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Impact of Moringa and Seaweed Priming on Seedling Establishment, Early Leaf Development, and Nodule Formation in Mung Bean (*Vigna radiata* L.)

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Abstract

Mung bean (Vigna radiata L.) productivity in Pakistan remains low due to weak crop establishment and frequent early-season stresses. Seed priming with botanical and seaweed-based biostimulants offers a simple way to boost early vigor without relying entirely on synthetic inputs. This field study was performed to examine the effect of moringa leaf extract (MLE) and Ascophyllum nodosum seaweed extract (SWE), on early growth of mung bean under semi-arid conditions. Seven seed priming treatments including MLE 1.5%, MLE 3%, SWE 0.2%, SWE 0.5%, MLE (1.5%) + SWE (0.2%), MLE (3%) + SWE (0.5%) and control (unprimed seed), each with three replications were evaluated to check their impact on germination (%), field emergence (%), seedling vigor index (SVI), nodules per plant, and leaf area at 20 days after sowing (DAS). All priming treatments increased germination compared to control (91.33%), with the highest values observed in MLE (3%) + SWE (0.5%) (97.00%) and MLE 1.5% (96.33%). MLE 3% and combination of both MLE (3%) + SWE (0.5%) improved seedling vigor index (SVI) up to 1747 and 1757, respectively as compared to control (1519). Emergence followed a similar trend, rising to 94.33% under MLE (3%) + SWE (0.5%). SWE positively impacted early nodulation and SWE 0.5% produced maximum (17.33) nodules plant^{-1}. MLE 1.5% demonstrated highest Leaf area at 20 DAS (200.44 cm^2), while most other priming treatments slightly improved leaf area relative to the control. Given the findings of this study, seed priming with MLE and SWE without foliar application significantly enhances early growth, nodulation, and canopy development in mung bean.

1. Introduction

Mung bean (*Vigna radiata* L.) is a vital grain legume extensively cultivated in tropical and subtropical regions like Pakistan, South Asia and Africa (Ahmed et al., 2024). It fits well into short cropping windows, requires relatively little water, and produces nutritious grain rich in protein and minerals. As a legume, it also fixes atmospheric nitrogen, improving soil health and boosting yields of subsequent crops like wheat or rice. (Li et al., 2023). Due to its short growth cycle and adaptability to diverse agro-climatic conditions, mung beans are widely integrated into cereal-based cropping systems, particularly in rainfed and semi-arid regions. Its inclusion in crop rotations enhances soil organic matter, improves nutrient cycling, and contributes to sustainable intensification of agricultural systems (Shah et al., 2021). However, the yield achieved by farmers remain far below than potential in Pakistan. Different factors like high temperatures, intermittent drought, poor soil fertility, low seed quality and late sowing weaken crop establishment and

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reduce yield potential (Nawaz et al., 2021). Climate variability has further intensified these constraints by increasing the frequency of heat waves and irregular rainfall during early growth stages. Poor stand establishment caused by weak or uneven germination ultimately limits nodulation, canopy development, and biomass accumulation in mung bean (Raza et al., 2024). In response, many have increased synthetic fertilizer and pesticide use, raised costs and risked environmental harm. (Ademe, 2023). Excessive reliance on chemical inputs has also contributed to soil degradation, nutrient imbalances, and declining soil microbial diversity, particularly in smallholder farming systems (Meena et al., 2020). These challenges highlight the need for eco-friendly and cost-effective alternatives that enhance crop productivity without compromising environmental sustainability.

