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# Bio-Stimulants Humid Priming: A Technology for Improving Seed Vigour of Annual Moringa cv. PKM 1

**Geetharani. P [a*], C. Parameswari [a], M.P. Kavitha [a], G. Sudhakar [a] and M. Madhan Mohan [a]**

*[a] Agricultural Research Station, TNAU, Vaigai Dam, India.*

***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

*Moringa oleifera* Lam. is a multiple purpose tree used as human and animal food, cosmetic production and water purification. Seed priming combined with organic bostimulants has been used to improve plant performance. This study aimed to verify the efficiency of humid seed priming using bio stimulants such as jeevamirtham, panchagavya, moringa leaf extract, seaweed extract, egg amino acid and liquid-based bio formulations of *Bacillus subtilis* on seed germination and seedling growth of *Moringa oleifera.* The humid priming technique involves soaking seeds in a loosely tied cloth bag for a predetermined duration, then placing the bags in a closed container on an elevated platform to provide dark, humid conditions that facilitate the invigoration process. Seeds are soaked for 12 hours, exposed to dark, humid conditions for another 12 hours, and then shade dried to the original moisture content for proper handling. The experiment on seed invigoration with humid

**Corresponding author: E-mail: geetharani.p@tnau.ac.in, geetharanitnau@outlook.com;*

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priming was conducted at Agricultural Research Station, Vaigai Dam, during 2023 with annual moringa variety PKM1. The annual moringa seeds were subjected to humid priming with various bio-stimulants in different concentrations to determine the seed quality parameters *viz.*, speed of germination, germination percentage, seedling length, dry weight and seedling vigour. All the humid priming treatments with bio stimulants increased the emergence rate and enhanced the seedling vigor. However, humid priming with 1% liquid formulations of *Bacillus subtilis* had a significantly positive influence on speed of germination (6.64), days for 50% emergence (4.5 days), maximum germination percentage (94%), root length (15.7 cm), shoot length (29.2 cm), dry matter production (3.6 g/10 seedlings), and vigour index value (4220) over the control which recorded only 5.14,8.0,74 %,9.3cm, 18.9cm,2.05g, 2086 respectively. The germination percentage increased by 27% in humid priming with 1% *Bacillus subtilis* compared to control.

*Keywords: Moringa; humid priming; bio-stimulants.*

## 1. INTRODUCTION

*Moringa oleifera* is a multipurpose plant which is now being promoted as a fodder crop. It is a highly nutritious tree preferred by consumers for its edible parts. Moringa grows best in temperatures ranging from 25˚C to 38˚C and requires an annual rainfall of at least 500 mm. As a fast-growing tree, it has various uses, including as a food source (Hsu *et al*., 2006) and for medicinal purposes (Fuglie, 2001). Its fresh leaves are particularly beneficial for livestock, containing 19.3% to 26.4% crude protein (Aregheore, 2002). The seeds yield a colorless oil known as "Ben oil," valued similarly to olive oil and often used as a lubricant (Booth and Wickens, 1998)

Propagation of moringa is primarily through seeds, a highly effective and commercially advantageous method. Successful cultivation requires high-quality seeds, which generally germinate within two weeks when sown at a depth of 2 cm. Moringa seeds can also be sown in poly bags, and the seedlings can be transplanted once they reach a height of 30 cm. The tree is prolific, with a single tree yielding 500 g to 1 kg of seeds, approximately 3,000 seeds per kilogram (Ahmeda & El-Mahdya, 2022; Bahir et al., 2014; Zhang & Ervin, 2004).

Seed degeneration is influenced by the seed's physiological state, genetic makeup, and storage circumstances (Rajinder Singh and Bassi, 2016). The necessity for a sufficient supply of high-quality seed is critical for the foundation of a successful seed production programme as well as the continued viability and profitability of agriculture. Unexpected seed viability losses would have a detrimental impact on production schedules and seed sales, resulting in large losses for agricultural sectors every year (Walters *et al.*, 2010).

The advantageous effect of botanicals like moringa leaf extract on growth of tomato was reported by Bashir *et al.* (2014). Application of sea weed extract resulted in increased endogenous cytokinin levels, thereby leading to improved drought resistance (Zhong and Ervin, 2004). Application of jeevamrutham and panchagavya significantly enhanced the seed yield in cowpea (Sutar Reshma *et al.*, 2019)

However, seed invigoration treatments can improve the physiological quality of low-vigor seed lots, leading to higher germination rates. One effective approach to seed invigoration is seed priming, a widely researched technique over the past two decades for boosting germination and seedling vigor. This study examines a seed priming technique called humid priming, applied to moringa seeds using biostimulants.

