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Moringa Leaves Extract an Eco-friendly Priming Agent for Bitter Gourd Seed Germination, Early growth and Seed vigor

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Abstract

Bitter gourd (Momordica charantia L) is one of the Pakistan's most famous summer vegetable crop. The thick seed coat of bitter gourd encloses the embryo, hindering germination. Thick seed coat can hinder water absorption that delay germination. An experiment was conducted in Horticulture laboratory of UAF Sub campus Burewala to assess the effect of Moringa leaves extract priming on Bitter Gourd seed germination at different levels. Moringa leaf extract (MLE) is rich in growth-regulating hormones. Treatments included Control, Hydro priming and varying concentrations (3.1%, 3.7%, 4.4%, and 5%) of Moringa leaves extract under completely randomized design (CRD) layout with twelve treatments and two replications. The studied parameters (energy of germination, germination percentage, root shoot ratio, germination rate, shoot length, root length, dry matter contents, and rate vigor index) were recorded and analyzed using standard statistical procedures. Results of the experiment showed that moringa leaves extract (3.7%) for 24 hours significantly improved seed and seedling quality attributes, including higher germination (95%), root length (4.58 cm), shoot length (6.53 cm), and vigor index (10.64) compared to other treatments. Keeping in view the results of experiment, Bitter Gourd seed priming with 3.7% Moringa leaves extract recommend for early seed germination and healthy seedling growth due to its chemical nature.

1. Introduction

Bitter gourd (*Momordica charantia* L.) belongs to the Cucurbitaceae family is a delicious vegetable with high nutrient and medicinal value. It is generally grown as a summer vegetable crop in Pakistan. This vegetable is very famous in Asian countries; however, it is cultivated throughout the globe (Thriveni *et al.,* 2015). In the plain regions it is usually cultivated as a summer crop between the months of January and June (Singh *et al.,* 2006). The plant thrives in sandy loam soil with a pH range of 6.0 to 6.7, and it can withstand temperatures between 25 and 28°C (Saha *et al.,* 2022). The crop has significant nutritional and therapeutic value. Its immature fruit is a great source of vitamins C and A, minerals, and dietary fiber. It also purifies blood and is very helpful for diabetics (Asna *et al.,* 2020). Likewise, it also has anti-carcinogenic properties and can be used against multiple cancer forms as a cytostatic agent.

Bitter gourd seeds have thick seed coverings that make it challenging for even seeds with excellent germination rates to sprout. Because the embryo is enclosed by a thick seed coat, this mechanical limitation prevents germination and poor seedling vigor, which decreases crop production. This issue can be resolved by seed priming (Adhikari *et al.,* 2021). MLE is rich in growth hormones like cytokinins and auxins,

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which have been shown to stimulate enzymatic activity and enhance cell elongation, contributing to the breakdown of seed dormancy and accelerating germination.

Seed priming is pre-sowing treatment that aims to enhance seed germination and seedling growth. Enables seed to germinate and emerge under adverse climatic conditions, increase yield potential and vigor for fast, reduces the amount of time needed for imbibition (Gong, H *et al.,* 2015) and strong plant development. Hydro priming, Moringa leaves extract, and chemicals can be used for seed boosting. Priming methods use synthetic chemicals (e.g., KNO_3, PEG), but these may be costly or environmentally harmful (Nouman *et al.,* 2012). *Moringa oleifera*, known as the "miracle tree," contains abundant minerals, phytohormones, antioxidants growth-promoting compounds such as cytokinins, auxins, zeatin, and flavonoids. Moringa leaf extract (MLE) has been successfully used as a bio stimulant in crops like citrus, wheat, and tomato (Khan *et al.,* 2017), but its potential in bitter gourd seed priming remains underexplored. Bitter gourd was chosen for this study due to its rising economic and medicinal importance in Pakistan, where it is a widely consumed vegetable with strong local market demand and health benefits, especially for managing diabetes.

The study aims to evaluate the MLE concentrations on Bitter gourd seed germination. Compare MLE priming with hydro priming.

2. Materials and Methods

The pot experiment was conducted in the Horticulture Lab of UAF Sub Campus Burewala, with an average temperature ranging from 25°C to 28°C. The study aimed to assess the impact of various doses of moringa leaf extracts on bitter gourd germination and growth characteristics using a completely randomized design (CRD) with two replications, each containing 10 seeds.

2.1. Materials

Bitter Gourd seeds and Twelve Petri plates acquired from Horticulture Laboratory of UAF Sub-campus Burewala Vehari. Glass Petri plates of 9cm diameter and glass conical flask of 500 ml, 100ml was used in this study. Six flasks acquired from Horticulture Laboratory. Miscellaneous items like tissue paper were used as a growing medium. Forceps were used for picking up seeds. Ethanol was used for sterilization of hands, forceps, Petri plates and tissue papers. Distilled water was used for irrigation. Sterile scissors were used to cutting tissue papers according to Petri plates.

