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# Comparative Evaluation of *Azolla*-Based Biofertilizers and Inorganic NPK Fertilizers on Soil Physico-Chemical Properties, Nutrient Dynamics and Early Plant Growth

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## Abstract

The growing reliance on chemical-intensive fertilizers has created severe concerns about soil deterioration, nutritional imbalance, and long-term agricultural viability. Organic remedies, particularly biofertilizers like *Azolla*, present a promising way to restore soil health while maintaining crop output. The current study compared the effects of *Azolla*-based biofertilizers, alone and combined with cow dung, to inorganic NPK fertilizers on soil physicochemical parameters, nutrient dynamics, and early plant growth under pot culture conditions. A Randomized Complete Block Design (RCBD) was used, with five treatment options: control (no fertilization), *Azolla*, cow dung, *Azolla* plus cow dung, and NPK fertilizer. Soil pH, organic content, moisture content, and NPK levels were measured up to 60 days after planting, as well as seed germination and seedling growth parameters. The results showed that the combined *Azolla* + cow dung treatment (T<sub>3</sub>) provided the most balanced improvement in soil quality, maintaining a stable pH, significantly increasing organic carbon (up to 16%), improving phosphorus availability (36 kg/ha), and increasing potassium content while supporting favorable moisture retention. The *Azolla*-only treatment (T<sub>1</sub>) had the highest soil moisture levels, resulting in improved root growth and 100% seed germination. Although the NPK treatment (T<sub>4</sub>) produced the highest soil nitrogen and potassium concentrations, it did not lead to superior plant growth when compared to organic treatments. Organic amendments consistently boosted root and shoot development, indicating higher nutrient uptake efficiency and soil biological activity. Overall, the study shows that *Azolla*, especially when coupled with cow dung, can successfully increase soil fertility, moisture retention, and plant development, providing a sustainable alternative or supplement to synthetic fertilizers. These findings encourage the use of *Azolla*-based biofertilization techniques in environmentally responsible and sustainable agricultural systems.

**Keywords:** Climate resilience, Organic fertilizers, Potash, Phosphorus, Nitrogen, Moisture retention, Organic content

## Introduction

The evolution of fertiliser use, from conventional organic sources to contemporary chemical-intensive techniques, is affecting agricultural production in various climate zones.



It draws attention to the increasing demand for sustainable methods that support biodiversity preservation, soil health, ecological balance, and less environmental impact. Egyptian cotton farming and the use of *Azolla* are two examples of organic farming, which has its roots in early 20th-century concerns about the health and environmental impacts of conventional agriculture. (van Bommel et al., 2021) In contrast to contemporary chemically driven systems, agriculture that only used organic inputs historically delivered better food and steady yields (De Britto and Girija, 2021.) However, the sustainability of agroecosystems has been severely strained by the transition to intensive farming, which involves the widespread use of synthetic fertilizers, pesticides, and monoculture techniques (Punia and Khetarpaul, 2008).

Nutrient dynamics, soil health, and biodiversity all change significantly between conventional and organic farming, according to comparative research. (Chausali and Saxena, 2021; Rathore et al., 2018) According to Conventional systems, frequently increase yields through synthetic inputs but deteriorate soil and water quality. In contrast, organic systems stimulate microbial activity, repair soil structure, and promote long-term sustainability. According to research on phosphorus dynamics, plant absorption and nutrient availability are controlled by mineral interactions and soil pH (Penn and Camberato, 2019b; Tabatabai, 1996).

Crop productivity still depends on balanced fertilization techniques. Humic acid combined with lower NPK rates (75 percent of the recommended dose) maintains growth and production while reducing fertilizer inputs, according to studies on Egyptian cotton (Seadh et al., 2012; Soomro et al., 2000). The significance of combining organic amendments with inorganic fertilizers for sustainable pest and nutrient control is highlighted by the fact that too much nitrogen might increase pest pressure (Reddy et al., 2020). The nitrogen-fixing water fern *Azolla* is one of the biofertilizers that has drawn interest due to its ecological and agricultural adaptability. It has been shown to increase crop yields, improve soil fertility, and be used as animal feed and green manure (Rehman Raja et al., 2012; Sadeghi et al., 2013). *Azolla*-based systems support sustainable agriculture and environmental rehabilitation in addition to lowering reliance on synthetic fertilizers. The floating fern known as azolla may thrive in waters devoid of nitrogen (Kösesakal and Yildiz, 2019). *Azolla pinnata* is the species most seen in India and other tropical and temperate regions of the world. Thus, the current study is to evaluate the levels of vital elements like nitrogen, phosphorus, and potassium, compare inorganic fertilizers with *Azolla*-based biofertilizers, and explore the effects of *Azolla* inclusion on the physical characteristics of soil.

