Journal of Agriculture and Biology
ISSN: 3007-1755 (Print), 3007-1763 (Online)

Research Article

Effect of Farmyard Manure Seed Priming on Germination and Growth of Spinach (*Spinacia oleracea* L.)

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ABSTRACT

Spinach production is often constrained by poor seed germination and excessive reliance on chemical fertilizers, while the potential of organic seed priming, particularly with farmyard manure, to enhance seedling establishment and growth remains largely underexplored. A study was conducted at the Department of Horticulture, Sindh Agriculture University, Tandojam, during the winter season of 2024-2025 to investigate the impact of farmyard manure (FYM) seed priming at different concentrations on the germination and growth of spinach (*Spinacia oleracea* L.). The experiment was laid out in a Completely Randomized Design (CRD) with three replications. The treatments included: different concentration levels of farmyard manure. T_0 = Control (unprimed seeds) T_1 = Hydropriming (Distilled water), T_2 = 1% FYM T_3 = 10% FYM, T4 = 20% FYM, T_5 = 30% FYM. The results showed that seeds primed with 30% FYM resulted in the maximum values for all parameters, including seed germination (18.60%), germination index (5.96), number of leaves per plant (24.87), plant height (26.38 cm), plant fresh weight (17.75 g), shoot fresh biomass (14.24 g), root fresh biomass (3.38 g), shoot dry biomass (2.52 g), and root dry biomass (1.44 g). In contrast, the lowest seed germination percentage (11.30%) and germination index (3.05) were recorded in the 10% FYM treatment (T_3). Hydropriming (T_1) resulted in the lowest values for several growth parameters, including the number of leaves per plant (13.77), plant height (17.95 cm), shoot fresh biomass (4.40 g), and root dry biomass (0.10 g). Meanwhile, the control treatment (T_0) produced the lowest plant fresh weight (5.52 g) and fresh root biomass (1.13 g). In conclusion, seed priming with 30% farmyard manure significantly enhanced the germination and growth performance of spinach and is recommended over hydropriming as well as lower FYM concentrations.

Keywords: Spinach, Farmyard manure, Chemical fertilizer, Seed priming, Seed germination

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Article History
Received: May 26, 2025
Accepted: September 23, 2025
Published: October 09, 2025

INTRODUCTION

Spinach (*Spinacia oleracea* L.) is a cool-season, shallow-rooted green vegetable belonging to the Amaranthaceae family (which includes the former Chenopodiaceae). It originated in Southwest Asia, was domesticated by Arabs, transferred to Spain by the Moors, and has since been disseminated globally (Zhou et al., 2018; Aamur et al., 2025). It is exceptionally nutritious, rapidly growing, and is typically direct-seeded (Vignesh et al., 2012; Van Rensburg et al., 2007). It is one of the most nutrient-dense green vegetables, rich in vitamins (A, B_2, B_6, B_9, C, E, K, folate) and minerals (potassium, calcium, magnesium, iron, manganese, selenium), while being low in fat and calories and high in dietary fiber (Toledo et al., 2003; Umar et al., 2007; Rabie et al., 2014). It also contains bioactive compounds such as flavonoids, p-coumaric acid, and uridine, which possess antioxidant, anti-tumor, hypoglycemic, anti-inflammatory, and digestive health properties (Ismail et al., 2004; Ahmadi et al., 2010; Roughani and Miri, 2019; Murcia et al., 2020; Isaza et al., 2025). Regular consumption can reduce the risk of chronic diseases such as cardiovascular diseases and cancer, qualifying it as a functional food (Isaza et al., 2025). Due to its high nutritional value, short production cycle, and adaptability, spinach is a crucial crop for both commercial and smallholder farmers in Asia and Africa, contributing to food security and livelihoods (Abu et al., 2011; Kibirige et al., 2013), However, yields are often suboptimal due to inadequate agronomic and nutrient management practices (Aisha et al., 2013).