Plant biostimulants, such as moringa leaf extract (MLE) and seaweed extract (SWE) from *Ascophyllum nodosum*, offer an alternative. These substances enhance plant growth and stress tolerance by stimulating physiological processes, rather than just supplying nutrients (Ali et al., 2021). *Moringa oleifera* leaves contain high levels of minerals, vitamins, amino acids and secondary metabolites, as well as zeatin-type cytokinins, which are known to influence cell division, leaf expansion and senescence (Mashamaite et al., 2022). Recent studies have also demonstrated that moringa leaf extract contains antioxidant compounds that reduce oxidative damage and improve seedling vigor under abiotic stress conditions such as heat and drought (Rashid et al., 2020). Seaweed extracts contain a mixture of auxin-like and cytokinin-like compounds, betaines, polysaccharides (e.g. alginates), and a wide spectrum of nutrients, including essential micronutrients (Shukla et al., 2019). These bioactive substances enhance root development, water uptake, and hormonal regulation, thereby improving plant resilience to environmental stresses (Teklić et al., 2021). In legumes, seaweed extracts have also been reported to stimulate beneficial soil microorganisms, indirectly supporting nodulation and biological nitrogen fixation (Nanda et al., 2022). When used as seed-priming agents, biostimulants can help repair membranes and macromolecules damaged during seed storage and activate enzymes involved in reserve mobilization (Arun et al., 2022). Seed priming is considered a low-cost and farmer-friendly technique that improves germination rate, uniform emergence, and early seedling establishment under suboptimal conditions (Jarrar et al., 2024).

Furthermore, successful crop establishment during the early growth stages is a critical determinant of final crop performance in mung bean. Early seedling vigor promotes rapid root growth, improves nutrient and water acquisition, and supports the establishment of an efficient symbiotic relationship with nitrogen-fixing rhizobia (Chen et al., 2024). Uniform emergence also enhances crop competitiveness against weeds and enables plants to better tolerate environmental stresses such as heat and moisture deficits. In semi-arid environments, where fluctuations in temperature and soil moisture frequently impair stand establishment, the use of effective seed-priming agents may provide a practical strategy to improve crop productivity and resource-use efficiency (Devika et al., 2021). Previous studies have shown that MLE and SWE used for seed priming can improve germination and early growth under both normal and stress conditions in crops such as rice, wheat and some legumes (Farooq et al., 2019). More recently, improvements in nodulation efficiency and nitrogen fixation have been reported in legume crops following biostimulant-based seed priming, indicating a positive influence on plant–rhizobia interactions (Parihar et al., 2022). However, most studies focus on one biostimulant at a time, on foliar applications, or on crops other than mung beans. Although the benefits of MLE and SWE are widely reported, direct comparisons between MLE, SWE and their combination as priming agents for mung bean are limited. Therefore, the present study was conducted to evaluate the dose-dependent effects of MLE, SWE, and their combination as seed-priming agents on early crop establishment and nodulation in mung bean.

2. Materials and Methods

The experiment was carried out at the Agronomic Research Field of the Department of Agronomy, Faculty of Agricultural Sciences, University of the Punjab, Lahore, Pakistan (31.49° N, 74.30° E). Climatic data on temperature and rainfall during the experimental period were recorded. No rainfall was observed during the experimental period, while maximum and minimum air temperature are presented in Figure1.

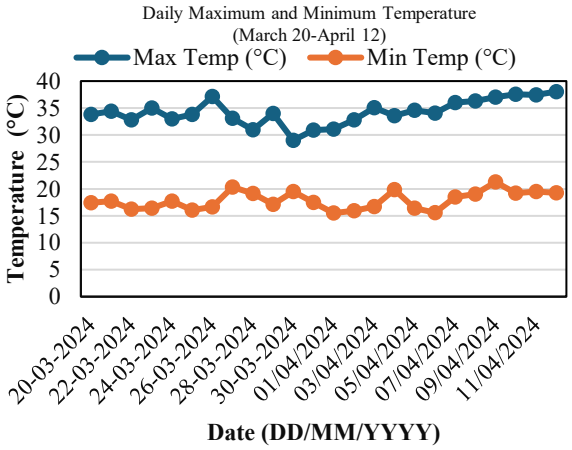


Figure 1. Variation in Daily Maximum and Minimum Air Temperature During the Early Growth Phase of Mung Bean

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Composite soil samples collected from the 0–15 cm soil layer prior to sowing were analyzed for physicochemical characteristics, and the results are shown in Table 1.