## Material and Methods

### Conducting and designing the experiment

The plant material was collected from Artificial Pond created for *Azolla* cultivation from JSS Academy of Higher Education & Research, Mysuru (12°20'39.5"N 76°39'05.8"E) This experiment consisted of 5 treatments with fertilizer applied in the experimental pots. All 30 pots were filled with 2kg of soil, and the respective number of fertilizers was added to each pot as shown in **Table 1**. The pots were arranged in Randomized Complete Block Design (RCBD) and the experiment was conducted in triplets.

**Table 1:** Treatments used for the current study

Symbol	Treatments
T <sub>0</sub>	Control (No Fertilization)
T <sub>1</sub>	<i>Azolla</i> (50 Grams per 2kg soil)
T <sub>2</sub>	Cow dung (50 Grams per 2kg soil)
T <sub>3</sub>	<i>Azolla</i> + Cow dung (50 Grams per 2kg soil)
T <sub>4</sub>	NPK (15 Grams per 2kg soil)

### Soil Analysis

A soil solution was prepared in the ratio of 1:5 using wrist action shaker to measure the pH of the soil. pH was measured using Labman LMPH-9 pH meter (Shi et al., 2009). To measure organic matter of soil 1 gram of soil is taken in a conical flask to which 10 ml of Potassium dichromate added. 20 ml of Sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) is added to the flask slowly. After 30 minutes, 200 ml of distilled water is added to the flask and back titrated with Ferrous Ammonium Sulphate (FAS) with diphenylamine indicator. The soil organic content is then calculated using the given formula (Schulte, 2012). To analyse the Moisture Content, 10 Grams of soil is taken in a pre-weighed crucible. The crucible is then kept in Hot air oven at 110°C for 16 hours. The crucible is then taken out and kept in a desiccator for cooling. The weight is then checked, and the difference in weight is calculated to measure the moisture content (Rowe, 2018).

$$\text{Moisture content} = \frac{W_2 - W_3}{W_3 - W_1} \times 100$$

Weight of dry Crucible - W<sub>1</sub>

Weight of sample before drying - W<sub>2</sub>

Weight of sample after drying - W<sub>3</sub>

## Results

**Table 2:** Initial Soil Parameters

Characteristics	Initial value
Soil pH (pH)	6.8
Organic carbon (%)	19.2
Nitrogen (N) (Kg/ha)	140
Phosphorous (P <sub>2</sub> O <sub>5</sub> ) (Kg/ha)	14
Potash (K) (Kg/ha)	65
Moisture content in the soil (%)	1.47

### pH Content in Soil

In T<sub>3</sub> treatment, the observed pH range was more stable. Treatment T<sub>3</sub>, which includes *Azolla* and cow manure, has the most consistent pH levels and optimal nutrient availability for plant uptake.

**Table 3:** pH Content Soil

### Organic Content in Soil

Across treatments, there are substantial differences in the soil's organic content (%) from days after planting (DAP) 10 to DAP 60. With an initial high of 13.5% and a subsequent stabilization at 11.6%, the control group exhibits expected fluctuations. Significant increases in organic content are shown by treatments T<sub>1</sub> and T<sub>3</sub>, with T<sub>1</sub> increasing gradually from 12.9% to 16.8% and T<sub>3</sub> continuing to rise at a constant pace, reaching a peak of 16%.

**Table 4:** Organic content in Soil

Organic content	DAP 10	DAP 20	DAP 30	DAP 40	DAP 50	DAP 60
T <sub>0</sub> (%)	10.7	8.2	8.5	12	13.5	11.6
T <sub>1</sub> (%)	12.9	15.6	12.4	14	14.5	16.8
T <sub>2</sub> (%)	12.6	13.9	10.3	12.4	13	11.8
T <sub>3</sub> (%)	13	15.3	12.9	12.1	13.6	16
T <sub>4</sub> (%)	9.4	10.7	11	8.2	10.2	9.7

**Footnote:** All values are expressed as organic content in percentage (%)

### Moisture Content in Soil

T<sub>1</sub> consistently maintained higher soil moisture levels (20.4% to 30.5%) than other treatments, suggesting that it would be the best choice. Conversely, T<sub>4</sub> consistently has a lower moisture content (4.2% to 11.6%), which might indicate challenges for optimal plant development if not managed correctly.

