	7.19	7.26
T9 - (K_2HPO_4) + ($Zn_3(PO_4)_2$) + Cowdung+ Vermicompost	4.55	4.72	4.77	4.87	0.78	0.81	0.84	0.94	1.55	1.62	1.70	1.77	7.51	7.61	7.71	7.81
T10 - Control	3.58	3.78	3.89	3.72	0.36	0.42	0.48	0.45	1.06	1.12	1.16	1.23	5.57	5.67	5.77	5.87
F test	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig
SE $\pm$ (m)	0.03	0.04	0.02	0.03	0.02	0.03	0.02	0.02	0.01	0.03	0.01	0.02	0.13	0.15	0.09	0.15
C.D (P=0.01)	0.12	0.16	0.10	0.12	0.08	0.11	0.07	0.08	0.05	0.12	0.04	0.08	0.52	0.62	0.37	0.61

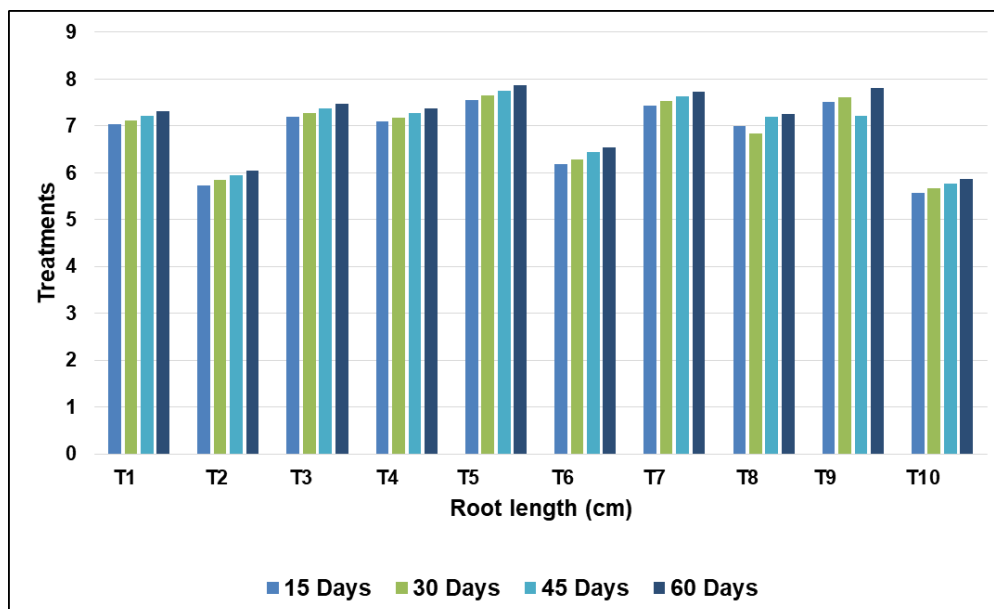


Fig 2: Root length of Azolla as influenced by different treatments at various intervals

The present investigation revealed that varying levels of organic matter had a significant effect on the growth, yield, and physiological characteristics of Azolla. Among the treatments evaluated, T5 (Cowdung+ (K_2HPO_4)) exhibited the best overall performance throughout the observation period. This treatment produced the maximum fresh and dry biomass, chlorophyll content, heterocyst percentage, nitrogen and phosphorus content, along with greater frond size at all observation intervals. The improved growth and physiological attributes may be associated with enhanced nutrient availability, which promoted efficient nutrient assimilation and symbiotic activity. These results are consistent with earlier studies that highlighted the beneficial role of organic manure and micronutrient amendments in improving Azolla production. Hence, the application of Cowdung+ (K_2HPO_4) at an appropriate level may be recommended as a suitable practice for sustainable Azolla cultivation and effective nutrient management. The long-term effectiveness of cowdung + K_2HPO_4 on Azolla growth, nutrient accumulation, and its potential as a biofertilizer may also be assessed.

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