2.2. Methods

Sterilization of equipment's Twelve Petri plates were washed first with tap water, then distilled water. Further the tissue paper was kept in the Petri plates and a set of six plates was wrapped with newspaper and kept in the autoclave in lab for 45 minutes at 121 ℃, 15 psi. After 45 minutes petri plates kept in fume Hood first cover hands by wearing gloves. Petri plates again sterilized with 70% ethanol. Forceps were sprayed with 70% ethanol and passed over a flame for 5 to 7 seconds for sterilization.

2.2.1. Moringa leaves extract preparation

Fresh moringa leaves were collected from the UAF Sub Campus Burewala. The leaves were washed with water. Pestle and mortar are used to crush the leaves into paste. The paste was prepared on a fresh weight basis. Priming solutions of Moringa leaf extract were prepared in different concentrations. In this experiment priming solution of moringa leaf extract 5%, 4.4%, 3.7% and 3.1% prepared with deionized distilled water. The extract was passed through muslin cloth to remove coarse particulates and ensure a uniform solution before priming. Different concentrations of moringa leaf extract (MLE) were used for priming of bitter gourd seeds, with the following treatments: T6: Untreated seeds (Control), T5: Priming with distilled water, T4: Priming with 3.1% MLE, T3: Priming with 3.7% MLE, T2: Priming with 4.4% MLE, T1: Priming with 5% MLE. Bitter gourd seeds were primed for 24 hours. Solution was constantly swirled at 120 revolutions per minute on Orbital Shaker (Model No: OS-300) for proper secretion of the solution. After priming, seeds washed with distilled water and were air-dried. Germination trial Petri plates with tissue paper were used as growing media. Ten (10) seeds were sown in each Petri plate with the help of forceps. After sowing petri plates moistened with distilled water. Unprimed seeds were used as a control. Petri plates inspected and data recorded for seed germination on daily basis. The experiment was run and the different parameters (energy of germination, germination percentage, Root Shoot ratio, Germination rate, Shoot length, Root length, Dry matter contents, and rate vigor index) were recorded and analyzed using standard statistical procedures.

2.2.2. Parameters studied

The parameters regarding seed germination and seedling growth were recorded as follows

Energy of Germination (EG)

Energy of germination was calculated by using the following formula

$$EG = \frac{\text{Total no of seed germinated at 4th day}}{\text{Total no of seed sown}} \times 100$$

Final Germination Percentage (FGP)

Final germination percentage was calculated by using the following formula (Goodchild and Walker, 1971).

$$FGP = \frac{\text{Total no of seeds germinated}}{\text{Total no of seed sown}} \times 100$$

Germination rate index (GRI)

The germination rate index gives an indication of the percentage of seeds germination per day of the test run period. The percentage of seeds that germinate each day throughout the test run time is shown by the germination rate index (Esechie, 1994).

$$GRI = \frac{G_1}{1} + \frac{G_2}{2} + \ldots \frac{G_x}{x}$$

G_1=Germination percentage at 1st Day

G_2=Germination percentage at 2nd Day

G_x=Germination percentage on Final Day

Mean Germination Time (MGT)

A seed lot's mean germination time indicates how long it takes on average to start and finish germination (Al-Mudaris, 1998).

$$MGT\ (days) = \frac{\text{Seed germination at last day}}{\text{Total Days}} \times 100$$

Vigor Index (VI)

Vigor Index was calculated from Seedling length and Final Germination percentage as suggested by ISTA (1999).

VI = (Avg. Root Length + Avg. Shoot Length) x FGP (%)

Statistical Analysis

The experiment was laid in Completely Randomized Design (CRD) with replications. Fisher Analysis of Variance Technique was used to analyze experimental treatments. The Tukey HSD test at $\alpha = 0.05$ was used to find out differences among treatment means.

3. Results

Results of the experiment showed that Moringa leaves extract (3.7%) for 24 hours was most effective in boosting almost all seeds and seedling quality attributes. Keeping in view the results of experiment Bitter Gourd seed priming with 3.7% Moringa leaves extract recommend for early seed germination and healthy seedling growth. Energy of germination, Germination percentage, Root Shoot ratio, Germination rate, shoot length, root length, dry matter contents, and rate vigor index were recorded and analyzed using standard statistical procedures.