Table 1. Soil properties of the experimental site

Soil parameter	Value
Soil texture	Sandy loam
Soil pH	7.7
Electrical conductivity (EC)	0.42 dS m^{-1}
Organic matter	0.74 %
Cation exchange capacity (CEC)	6.3 $cmol_c$ kg^{-1} soil
Available nitrogen (N)	0.038 %
Available phosphorus	6.32 mg kg^{-1} soil
Extractable potassium	160 mg kg^{-1} soil

2.1. Experimental design and treatments

The experiment was carried out in randomized complete block design (RCBD) with three replications. Each plot size was 4.5 m × 1.5 m (6.75 m²). Rows were spaced 75 cm apart, and plants within the row were approximately 10 cm apart. Seven seed-priming treatments were applied (Table 2).

Table 2. Table representing Treatment Name, Bio stimulant constitution and their Dose

Treatment Name	Bio stimulant extract	Dose
Control	–	–
MLE1	Moringa	1.5%
MLE2	Moringa	3%
SWE1	Seaweed	0.2%
SWE2	Seaweed	0.5%
COMB1 (MLE1 + SWE1)	Moringa + Seaweed	1.5% + 0.2%
COMB2 (MLE 2+ SWE2)	Moringa + Seaweed	3% + 0.5%

2.2. Preparation of priming solutions

2.2.1. Moringa leaf extract

Fresh, fully expanded leaves of *Moringa oleifera* were collected, washed and air-dried briefly to remove surface moisture. For the stock extract, 100 g of leaves were blended with 1000 mL distilled water (1:10 w/v), and the slurry was filtered first through muslin cloth and then through Whatman No. 1 paper to produce a clear solution. Working solutions of 1.5% and 3% (v/v) MLE were prepared by diluting the stock with distilled water.

2.2.2. Seaweed extract

A commercial *Ascophyllum nodosum* product (Seamaxx®) was used as the seaweed source. The manufacturer's stock solution was diluted with water to obtain 0.2% and 0.5% (v/v) SWE for priming of seeds.

2.2.3. Combined MLE + SWE solutions

For the combined treatments, appropriate volumes of the MLE and SWE working solutions were mixed to obtain 1.5% MLE + 0.2% SWE and 3% MLE + 0.5% SWE. These mixtures were prepared fresh on the day of priming.

2.2.4. Seed priming procedure

For each treatment and replication, the quantity of seed required for a plot was weighed and placed in labelled containers. Seeds were covered with the respective priming solution for 2 hours, maintaining a solution: seed ratio that allowed full imbibition without leaving seeds exposed (Farooq et al., 2019). After soaking, seeds were drained and spread in a single layer under shade until they regained a free-flowing state. Seeds were then sown on the same day. Control seeds were not soaked; they were sown dry, but all other aspects of their handling were the same as for primed seeds.

2.3. Crop management

The land was ploughed and harrowed to prepare a fine seedbed, followed by ridge formation. Mung bean cultivar PRI Mung-2018 was sown on 20 March 2024. Seeds were sown at a rate of 12 kg per acre using the traditional "chopa" method on ridges. Nitrogen, phosphorus, and potassium were applied at recommended rates of 9, 23, and 13 kg per acre, respectively. Irrigation was applied at sowing to ensure uniform soil moisture for germination and was the only irrigation provided during the first 20 days after sowing. Weeds were removed manually throughout the growing period. No fungicides, insecticides, or herbicides were applied.

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2.4. Data collection

All observations were recorded from plants within a 1 m² quadrat placed at the center of each plot to avoid border effects.

2.4.1. Germination percentage

Germination percentage was recorded seven days after sowing by counting the number of germinated seeds (normal seedlings with emerged radicles and shoots) within the 1 m² area. Germination percentage was calculated as:

Germination (%) = (Number of germinated seeds / Total seeds sown) × 100

2.4.2. Emergence percentage

Field emergence was recorded at 10 DAS by counting the number of seedlings emerged above the soil surface within the 1 m² sampling area. Emergence percentage was calculated as:

Emergence (%) = (Number of emerged seedlings / Total seeds sown) × 100

2.4.3. Seedling vigor index

At 20 DAS, randomly selected seedlings were carefully uprooted from each plot. Seedling length (root + shoot length from root–shoot junction to shoot tip) was measured, and the mean value was used for calculations. Seedling vigor index (SVI) was calculated as:

SVI = Germination (%) × Seedling length (cm)

2.4.4. Nodules per plant (20 DAS)

At 20 DAS, 3–5 plants per plot were randomly uprooted, and the root systems were gently washed under running water to remove soil particles. All visible nodules on the roots were counted manually, and the mean number of nodules per plant was calculated.