Biostimulants, whether natural or synthetic, can be applied to seeds, plants, or soil, and they promote growth by improving tolerance to abiotic stress and enhancing seed and grain yield and quality. These substances are effective even in small concentrations, improving nutrient efficiency, stress tolerance, and crop quality traits.

## 2. MATERIALS AND METHODS

The experiment was conducted using medium vigour seedlot of *Moringa oleifera* cv. PKM1 at the Agricultural Research Station, Vaigai Dam, under Tamil Nadu Agricultural University, India. The main objective of the study was to evaluate the effectiveness of humid priming seed invigoration treatment with various bio stimulants.

The moringa seeds underwent humid priming using the following treatments: T1 - Jeevamirtham (0.5%), T2 - Jeevamirtham (1.0%), T3 - Panchagavya (0.5%), T4 - Panchagavya (1.0%), T5 - Moringa leaf extract (0.5%), T6 - Moringa leaf extract (1.0%), T7 - Seaweed extract (0.5%), T8 - Seaweed extract (1.0%), T9 - Egg amino acid (0.5%), T10 - Egg amino acid (1.0%), T11 - liquid-based bio formulation of *Bacillus subtilis* (0.5%), T12 - liquid-based bio formulation of *Bacillus subtilis* (1.0%), T13 - hydro priming, and T14 - control (dry seeds). The seeds were soaked for 12 hours, followed by exposure to dark, humid conditions for an additional 12 hours, and then shade-dried to their original moisture content.

The germination test was conducted using the roll towel method with 100 seeds per treatment, replicated four times. The speed of germination, number of days taken for 50% germination in each treatment were recorded. At the final count, the number of normal seedlings was determined, and the mean was expressed as the germination percentage. Speed of emergence was recorded daily and calculated by using the formula proposed by Maguire (1962). The lengths of the roots and shoots were measured in centimeters. After measuring root and shoot lengths, the seedlings were placed in paper bags, shade-dried for 24 hours, and then dried in a hot air oven at 80 ± 2°C for another 24 hours. The dried samples were cooled in a desiccator, weighed, and the weight was expressed in gram per 10 seedlings. The vigour index was calculated using the formula suggested by Abdul-Baki and Anderson (1973), with the mean values expressed as whole numbers.

## 3. RESULTS AND DISCUSSION

The effects of different duration of humid priming treatments on seed germination was first studied under laboratory conditions. The humid priming technique of soaking seeds in a loosely tied cloth bag for a predetermined duration, then placing the bags in a closed container on an elevated platform to provide dark, humid conditions that facilitate the invigoration process.

All the durations of humid priming treatments exhibit significantly high speed of germination, maximum germination percentage, seedling length, dry weight of seedlings and vigor index (Table 1). Seeds soaked for 12 hours, exposed to dark, humid conditions for another 12 hours, and then shade dried to the original moisture content was the best for germination of *M. oleifera* and reached 87% compared to unprimed seeds (74%).

Statistically significant difference was observed among the different bio- stimulants of humid priming with respect to seed germination and seedling vigour compared to control. In the present study, moringa seeds treated with liquid-based bio-formulations of 1% *Bacillus subtilis* achieved the maximum speed of germination (6.64), 50% germination in 4.5 days, while untreated control seeds took the longest, with 50% germination recorded at 8 days. The treatment with 1% *Bacillus subtilis*

**Table 1. Standardization of duration of humid priming for seed invigoration in annual moringa cv. PKM1**

| Soaking & Incubation duration (h) | Speed of emergence | Germination (%) | Root length (cm) | Shoot length (cm) | DMP (g /10 seedlings) | Vigour Index |
|---|---|---|---|---|---|---|
| T1 (12h+12h) | 6.28 | 87 | 18.1 | 28.9 | 4.62 | 4089 |
| T2 (12h+24h) | 5.34 | 85 | 17.0 | 18.0 | 4.19 | 2975 |
| T3 (12h+36h) | 5.28 | 82 | 13.6 | 22.6 | 3.48 | 2968 |
| T4 (12h+48h) | 5.93 | 84 | 14.0 | 18.3 | 3.61 | 2713 |
| T5 (24h+12h) | 5.12 | 80 | 16.2 | 21.8 | 3.75 | 3040 |
| T6 (24h+24h) | 5.52 | 78 | 15.0 | 26.3 | 3.21 | 3221 |
| T7 (24h+36h) | 5.06 | 76 | 14.4 | 21.8 | 3.22 | 2751 |
| T8 (24h+48h) | 5.14 | 76 | 13.8 | 25.0 | 3.33 | 2948 |
| T9 (12h+0) | 5.60 | 84 | 12.0 | 16.0 | 3.69 | 2352 |
| T10 (Control) | 5.10 | 74 | 9.3 | 14.3 | 3.20 | 1794 |
| **SEd** | | **4.0194** | **0.131** | **1.7325** | **0.1721** | **146.221** |
| **CD (P=0.05)** | | **8.4446** | **0.275** | **3.6399** | **0.3615** | **307.204** |