Table 1. Seedling Attributes as Affected by Experimental Treatments

Treatments	Root length (cm)	Shoot length (cm)	Root Shoot Ratio	DMC (%)
T1 (MLE 5%)	2.00b	6.25ab	0.31b	12.22bc
T2 (MLE 4.4%)	3.33ab	7.52a	0.43ab	15.75ab
T3 (MLE 3.7%)	4.58a	6.53ab	0.69a	14.42ab
T4 (MLE 3.1%)	2.62ab	7.52a	0.30b	16.08a
T5 (Hydro-priming)	3.62ab	7.35ab	0.49ab	13.12abc
T6 (Control)	2.29ab	5.09b	0.32b	10.38c

Note: Means within the same column followed by the same letter are not significantly different at $p \leq 0.05$ according to Tukey's HSD test.

All Seedling parameters were Significant at 5% level of significance (Table 1). Maximum Root length (4.58 cm) and Root shoot ratio (0.69), shoot length (6.53cm) and dry matter content (14.42%) were observed in T3 (MLE 3.7%). Minimum Root length (2.00cm), shoot length (5.09cm), Root shoot ratio (0.30) and dry matter contents (10.38%) of bitter gourd seedling were observed in T1, T6, T4 and T6 respectively. These improvements under 3.7% MLE may be attributed to the optimal balance of auxins and cytokinins in the extract, which are known to regulate cell division, elongation, and differentiation during early seedling growth.

All Seed quality parameters were non-significant at 5% level of significance Except Germination rate index that have significant results at 5% level of significance (Table 2). Maximum Energy of germination (75.00%), Final germination percentage (95.00%), Vigor index (10.64) and germination rate index (9.97) of bitter gourd seed quality were observed in T3 (MLE 3.7%). Minimum Energy of germination (55.00%), Final germination percentage (80.00%), Vigor index (6.56) and Germination rate index (6.55b) of bitter gourd seed quality were observed in T4 (MLE 3.1%) and T6 (control), respectively.

Table 2. Seed Quality Attributes as Affected by Treatments

Treatments	Energy of Germination (%)	Final Germination (%)	Vigor Index	Germination Rate Index
T_1 (MLE 5%)	69.44	90.00	7.55	9.48ab
T_2 (MLE 4.4%)	70.00	90.00	9.96	9.63ab
T_3 (MLE 3.7%)	75.00	95.00	10.64	9.97a
T_4 (MLE 3.1%)	55.00	80.00	7.83	7.41ab
T_5 (Hydro-priming)	60.00	83.89	9.28	8.68ab
T_6 (Control)	65.00	80.00	6.56	6.55b

Note: Means within the same column followed by the same letter are not significantly different at $p \leq 0.05$ according to Tukey's HSD test.

4. Discussion

Moringa leaf extract is beneficial seed priming agent due to its high nutritional and bioactive content (Mashamaite *et al.,* 2022). Rich in multiple nutrients and growth hormones; readily biodegradable locally available in tropical regions. Our findings confirm that 3.7% MLE significantly improved seedling vigor and germination traits in bitter gourd, which aligns with the chemical profile of MLE being rich in auxins, cytokinins, and antioxidants. These results are consistent with Khan *et al.* (2017), who observed enhanced seedling growth and yield in tomato primed with MLE, attributing improvements to the presence of growth regulators like zeatin and IAA. Similarly, Mahboob *et al.* (2015) reported improved germination and early vigor in maize treated with Moringa extracts. In both cases, MLE contributed to increased enzymatic activity and hormonal stimulation, supporting our observations in bitter gourd. Auxins were the first class of growth regulators found. Auxins are the chemicals that have a favorable effect on the production of buds (Thimann 1993), they enhance cell and root initiation and help to generate other growth hormones. IAA is a naturally occurring hormone, while NAA, IBA, and 2-4D are produced in nature (Gupta *et al.,* 2010). Apical dominance, root induction, fruit drop control, blooming regulation, parthenocarpy, phototropism, geotropism, herbicides, prevent abscission, sex determination, xylem differentiation, and nucleic acid activity (Tanimoto, E., 2005). Gibberellins is the growth regulator (Leite *et al.,* 2003). GA promotes seed germination, flowering, and fruit maturation. Encourage cell division and elongation, seed germination, bolting/flowering in response to long days, and the prevention of hereditary dwarfism, enlargement of fruit and blossom size, dormancy and prolongation of shelf life. Cytokinins promotes cell division (Khan *et al.,* 1971), cell growth and differentiation. Also promote root development and bud initiation, facilitate nutrient transfer, extend the shelf life of fruits and vegetables, and stop chlorophyll deterioration. Other research using MLE as a growth stimulator showed yield and nutrient uptake enhancements in different agronomic and horticultural crops. (Mvumi *et al.,* 2013).

Thus, the efficacy of MLE in our experiment is not only supported by its phytochemical composition but is also justified through consistent trends seen in other horticultural and agronomic crops. This strengthens the recommendation of using 3.7% MLE as an eco-friendly seed priming treatment for bitter gourd.