2.4.5. Leaf area (20 DAS)

Leaf area was measured using a leaf area meter, and the mean leaf area per plant (cm²) at 20 DAS was used for statistical analysis.

2.5. Statistical analysis

Data were analyzed using analysis of variance (ANOVA), and treatment means were compared using the least significant difference (LSD) test at $p \leq 0.05$ using Statistix 8.1 software.

3. Results

3.1. Effects of seed priming on germination and emergence

Seed priming significantly affected germination percentage ($p \leq 0.05$) (Table 1). The unprimed control recorded a germination of 91.33%. All priming treatments improved germination over control. The highest germination was observed in COMB2 (97.00%), followed by MLE1 (96.33%). Other treatments (MLE2, SWE1, SWE2, and COMB1) ranged between 95.00% and 96.00%, all remaining higher than the control (Figure 2). Field emergence at 10 DAS showed a similar pattern. The control recorded 91.33% emergence. The highest emergence was observed in COMB2 (94.33%), followed by MLE2 (93.33%). MLE1, SWE1, and COMB1 recorded identical emergence (92.33%), while SWE2 showed emergence similar to the control (91.33%) (Figure 2b).

3.2. Seedling vigor index

Seedling vigor index (SVI) was significantly influenced by seed priming treatments. The control recorded the lowest SVI (1519.2). The highest SVI was recorded in COMB2 (1756.7), closely followed by MLE2 (1746.9), representing approximately a 15% increase over the control. Treatments SWE1, SWE2, MLE1, and COMB1 showed intermediate responses and were statistically similar to each other, with an average increase of about 8% over the control (Figure 2c).

3.3. Nodulation at 20 DAS

Seed priming significantly affected nodulation at 20 DAS. The control recorded 9.33 nodules plant^{-1}. The highest number of nodules was observed in SWE2 (17.33 nodules plant^{-1}), representing an 85% increase over the control. Other treatments showed variable responses; COMB2 recorded 10.00 nodules plant^{-1} and was statistically similar to the control, indicating limited effect of combined high-dose treatment on nodulation (Figure 2d).

3.4. Leaf area at 20 DAS

Leaf area was significantly affected by seed priming treatments. The highest leaf area was recorded in MLE1 (200.44 cm²), followed by COMB1 (195.59 cm²), SWE2, and MLE2 (194.32 cm²). COMB2 recorded the lowest leaf area, which was lower than COMB1 and slightly lower than the control (Figure 2e). Overall, most priming treatments improved leaf area compared to the unprimed control.

4. Discussion

The consistent improvement in germination and emergence across all primed treatments confirms that MLE and SWE are effective seed-priming agents for mung beans under field conditions. A small difference in germinability, even of 3–6%, can help achieve uniformity of crop stands and fewer gaps between plants, especially when sowing conditions are suboptimal (Nawaz et al., 2021). Seed priming likely enhanced the early metabolic processes of germination by