Seed priming, a pre-sowing technique that involves controlled hydration of seeds followed by re-drying, is a promising method for improving seed germination, seedling vigor, and crop stand establishment (Ashraf and Foolad, 2005; Abbasdokht, 2015; Chachar et al., 2025). Various priming techniques, such as hydro-priming (water), halo-priming (salts), osmo-priming (osmotic agents), thermo-priming (temperature), solid matrix priming and bio-priming (biological agents), have been extensively researched (Parera and Cantliffe, 2010; Yari et al., 2016). Priming enhances germination by stimulating pre-germinative metabolic activities, including nucleic acid repair, protein synthesis, and membrane restoration (Moradi et al., 2017), while also boosting antioxidative enzyme activity (Hsu et al., 2017) and glyoxysomal enzyme activity (Lin and Sung, 2001). These physiological advantages can translate into improved plant growth and yield, particularly under stress conditions. The effectiveness of priming, however, is highly dependent on the species, seed vigor, temperature, water potential, and the priming agent used (Parera and Cantliffe, 2010).

Traditional spinach cultivation often relies heavily on synthetic fertilizers, especially nitrogen and phosphorus, with poor potassium supplementation. This can lead to soil nutrient imbalance, degradation, and a loss of long-term soil fertility (Yousaf et al., 2017; Li et al., 2019; Chachar et al., 2024). Organic amendments, such as farmyard manure (FYM), offer a sustainable alternative for enhancing soil fertility and plant nutrition. FYM, one of the oldest and most common organic fertilizers, is a mixture of livestock excreta and bedding material (Lampkin, 2002). It provides macro- and micronutrients, improves soil physical properties, cation exchange capacity, water retention, aeration, and stimulates microbial activity (Watson et al., 2002; Soyergin, 2006; Mercik and Stepien, 2006; Schoenau, 2006; Adiloğlu and Eraslan, 2012; Prado et al., 2022; Shafique et al., 2023). The nutrient content of FYM, particularly nitrogen, phosphorus, and potassium, varies with animal type and age, bedding material, and storage conditions (Lampkin, 2002; Shafique et al., 2023). Incorporating FYM into seed priming regimens could potentially increase seed nutrient uptake, accelerate germination, improve early seedling development, and promote soil health in subsequent growth stages.

Given the established role of seed priming in improving germination and the benefits of FYM in nutrient supply and soil health, integrating FYM-based seed priming may represent a sustainable and cost-effective strategy for enhancing spinach production. Therefore, this study was conducted to investigate the effect of farmyard manure seed priming at different concentrations on the germination and early growth of *Spinacia oleracea* L.

MATERIALS AND METHODS

The experiment was conducted during the winter season of 2024–2025 in the Department of Horticulture, Sindh Agriculture University, Tandojam, Pakistan, located at approximately 25.45° N latitude and 68.49° E longitude, at an altitude of about 24 m above sea level. The region experiences average winter temperatures ranging from 10 °C to 20 °C. Well-rotted farmyard manure (FYM) was collected from the Department of Livestock Management, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam. The growing medium for pot filling was prepared by mixing soil, sand, and FYM in a 2:2:1 ratio.

Experimental Design and Treatments

The experiment was arranged in a Completely Randomized Design (CRD) with six treatments and three replications. Each treatment in every replication was represented by one pot, giving a total of 18 pots. Each pot contained 40 seeds T_0 = Control (unprimed seeds), T_1 = Hydropriming (Distilled water), T_2 = 1% FYM solution, T_3 = 10% FYM solution, T_4 = 20% FYM solution, T_5 = 30% FYM solution.

Seed Priming and Crop Establishment

FYM solutions were prepared by mixing the required quantity of composted FYM in distilled water and filtering through a double-layer muslin cloth to remove coarse particles. Seeds were soaked in their respective priming solutions for 12 hours at room temperature (25 ± 2 °C). After priming, the seeds were rinsed with distilled water, surface-dried on sterile filter paper for 30 minutes, and sown immediately in the pots filled with the 2:2:1 soil–sand–FYM mixture.

The pots were placed under natural outdoor conditions at Sindh Agriculture University, Tandojam, where the average winter temperature ranges between 10–20 °C, Irrigation was applied uniformly to all pots with canal water at two-day intervals to maintain adequate soil moisture. Thinning was performed within each pot at the 2–3 true leaf stage to maintain optimum plant population, while manual weeding was carried out regularly in the pots to avoid competition.

Data Collection and Methodology

The data of the germination and growth-related attributes were recorded for further statistical analysis. The observations were recorded, Seed germination (%), germination index, number of leaves per plant, plant height (cm), plant weight (g), fresh biomass of shoot (g), fresh biomass root (g), dry biomass of shoot (g), dry biomass of root (g).