**Table 2. Effect of humid priming with bio-stimulants on seed quality parameters of annual moringa cv.PKM1**

| Treatments | Speed of emergence | Days to 50% emergence | Germination (%) | Root length (cm) | Shoot length (cm) | DMP (g /10 seedlings) | Vigour Index |
|---|---|---|---|---|---|---|---|
| T1 | 5.31 | 6.0 | 76 | 09.7 | 22.0 | 2.32 | 2409 |
| T2 | 5.28 | 6.8 | 76 | 13.6 | 20.8 | 2.64 | 2614 |
| T3 | 5.45 | 7.0 | 76 | 11.7 | 27.0 | 3.14 | 2941 |
| T4 | 5.90 | 7.5 | 78 | 12.8 | 21.4 | 2.12 | 2667 |
| T5 | 6.35 | 5.0 | 88 | 14.4 | 25.8 | 3.02 | 3537 |
| T6 | 6.49 | 4.5 | 90 | 15.6 | 25.6 | 3.40 | 3708 |
| T7 | 6.44 | 5.5 | 90 | 11.3 | 24.2 | 3.00 | 3195 |
| T8 | 5.77 | 6.2 | 76 | 12.4 | 22.6 | 2.10 | 2660 |
| T9 | 5.67 | 6.5 | 76 | 09.1 | 26.4 | 2.28 | 2698 |
| T10 | 5.56 | 7.5 | 78 | 14.3 | 11.8 | 2.19 | 2035 |
| T11 | 6.13 | 5.5 | 80 | 15.4 | 28.6 | 3.15 | 3520 |
| T12 | 6.64 | 4.5 | 94 | 15.7 | 29.2 | 3.60 | 4220 |
| T13 | 5.89 | 6.5 | 78 | 08.9 | 24.8 | 3.00 | 2628 |
| T14 | 5.14 | 8.0 | 74 | 09.3 | 18.9 | 2.05 | 2086 |
| **SEd** | **0.048** | **0.551** | **2.225** | **1.704** | **1.860** | **0.154** | **68.61** |
| **CD (0.05)** | **0.134** | **1.595** | **6.446** | **4.937** | **5.395** | **0.446** | **198.77** |

was the most effective, resulting in the highest germination rate (94%), followed by 1% moringa leaf extract (90%). An increase of 27% and 22% in germination was observed when the moringa seeds were subjected to 1% bacillus humid priming and 1% MLE, respectively. In contrast, untreated seeds showed the lowest germination percentage (74%), consistent with the findings of Teresa *et al*. (2017) in tomatoes.

Seedling growth characteristics, such as shoot length, root length, and dry weight, were recorded 11 days after sowing. The treatment with 1% liquid-based *Bacillus subtilis* (T12) showed the highest values for root length (15.7 cm), shoot length (29.2 cm), seedling dry weight (3.6 g/10 seedlings), and seedling vigour index (4220). This was followed by the treatment with 1% moringa leaf extract (T6), with the lowest values recorded in the control. These results suggest that germination characteristics are directly correlated with seedling growth and vigour.

The positive effects of *Bacillus subtilis* are attributed to its ability to reduce ethylene levels through the ACC deaminase activity of the bacteria, which enhances germination. Additionally, the production of IAA by the bacteria stimulates cell division, promoting embryo growth (Jalili *et al*., 2009). The plant hormones present in moringa leaf extract (MLE) also promote seed germination (Abeer and Amal, 2022).

## 4. CONCLUSION

The results of the present study demonstrated beneficial effects on improving germination in moringa. Among all the seed invigoration treatments, seeds treated with 1% liquid-based *Bacillus subtilis* and 1% moringa leaf extract showed significantly higher germination rates and seedling vigor compared to all other treatments.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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