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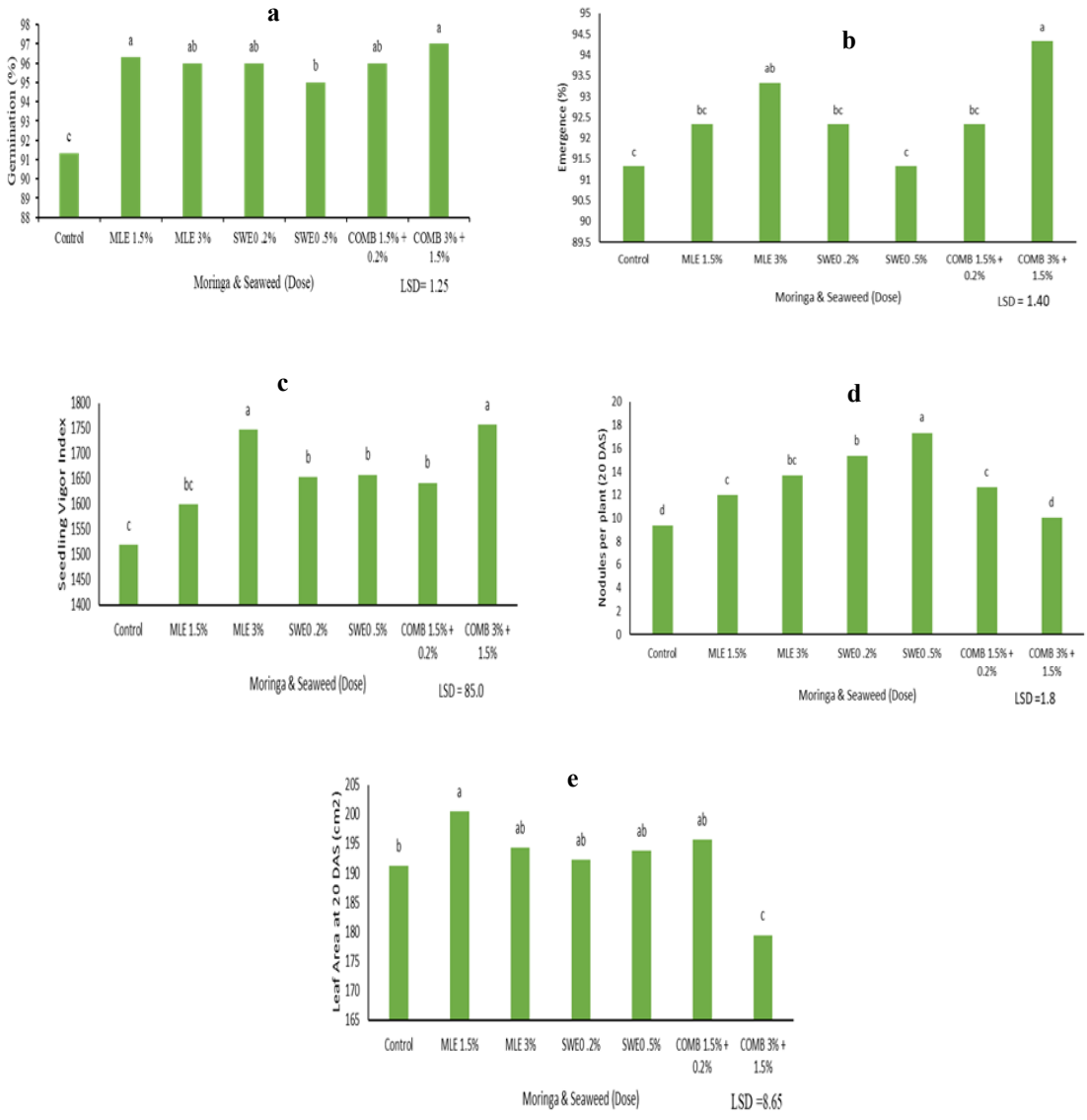


Figure 2. Effects of different biostimulant treatments on mung bean (*Vigna radiata* L.) performance, including germination percentage, emergence percentage, seedling vigor index (SVI), nodulation (nodules per plant at 20 DAS), and leaf area at 20 DAS. Bars represent mean values, and different letters indicate significant differences among treatments at $p \leq 0.05$."

accelerating enzyme activation (e.g., α-amylase), membrane rehydration, and repair of cellular damage accumulated during seed storage (Khan et al., 2022). Moringa leaf extract (MLE) and seaweed extract (SWE) contain bioactive compounds including cytokinins, growth promoters like auxins, vitamins, phenolics, and betaines, which play a key role in controlling cell division, osmolyte accumulation, and responses to oxidative stress during germination (Irshad et al., 2022). Cytokinins in MLE stimulate cell division and shoot development, and SWE has been reported to improve root growth and stress resistance. The same effect of MLE and SWE on germination and seedling growth were also observed in cereals and legumes under normal and stress situations (Verma et al., 2021). In addition, biostimulant-based seed priming has been reported to enhance antioxidant defense systems by increasing the activity of enzymes such as superoxide dismutase (SOD) and catalase (Campobenedetto et al., 2020). This helps to decrease the