Seed germination (%)= Seed germination percentage was calculated by using the following formula:

$$SG(\%) = \frac{Ng}{Nt} \times 100$$

Where,

Ng= Number of seeds germinated
Nt= Total number of seeds sown

Germination Index (GI): The germination index was calculated following the standardized method outlined by the Association of Official Seed Analysts (AOSA, 1983).

$$GI = \sum \frac{Gt}{Dt}$$

Where:
Gt = Number of seeds germinated on day t
Dt= Number of days after sowing (day t)

Number of Leaves Per Plant

The number of true leaves was recorded from five randomly selected plants in each treatment. Leaves were counted manually by visual observation. The mean number of leaves per plant was then calculated for each treatment and replication to obtain the average value.

Plant Height (cm)

Plant height was measured from the soil surface to the tip of the longest leaf using a measuring scale. Observations were recorded every 15 days during the growth period from the plants randomly selected in each treatment. The mean plant height was then calculated for each treatment and replication.

Plant Weight (g)

Entire plants were carefully uprooted from each treatment, washed gently to remove adhering soil particles, and weighed immediately using an electronic weighing balance. The mean plant weight was then calculated for each treatment and replication, and the results were expressed in grams (g).

Fresh Biomass of Shoot (g)

Shoots from five randomly selected plants in each treatment were separated from the roots, and their fresh biomass was measured immediately using an electronic weighing balance. The mean shoot fresh weight was calculated for each treatment and replication, and the results were expressed in grams (g).

Fresh Biomass of Root (g)

Roots from five randomly selected plants in each treatment were carefully separated from the shoots, washed gently to remove adhering soil particles, and weighed immediately using an electronic weighing balance. The mean root fresh weight was calculated for each treatment and replication, and the results were expressed in grams (g).

Dry Biomass of Shoot (g)

After recording the fresh biomass, shoot samples were oven-dried at 60 °C for 24 hours in a hot-air oven until a constant weight was obtained. The dry weight was then measured using an electronic weighing balance, and the values were expressed as mean dry biomass per plant in grams (g).

Dry Biomass of Root (g)

After recording the fresh biomass, root samples were oven-dried at **60 °C** for 24 hours in a hot-air oven until a constant weight was achieved. The dry weight was then measured using an electronic weighing balance, and the mean values were expressed as dry biomass per plant in grams (g).

Statistical Analysis

All collected data were subjected to a one-way analysis of variance (ANOVA) using Statistix 8.1 software (Statistix, 2006). The significance of treatment means was compared using the Least Significant Difference (LSD) test at a 5% probability level ($p \leq 0.05$).

RESULTS

Seed Germination and Early Growth Parameters

The effect of farmyard manure (FYM) seed priming at varying concentrations on seed germination (%), germination index, and number of leaves per plant in spinach was evaluated. Data are presented in Figure 1, with the analysis of variance in Table 1.

The results show a clear positive trend with increasing FYM concentrations, particularly at higher levels. Seed germination percentage was highest in T_5 (30% FYM) at 18.60%, followed closely by T_4 (20% FYM) at 13.3%. The lowest germination was recorded in T_3 (10% FYM) with 11.30%, while control treatment (T_0) and distilled water treatment (T_1) exhibited moderate germination levels (15.60% and 16.00%, respectively). Although differences in seed germination percentage were not statistically significant ($P = 0.2506$), the numerical data indicate a trend of improved germination with higher FYM concentrations.

A comparable trend was recorded for the germination index, with treatment T_5 (30% FYM) exhibiting the highest value (5.96), followed by T_4 (20% FYM) with a value of 4.95. The lowest germination index (3.05) was observed in T_3 (10% FYM). Although these variations were statistically non-significant ($P = 0.1208$), the numerical trend indicates that higher concentrations of farmyard manure (FYM) promote more rapid and uniform seed germination, likely due to improved soil nutrient availability and moisture retention.

The number of leaves per plant exhibited a statistically significant variation among treatments ($P = 0.0019$). The highest mean leaf count was recorded in T_5 (30% FYM) with 24.87 leaves per plant, which was significantly greater than all other treatments. In contrast, the control (T_0) and distilled water (T_1) treatments produced the lowest numbers of leaves (14.77 and 13.77, respectively).

Overall, the data indicate that higher FYM concentrations (20–30%) are more effective than lower concentrations or no priming in enhancing spinach seedling establishment and early growth, with the most pronounced and statistically significant effects observed on the number of leaves per plant.