**Table 5:** Moisture content in Soil

Treatments	DAP 10	DAP 20	DAP 30	DAP 40	DAP 50	DAP 60
T <sub>0</sub> (%)	24.2	11.9	22.1	22.2	19.7	20.9
T <sub>1</sub> (%)	25.9	20.4	26.7	27.5	30.5	22.6
T <sub>2</sub> (%)	14.4	15.1	21.8	14.6	16.5	19.6
T <sub>3</sub> (%)	17.2	11.6	13.1	15.6	23.9	20.7
T <sub>4</sub> (%)	5.2	4.7	4.2	9.8	5.6	11.6

### NPK Content in Soil

All treatments had nitrogen levels before planting that varied from 5.4 to 5.7 units, indicating a generally stable starting state. Treatment T<sub>4</sub> had the maximum nitrogen absorption at 344 units, with the nitrogen concentration varying from 336 to 344 units after 60 days. Initial phosphorus levels ranged from 0.5 to 0.7 units. After 60 days, all treatments showed a steady rise in phosphorus content, with Treatment T<sub>3</sub> demonstrating the largest increase at 36 units. The initial potassium readings, which ranged from 2.6 to 2.68 units, were comparatively constant. Following planting, potassium levels rose consistently, with Treatment T<sub>4</sub> having the greatest potassium concentration (160.8 units). Table 6 summarizes the NPK content for each treatment both before planting and 60 days thereafter. Soil fertility categories from (Potdar et al., 2021) were utilized to interpret the nutrient status in Table 7.

The treatments had a noticeable impact on seed germination. With a germination index of 50%, the Control group showed that fewer seeds germinated are 3. As opposed to the Control, Treatments T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, and T<sub>4</sub> all had greater germination rates with 100% germination indices, suggesting that these treatments were successful in promoting seed germination. Compared to the Control (12 cm), the roots of the T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, and T<sub>4</sub> treatments were longer. With roots that were 18.9 cm long, T<sub>1</sub> was the longest, followed by T<sub>2</sub> (17.6 cm), T<sub>3</sub> (16.3 cm), and T<sub>4</sub> (15.3 cm). This demonstrates that both treatments enhanced root formation when compared to the untreated Control. Treatments T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, and T<sub>4</sub> encouraged larger shoot lengths in comparison to the Control (10.5 cm). T<sub>2</sub> exhibited

the longest shoots (16.9 cm), with T<sub>1</sub> (16.8 cm), T<sub>3</sub> (15.6 cm), and T<sub>4</sub> (10.8 cm) following closely after. This suggests that these treatments promoted considerable shoot elongation in addition to stimulating root development relative to other treatments.

**Table 6:** NPK Content in Soil

Treatments	Before plantation			Soil fertility level		After 60 days of plantation		
	N (Kg/ha)	P (Kg/ha)	K (Kg/ha)	Before	After	N (Kg/ha)	P (Kg/ha)	K (Kg/ha)
T <sub>0</sub>	5.6	0.56	2.6	Low	High	336	33.6	156
T <sub>1</sub>	5.4	0.70	2.64	Low	High	338	35	158.4
T <sub>2</sub>	5.5	0.58	2.63	Low	High	336	34.7	158
T <sub>3</sub>	5.6	0.5	2.65	Low	High	341	36	158.9
T <sub>4</sub>	5.7	0.6	2.68	Low	High	344	38	160.8

**Table 7:** Soil fertility levels of nutrients N, P and K (Potdar et al., 2021).

Soil fertility levels	Nitrogen (kg/ha)	Phosphorus (kg/ha)	Potassium (kg/ha)
Very low	Less than 140	Less than 7	Less than 100
Low	141-280	7.1-14	101-150
Medium	281-420	14.1-21	151-200
Moderately high	421-560	21.1-28	201-250
High	561-700	28.1-35	251-300
Very high	Greater than 700	Greater than 35	Greater than 300