5. Conclusion

Based on our results, it may be concluded that Bitter gourd seed quality and vigor can be successfully improved via priming with Moringa Leaf Extract @ 3.7%. MLE proved effective in enhancing germination percentage, seedling growth, and vigor index under laboratory conditions. Follow-up studies are recommended to evaluate the effectiveness of MLE priming under field conditions and to compare its performance with other natural and commercial bio-stimulants for broader application in sustainable agriculture.

6. Funding

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8. Conflict of Interest

The authors declare no conflict of interest.

9. Authors' Contribution

Adeena Shahid conducted the research study, Areeba Tariq wrote the paper, Rana Binyamin critically reviewed the paper.

10. SDGs addressed

This study contributes to SDG 2: Zero Hunger by enhancing germination and seedling vigor of bitter gourd, leading to improved vegetable productivity and food security. It supports SDG 12: Responsible Consumption and Production by promoting eco-friendly, plant-based priming (moringa leaf extract) as a sustainable alternative to synthetic chemicals. The work also aligns with SDG 13: Climate Action through environmentally friendly practices that reduce reliance on harmful inputs.

References

Adhikari, B., Dhital, P. R., Ranabhat, S., & Poudel, H. (2021). Effect of seed hydro-priming durations on germination and seedling growth of bitter gourd (*Momordica charantia*). *PLOS ONE, 16*(8), e0255258.

Asna, A. C., Joseph, J., & John, K. J. (2020). Botanical description of bitter gourd. In *The bitter gourd genome* (pp. 7–31). Springer Nature.

Gong, H., Rafi, K., Gu, H., Ram, G. J., Starr, T., & Stucker, B. (2015). Influence of defects on mechanical properties of Ti–6Al–4V components produced by selective laser melting and electron beam melting. *Materials & Design, 86*, 545–554.

Gupta, P., Sharma, S., & Saxena, S. (2010). Callusing in *Stevia rebaudiana* (natural sweetener) for steviol glycoside production. *International Journal of Agricultural and Biological Sciences, 1*(1), 30–34.

Khan, S., Basra, S. M. A., Afzal, I., Nawaz, M., & Rehman, H. U. (2017). Growth promoting potential of fresh and stored *Moringa oleifera* leaf extracts in improving seedling vigor, growth and productivity of wheat crop. *Environmental Science and Pollution Research, 24*, 27601–27612.

Khan, A. A. (1971). Cytokinins: Permissive role in seed germination: With other plant hormones, cytokinins regulate germination and dormancy by a novel mechanism. *Science, 171*(3974), 853–859.

Leite, V. M., Rosolem, C. A., & Rodrigues, J. D. (2003). Gibberellin and cytokinin effects on soybean growth. *Scientia Agricola, 60*, 537–541.

Mashamaite, C. V., Ngcobo, B. L., Manyevere, A., Bertling, I., & Fawole, O. A. (2022). Assessing the usefulness of *Moringa oleifera* leaf extract as a biostimulant to supplement synthetic fertilizers: A review. *Plants, 11*(17), 2214.

Mvumi, C., Tagwira, F., & Chiteka, A. Z. (2013). Effect of moringa extract on growth and yield of maize and common beans. *Greener Journal of Agricultural Sciences, 3*, 59–62.

Nouman, W., Siddiqui, M. T., Basra, S., & Maqsood, A. (2012). *Moringa oleifera* leaf extract: An innovative priming tool for rangeland grasses. *Turkish Journal of Agriculture and Forestry, 36*(1), 65–75.

Saha, S., Chatterjee, P., Halder, A. K., Nasipuri, M., Basu, S., & Plewczynski, D. (2022). ML DTD: Machine learning based drug target discovery for the potential treatment of COVID-19. *Vaccines, 10*(10), 1643.

Singh, S. P., & Grange, T. (2006). Measuring pathways to care in first-episode psychosis: A systematic review. *Schizophrenia Research, 81*(1), 75–82.

Tanimoto, E. (2005). Regulation of root growth by plant hormones—Roles for auxin and gibberellin. *Critical Reviews in Plant Sciences, 24*(4), 249–265.

Thriveni, V., Mishra, H. N., Pattanayak, S. K., Sahoo, G. S., & Thomson, T. (2015). Effect of inorganic, organic fertilizers and biofertilizers on growth, flowering, yield and quality attributes of bitter gourd (*Momordica charantia* L.). *International Journal of Farm Sciences, 5*(1), 24–29.

Thimann, K. V. (1939). Auxins and the inhibition of plant growth. *Biological Reviews, 14*(3), 314–337.

Zulfiqar, F., Casadesús, A., Brockman, H., & Munné-Bosch, S. (2020). An overview of plant based natural biostimulants for sustainable horticulture with a particular focus on moringa leaf extracts. *Plant Science, 295*, 110194.