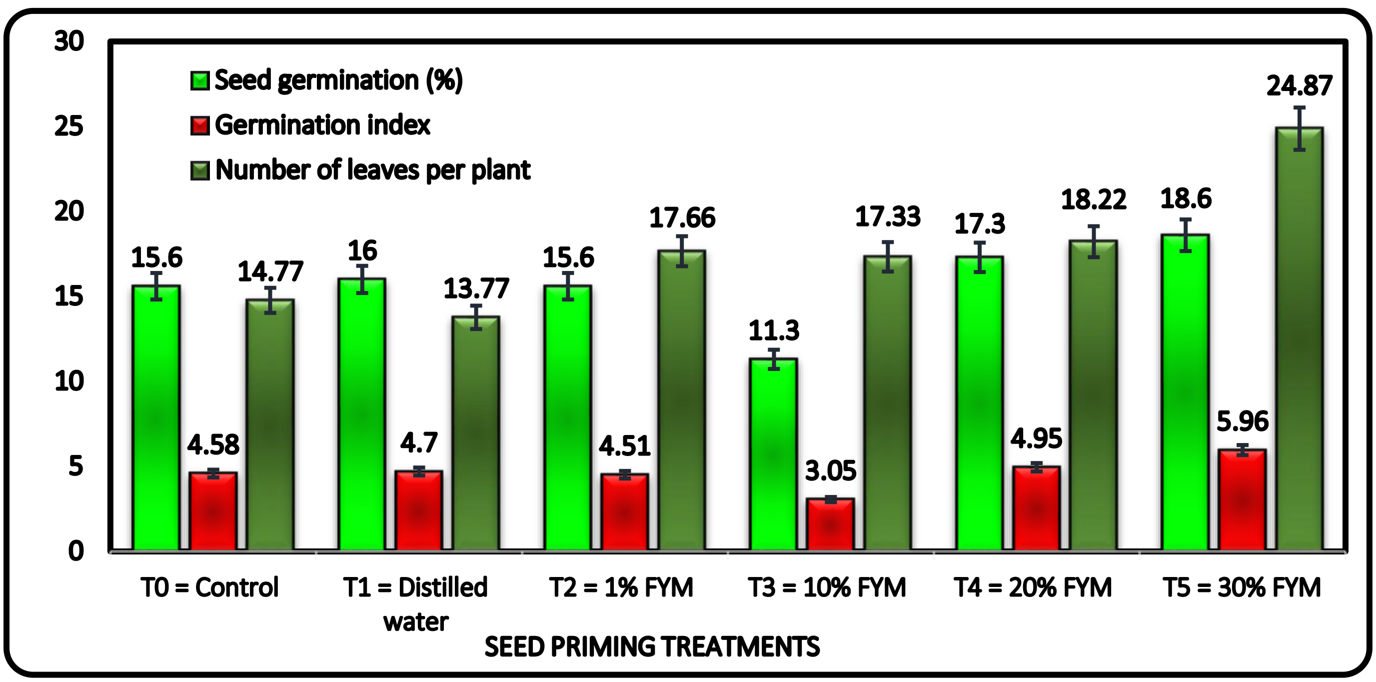

Figure 1. Effect of different concentrations of farmyard manure (FYM) used for seed priming on seed germination (%), germination index, and number of leaves per plant in spinach (*Spinacia oleracea* L.).

Table 1. Statistical analysis of FYM seed priming effects on seed germination (%), germination index, and number of leaves per plant in spinach

Level of Significance	Seed germination (%)	Germination index	Number of leaves per plant
P value	0.2506	0.1208	0.0019
SE	7.0547	0.8925	1.9861
LSD @ 5%	15.371	1.9455	4.3273

Plant Height (cm) and Biomass Accumulation

The effect of farmyard manure (FYM) seed priming at varying concentrations on plant height (cm) and plant weight (g) of spinach was examined. Data for these parameters are presented in Figure 2, with the analysis of variance in Table 2.