**Table 8:** Growth of the plant

Treatments	No of Seeds Germinated	Germination Index (%)	Length of roots (cm)	Length of shoots (cm)	No of Leaves
T <sub>0</sub>	3	50%	12	10.5	2
T <sub>1</sub>	6	100%	18.9	16.8	3
T <sub>2</sub>	6	100%	17.6	16.9	3
T <sub>3</sub>	6	100%	16.3	15.6	4
T <sub>4</sub>	3	50%	15.3	10.8	3

### Discussion

The results of this study are in line with earlier studies and provide compelling evidence for the significance of *Azolla* and other organic fertilizers in improving soil health, nutrient availability, and plant development. Organic matter, moisture retention, and phosphorus availability all significantly improved when *Azolla* and cow dung (T<sub>3</sub>) were applied together. This is in line with the findings of (Ahmad and Tariq, 2021), who stated that *Azolla*'s high nitrogen content and quick decomposition make it an effective biofertilizer, enhancing the soil with vital nutrients and organic carbon.

The enhancement of soil pH stability under T<sub>3</sub> is consistent with previous research by (Penn and Camberato, 2019), which highlighted the role of organic inputs in buffering soil pH and increasing phosphorus availability. This process is supported by the enhanced P uptake seen in T<sub>3</sub> (36 units), which demonstrates the critical role that organic-inorganic nutrient interactions play in crop absorption and nutrient solubility. Furthermore, the considerably greater moisture retention in *Azolla*-treated soils (T<sub>1</sub> and T<sub>3</sub>) supports the findings of (Lynch, 2022), who showed that increased microbial activity and organic carbon building in organically altered soils improve their structure and water-holding ability. The increased germination percentage and longer root lengths shown in organic treatments relative to the control can be explained by the necessity of moisture availability for seed germination and early development.

The results of (Rehman Raja et al., 2012) who described *Azolla* as a growth-promoting biofertilizer capable of enhancing nitrogen availability and improving physiological processes like chlorophyll development, were supported by the significantly better shoot and root development in organic treatments. Similarly, (Cevheri, 2021) found that by enhancing soil structure and microbial interactions, organic fertilizers increase cotton growth metrics in both saline and non-saline settings. These findings are consistent with the longer shoots seen in T<sub>1</sub>, T<sub>2</sub>, and T<sub>3</sub>. After 60 days, nitrogen and potassium levels were greater with inorganic fertilizer (T<sub>4</sub>), but plant development metrics were not higher than those of the organic treatments. This tendency is consistent with the findings of (Chausali and Saxena, 2021), who contended that although chemical fertilizers boost nutrient content, they may not always be as beneficial to long-term soil health or biological production as organic additions. Overall, the findings show that by increasing soil fertility, boosting nutrient absorption, encouraging plant

development, and supporting environmentally resilient farming, *Azolla*, either by itself or in combination with cow manure, may partially replace or supplement artificial fertilizers.

### Conclusion

In conclusion, addressing the environmental problems associated with conventional agriculture requires the use of organic farming practices. Because organic farming prioritises soil health via natural techniques like crop rotation, intercropping, and mulching, it fosters sustainability and biodiversity. It enhances soil fertility and microbial diversity while reducing pollutants and conserving water. The innovative use of *Azolla* as a biofertilizer shows that organic farming may boost agricultural productivity without the use of synthetic chemicals. The benefits of organic farming are illustrated by study results that centre on specific values: Treatment T<sub>3</sub>, which contained cow dung and *Azolla*, raised the quantity of organic carbon from 13% to 16% while keeping pH levels between 6.7 and 8.1, as opposed to the control group's decrease to 11.6%. Additionally, treatment T<sub>3</sub> showed improved moisture retention (20.4% to 30.5%), a significant increase in potassium (160.8 kg/ha), and phosphorus (36 kg/ha), all of which aided in the absorption of plant nutrients. While shoot length (16.9 cm) was superior for Treatment T<sub>2</sub>, germination (100%) and root length (18.9 cm) were also outstanding for Treatment T<sub>1</sub>.

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#### Author Contributions

HGL, KB, BG, HT, SHB and MSRS conceived the concept, wrote and approved the manuscript.

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