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build-up of ROS during early germination, leading to better seedling vigor and resistance to short-term abiotic stress like drought and heat stress (Choudhary et al., 2024). The higher seedling vigor index (SVI) observed in primed treatments indicates that seedlings were not only more numerous but also more vigorous in growth. This improvement may be attributed to enhanced mobilization of seed reserves and a favorable hormonal balance during early growth stages (Farooq et al., 2019). The root promoting effect of SWE and its ability to improve osmotic regulation may have contributed to its ability to delay early senescence, while the cytokinin component of MLE likely contributed to shoot growth (Hasanuzzaman et al., 2021). The combined application of both biostimulants at higher concentrations showed a synergistic effect, resulting in stronger seedling performance compared with the control.

The greater nodulation observed under SWE2 may be attributed to improved root development and enhanced plant–rhizobia interactions induced by seaweed extracts (Shukla et al., 2019). Seaweed-based biostimulants are known to stimulate root branching and root hair formation, increasing infection sites for rhizobia. Furthermore, micronutrients like cobalt and molybdenum found in *Ascophyllum nodosum* extracts could help with nodulation and nitrogenase activity. MLE also improved nodulation compared with the control, likely through its positive effects on root growth and metabolic activity, although its effect was less pronounced than SWE2 (Ding, 2022). The highest leaf area recorded under MLE1.5% may be linked to the high cytokinin content of MLE, which promotes cell division, leaf expansion, and chloroplast development while delaying senescence (Mashamaite et al., 2022). Greater leaf area improves light interception and photosynthetic efficiency, leading to better early biomass accumulation and vigor of seedlings.

Moreover, the observed early growth improvements can be attributed to better metabolic reprogramming of the seeds due to priming. In the seed priming method, partial hydration is applied for some time to activate pre-germinative metabolism without radicle emergence, which favors the activation of metabolism and enzyme activity when the seeds germinate in soil (Marthandan et al., 2020). This effect is a physiological "pre-conditioning" that enhances the energy metabolism by increasing respiration rates and more efficient ATP production, thereby enabling fast cell division and elongation in emerging seedlings. Moreover, the presence of osmolytes and phenolic compounds in MLE and SWE can provide protection of proteins and membranes to varying field temperatures, leading to low cellular damage during early growth stages (Mansinhos et al., 2022). Another aspect of these compounds is enhancing water-use efficiency at the seedling stage, in which mild moisture stress is typical in semi-arid environments. The synergistic effect of nutrient like components and plant growth regulators in both extracts probably may have established a better biochemical