The results revealed a consistent numerical increase in plant height across the treatments, although the differences were not statistically significant (P = 0.0620). The control (T_0) recorded an average plant height of 18.93 cm, while Hydropriming (T_1) resulted in a slightly lower value (17.95 cm). Seed priming with 1% FYM (T_2) and 10% FYM (T_3) enhanced plant height to 23.75 cm and 23.77 cm, respectively, though these values were statistically comparable to the control. The 20% FYM treatment (T_4) exhibited a plant height of 22.72 cm, similar to T_2 and T_3. The maximum plant height (26.38 cm) was recorded in T_5 (30% FYM), which numerically exceeded all other treatments but showed statistical overlap with T_2 and T_3. The gradual increase in plant height with higher FYM concentrations suggests a positive influence of organic priming on vegetative vigor, despite the absence of significant statistical differences.

Plant weight (g) exhibited a statistically significant response to various concentrations of farmyard manure (FYM) seed priming, as confirmed by the LSD test ($P < 0.05$). A progressive increase in plant weight was observed from T_0 to T_5 with rising FYM concentrations. The control (T_0) recorded the lowest mean plant weight (5.52 g), which was significantly lower than all other treatments. T_1 (distilled water priming) showed a moderate increase in plant weight (7.46 g), while T_2 (1% FYM) and T_3 (10% FYM) produced plants with weights of 10.50 g and 9.22 g, respectively. A further increase was recorded in T_4 (20% FYM) with a mean plant weight of 10.55 g. The maximum plant weight (17.75 g) was obtained from T_5 (30% FYM), which significantly outperformed all other treatments.

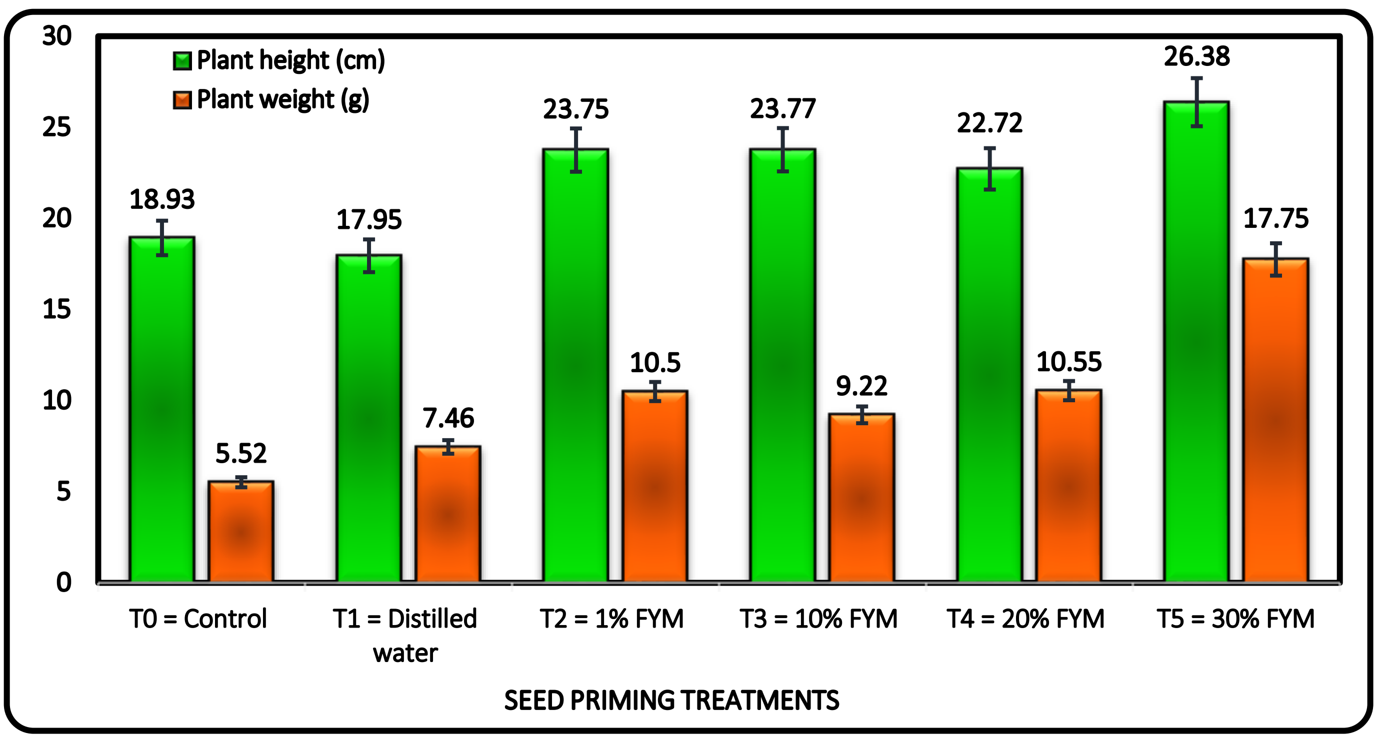

Figure 2. Effect of different concentrations of farmyard manure (FYM) used for seed priming on plant height (cm) and plant weight (g) in spinach (*Spinacia oleracea* L.).