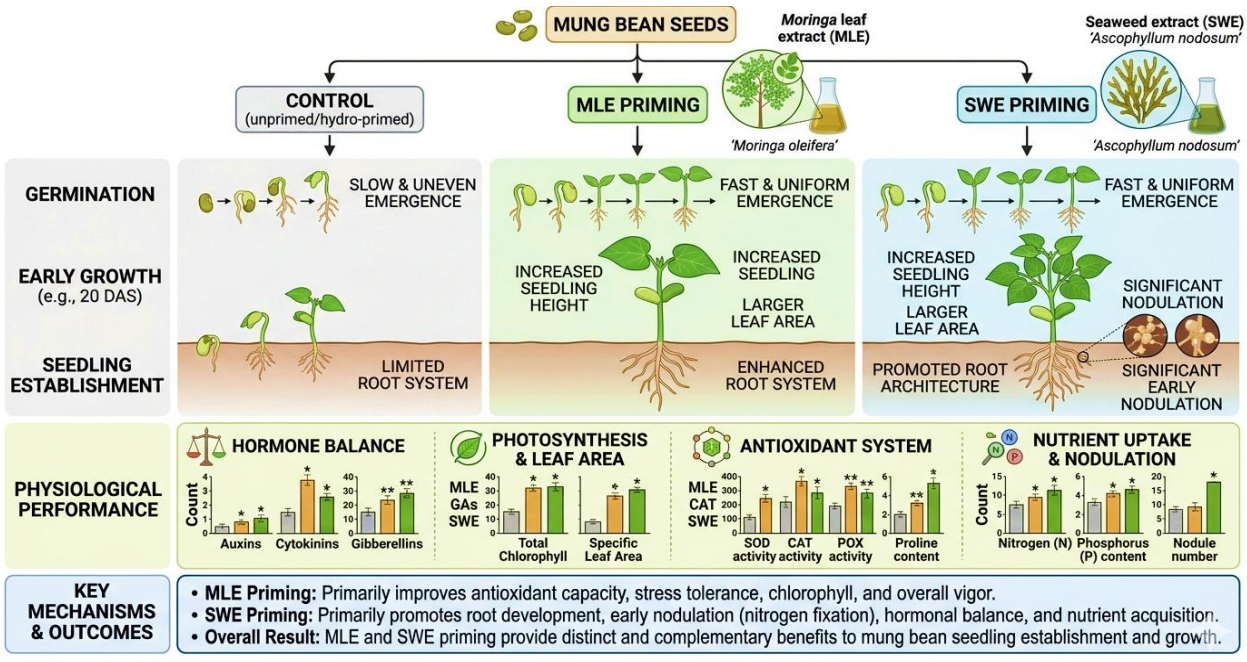


Figure 7. Schematic illustration showing the effects of seed priming with Moringa leaf extract and seaweed extract on early growth and physiological performance of mung bean (*Vigna radiata L.*) seedlings.

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environment for early root–shoot coordination, thereby enhancing stand establishment and early plant vigour in mung bean (Parveen et al., 2023).

Overall, seed priming with MLE and SWE improved early crop establishment through enhanced germination, seedling vigor, nodulation, and leaf development. The synergistic effect of these biostimulants could boost the efficiency of acquisition and utilization of nutrients and root–shoot coordination, thus favoring early balanced development and stress tolerance. The results demonstrate that MLE and SWE seed priming represents a simple, low-cost, and environmentally friendly strategy to improve early performance of mung bean under field conditions. The overall effects of Moringa leaf extract (MLE) and seaweed extract (SWE) on mung bean seedlings are represented in Figure 3.

5. Conclusion

Seed priming with moringa leaf extract (MLE) and *Ascophyllum nodosum* seaweed extract (SWE) significantly improved the early growth of mung bean in this field study. The combination of MLE and SWE enhanced seedling vigor, while seaweed extract improved nodulation in mung bean. Seed priming with MLE and SWE is practical for smallholder farmers because it requires only small extract quantities and simple preparation methods. Strengthening crop establishment during the critical 0–20 DAS period improves the crop's ability to cope with later stresses and potentially enhance yield, especially when combined with good agronomic practices. Additionally, the improved root and shoot development observed in primed seedlings can enhance nutrient and water use efficiency, providing a stronger foundation for subsequent growth stages. The use of natural biostimulants like MLE and SWE also reduces dependency on synthetic fertilizers and pesticides, supporting environmentally sustainable cultivation practices. By promoting uniform emergence and vigorous early growth, these priming treatments can help mitigate the negative effects of erratic weather and soil fertility constraints common in smallholder farming systems. Overall, seed priming with MLE and SWE offers a practical, sustainable and biologically sound way to enhance mung beans yield potential in legume-based cropping systems.

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8. Conflict of Interest

The authors declare no conflict of interest.

9. Authors' Contribution

Muhammad Bilal Chattha and Shahzaib Anwar were responsible for conceptualization and research design. Experimental work and preparation of the original draft were carried out by Shahzaib Anwar and Sohaib Ahmad. Data processing and analysis were performed by Muhammad Ajmal Rana and Farghab Ahmad Rana. Revision and proofreading of the manuscript were done by Muhammad Ali and Muhammad Ajmal Rana. Overall supervision of the study was provided by Muhammad Bilal Chattha.

10. SDGs Addressed

This study aligns with SDG 2 (Zero Hunger) by enhancing mung bean establishment, nodulation, and yield potential, improving food and nutritional security; SDG 12 (Responsible Consumption and Production) through the use of sustainable, plant- and seaweed-based biostimulants that reduce dependence on synthetic inputs; and SDG 13 (Climate Action) by improving crop resilience and early growth under semi-arid and stress-prone conditions. Overall, it supports climate-smart, low-input, and environmentally sustainable agriculture.

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