Table 2. Statistical analysis of FYM seed priming effects on plant height (cm) and plant weight (g) in spinach

Level of Significance	Plant height (cm)	Plant weight (g)
P value	0.0620	0.0006
SE	2.6725	1.8915
LSD @ 5%	5.8228	4.1211

Shoot and Root biomass (Fresh and Dry Weight)

The effect of farmyard manure (FYM) seed priming at varying concentrations on fresh shoot biomass, dry shoot biomass, fresh root biomass, and dry root biomass of spinach was recorded. Data for these parameters are presented in Figure 3, with the analysis of variance in Table 3. Significant variation was observed among treatments for fresh shoot biomass (P = 0.0002) and dry root biomass (P = 0.0286), whereas dry shoot biomass (P = 0.0936) and fresh root biomass (P = 0.0906) did not differ significantly at the 5% probability level.

For fresh shoot biomass, the highest value was obtained in T_5 (30% FYM) with 14.24 g, which was significantly greater than all other treatments and nearly 3.2 times higher than the control (T_0, 4.40 g). Treatments T_2 (1% FYM) and T_4 (20% FYM) also produced relatively high fresh shoot biomass (8.35 g and 8.25 g, respectively), whereas the lowest was recorded in the control treatment. Dry shoot biomass followed a similar numerical trend, with T_5 (30% FYM) again recording the highest value (2.52 g), followed by T_4 (1.86 g) and T_2 (1.66 g). However, the differences were not statistically significant at the 5% level, indicating variability within replicates. For fresh root biomass, T_5 (30% FYM) achieved the highest value (3.38 g), with a gradual increase observed from T_0 (1.13 g) through intermediate FYM concentrations. Although these differences were not statistically significant, the trend suggests a positive association between FYM concentration and root fresh mass. In terms of dry root biomass, T_5 (30% FYM) was again superior (1.44 g), significantly higher than all other treatments. The lowest dry root biomass was recorded in the control treatment (0.10 g), indicating that FYM priming, especially at higher concentrations, markedly enhances root dry matter accumulation.

The data demonstrate that FYM seed priming, particularly at 30% concentration, substantially improves both shoot and root biomass in spinach. The most pronounced and statistically significant responses were observed for fresh shoot biomass and dry root biomass, suggesting that elevated FYM levels enhance both above-ground and below-ground growth, likely due to improved nutrient availability and physiological vigor during early development.

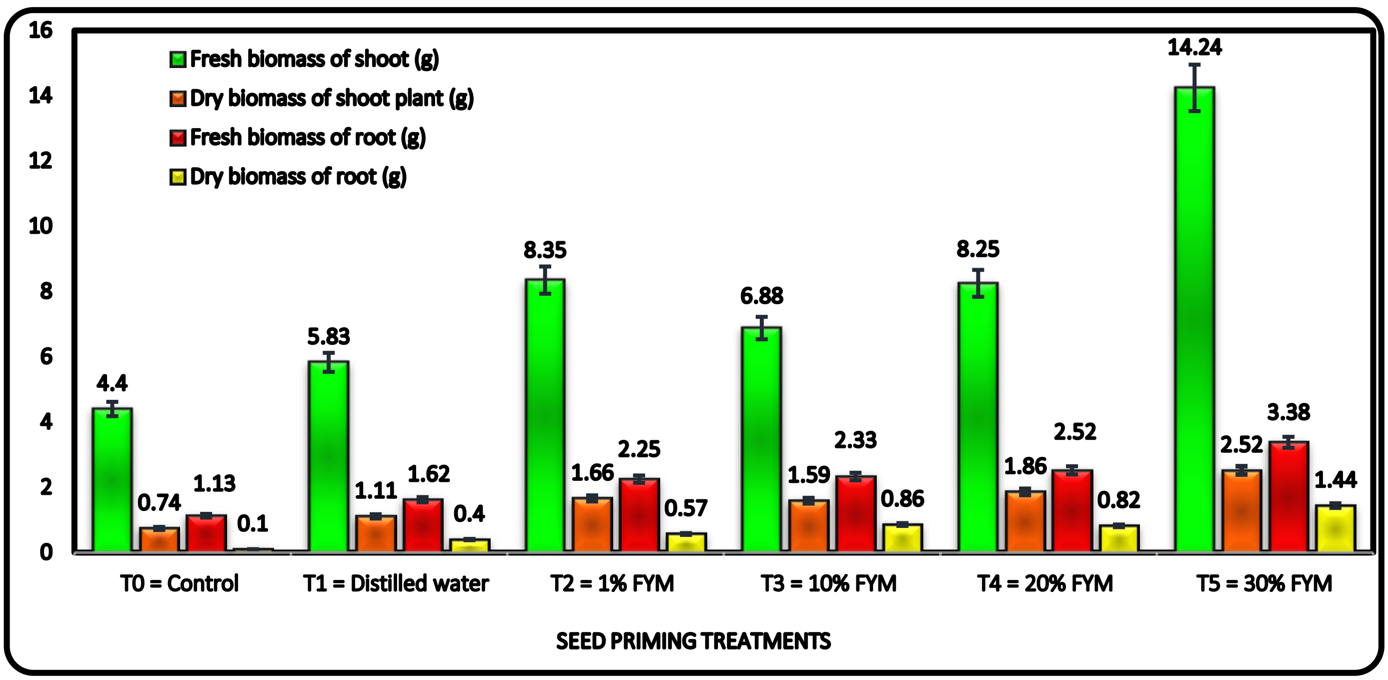

Figure 3. Effect of different concentrations of farmyard manure (FYM) used for seed priming on fresh biomass of shoot (g), dry biomass of shoot (g), fresh biomass of root (g) and dry biomass of root (g).

Table 3. Statistical analysis of FYM seed priming effects on fresh biomass of shoot (g), dry biomass of shoot (g), fresh biomass of root (g) and dry biomass of root (g).

Level of Significance	Fresh biomass of shoot (g)	Dry biomass of shoot (g)	Fresh biomass of root (g)	Dry biomass of root (g)
P value	0.0002	0.0936	0.0906	0.0286
SE	1.3699	0.5549	0.6924	0.3361
LSD @ 5%	2.9846	1.2090	1.5087	0.7323

DISCUSSION

Organic fertilizers are widely recognized for their positive influence on soil fertility and plant growth because they supply essential macro- and micronutrients, improve organic matter content, and enhance the activity of beneficial microorganisms (Soyergin, 2006; Mercik and Stepien, 2006; Kacar and Katkat, 2007). Their application improves soil physical, chemical, and biological properties by increasing cation exchange capacity, enhancing water-holding capacity, improving aeration and thermal regulation, and stimulating microbial activity (Watson et al., 2002; Schoenau, 2006; Prado et al., 2022). Organic amendments also promote the mineralization of nutrients, making them more accessible to plants, and improve soil structure and texture, thereby supporting sustainable crop production (Harris et al., 1996; Granata et al., 2024).

Farmyard manure (FYM), which consists of livestock excreta combined with bedding material, is among the most traditional and widely used organic fertilizers (Lampkin, 2002; Shafique et al., 2023). Its nutrient content particularly nitrogen (N), phosphorus (P), and potassium (K) depend on the species of livestock, their diet, age, bedding materials, and storage conditions. FYM enhances soil aeration, water retention, and cation exchange capacity, and increases microbial biomass, thereby improving nutrient uptake and root development (Watson et al., 2002; Schoenau, 2006; Dawar et al., 2022). Moreover, FYM contains humic substances and plant growth regulators that can stimulate enzymatic activity, enhance hormonal balance (e.g., auxins and cytokinins), and promote seed metabolic activity during germination (Akhtar et al., 2023; Zia-Ur-Rehman et al., 2023).

Seed priming with FYM can improve water imbibition and activate enzymes responsible for the mobilization of stored food reserves, which accelerates germination and uniform seedling emergence (Hasanović et al., 2025). The nutrients and bioactive compounds in FYM may also stimulate antioxidant enzyme activity, thereby protecting germinating seeds from oxidative stress (Rostaei et al., 2024). These mechanisms explain why higher FYM concentrations promote higher germination percentages and germination indices compared to lower concentrations or hydropriming treatments. The number of leaves per plant is an important trait associated with photosynthetic capacity and biomass accumulation. FYM improves nutrient availability and soil microbial activity, both of which influence leaf initiation and expansion (Rizar et al., 2023). Moreover, organic matter in FYM increases soil moisture retention, which supports leaf growth by maintaining turgor pressure and facilitating cell expansion. The progressive increase in leaf production with higher FYM concentrations demonstrates the positive influence of farmyard manure priming on early vegetative growth, likely due to enhanced nutrient availability and improved physiological activity (Wafaa et al., 2006).

Increased plant height due to FYM application is likely linked to improved nutrient supply, particularly nitrogen, which plays a central role in cell division and elongation (Dawar et al., 2022; Hellal et al., 2024). FYM also enhances the availability of micronutrients such as zinc and magnesium, which are important cofactors for enzymes regulating growth hormones like gibberellins. Additionally, better soil structure and aeration improve root growth, further supporting aboveground biomass development. FYM enhances nutrient uptake and water availability, both of which are critical for biomass production (Rehim et al., 2020; Zia-Ur-Rehman et al., 2023). Improved root growth due to FYM application enhances the plant's ability to access water and nutrients, leading to increased fresh biomass accumulation in both shoots and roots (Semenov et al., 2021; Akhtar et al., 2023). This is also linked to the supply of humic substances in FYM, which enhance photosynthetic efficiency and assimilate translocation to growing tissues (Rostaei et al., 2024).

Dry biomass accumulation reflects the ability of the plant to convert assimilates into structural tissues. FYM provides slow-release nutrients and organic compounds that stimulate growth and prolong metabolic activity, thus increasing dry matter accumulation (Dawar et al., 2022; Zia-Ur-Rehman et al., 2023). Enhanced root development due to FYM also contributes to higher root dry biomass, supporting sustained nutrient and water uptake. Organic amendments like FYM also improve soil microbial diversity, which may indirectly enhance plant dry matter production by promoting nutrient mineralization and stress resilience (Semenov et al., 2021; Akhtar et al., 2023).

The findings of present reserach collectively suggest that FYM seed priming, especially at higher concentrations such as 30%, can substantially improve early seedling vigor through mechanisms involving enhanced nutrient availability, improved water relations, stimulated enzyme activity, and better soil microbial function. Conversely, lower FYM concentrations or hydropriming may limit these benefits due to insufficient nutrient supply or reduced stimulation of physiological processes, while excessively low nutrient availability could limit enzyme activation and growth potential. Thus, the application of FYM as a seed priming agent not only improves germination and early growth performance but also contributes to sustainable crop production by reducing dependency on chemical fertilizers and improving soil health (Citak and Sonmez, 2010; Mufwanzala and Dikinya, 2010; Baliah and Muthulakshmi, 2017; Kumarpandit et al., 2017; Türkkan and Kibar, 2022).

CONCLUSIONS

Based on the findings of the present study, spinach seeds primed with 30% farmyard manure (FYM) exhibited superior germination and early growth performance compared to lower FYM concentrations and the control treatment. The enhanced growth attributes indicate that FYM-based seed priming improves nutrient availability, stimulates microbial activity, and supports better seedling vigor.

From a practical perspective, this technique offers a low-cost, eco-friendly approach that can be easily adopted by vegetable growers, particularly smallholder farmers, to enhance crop establishment and productivity while reducing dependence on synthetic fertilizers.

Future research should explore the optimal FYM concentration and soaking duration for different crops and environments, as well as investigate the physiological and biochemical mechanisms underlying FYM-induced improvements in growth and stress tolerance.

AUTHOR CONTRIBUTIONS

Shan Muhammad: Conducted research and data collection, Saba Ambreen Memon: Designing, supervision, write up and finalizing the manuscript, Muzafaruddin Chachar: Wrote first draft of paper and developed graphs, Memoona Islam Majeedano & Maira Raqeeb Tunio: References management, citations and proof read the manuscript, Sana Shazia Jiskani & Raheem Ullah: Statistical analysis and proof read the manuscript, Imran Ullah & Muhammad Iqbal: Data collection, review & editing.

COMPETING OF INTEREST

The authors declare no conflict of interest.

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