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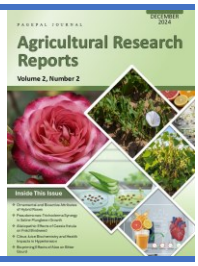

Aloe Vera Leaf Extract as a Biopriming Agent to Improve Germination Performance in Bitter Gourd (*Momordica charantia*) Seeds

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Abstract

Bitter gourd (Momordica charantia L.), locally known as "Karela," is an important summer vegetable in Pakistan belonging to the Cucurbitaceae family. Its cultivation is often constrained by a thick seed coat that restricts water uptake and gas exchange, leading to poor germination. Seed priming has emerged as an effective strategy to overcome this limitation and improve early seedling vigor. In this study, Aloe vera leaf extract (AVE), containing bioactive compounds such as vitamins, salicylic acid, enzymes, and amino acids, was evaluated as a biological priming agent. A Completely Randomized Design (CRD) was employed with treatments including non-primed control, hydropriming, and osmopriming with AVE (0.5 and 2.5%) for 24 and 48 hours. Germination parameters such as emergence speed, germination percentage, germination rate index, mean germination time, vigor index, root length, root-to-shoot ratio, and total biomass were recorded. Results showed that seed priming substantially enhanced germination and seedling growth. The most effective treatment was soaking seeds in 2.5% ascorbic acid for 48 hours, which achieved 90% germination energy, 88.15% seed moisture, and significantly higher root growth compared to untreated seeds. Shorter priming durations or lower concentrations (0.5% AVE or hydropriming for 24 hours) also improved results, but less consistently. Overall, priming with 2.5% ascorbic acid for 48 hours was identified as the most effective approach for improving bitter gourd seed germination and vigor, offering a practical method for growers to enhance crop establishment.

1. Introduction

The bitter gourd (*Momordica charantia* L.) is a member of the Cucurbitaceae family. It is additionally referred to as bitter melon or balsam pear. Southeast Asia, China, and India are among the primary regions of production for the crop (Behera *et al.* 2008). To mitigate the fruit's inherent harshness, it is typically cooked or salted before cooking (Saeed *et al.* 2018). According to study findings, bitter gourds possess an insulin-mimetic substance that influences blood and urine glucose levels (Janagal *et al.* 2018). In addition, it contains anticancer (Bai *et al.* 2016), antioxidant, and maybe dementia-related properties (Joshi *et al.* 2017).

Temperature, moisture content, soil type, and seed quality constitute a few of the variables that influence germination (Nath *et al.* 2018). Despite ideal conditions, bitter gourd seeds are unable to sprout. The seed coat or natural constraints may be the cause of this non-germination. For the sake of bitter gourd seeds to germinate, they must have minerals, including potassium, phosphorus, and nitrogen. Growth retardation may result from the shortage of

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certain elements (Rajkumar and Geetha, 2017). Seeds usually require pre-sowing treatment because of the hard shell. Methods include immersing in water for a whole day to loosen the shells. Also, scratching them with a knife or sandpaper can facilitate water absorption. Another efficient technique is to pre-germ seeds by bathing them in a moist towel for a few days before sprouting. They may be planted in both humid and well-draining soil once they have sprouted.

Seed Priming is the method that impacts the likelihood that seeds will germinate and develop into seedlings, involving initially hydrating and then dehydrating seeds just before planting. Significant benefits of priming include increased germination levels and synchronization. The vigor of seedlings and resilience to various stressors such as salinity, temperature stress, and heavy metal toxicity have been observed (Farooq *et al.* 2013; Singh *et al.* 2016).

Phytochemicals found in plant extracts are increasingly recognized for their ability to promote seed germination, boost early growth, and improve resistance to environmental stress. Aloe vera, well-known as a medicinal succulent, contains a rich array of bioactive compounds, including polysaccharides, anthraquinones, salicylic acid, gibberellins, and amino acids, that work together as anti-inflammatory antioxidants and mild antimicrobials. Growing evidence now points to benefits beyond human health, with Aloe vera leaf extract (AVE) being tested as a natural bio stimulant for seeds and seedlings. Imran *et al.* (2014) and Rauf *et al.* (2022) each reported faster germination, longer roots, and heavier seedlings in several crops treated with AVE before planting. René *et al.* (2024) showed similar gains in tomato, where AVE produced more vigorous, evenly sized plants.

Despite those promising results, very few studies have looked at using aloe vera extract, or AVE, to prime bitter gourd seeds (*Momordica charantia L.*), a crop whose germination is often stalled by seed coats that cause dormancy. Because of this gap, the present research tests AVE as a natural, eco-friendly biopriming agent and asks whether it can boost both germination rates and early seedling growth in bitter gourd. To answer that question, the team compared two concentrations of the extract (0.5 percent and 2.5 percent) soaked for either one or two days and then measured standard traits such as germination speed, seedling vigor, and total biomass under steady, controlled conditions.

2. Materials and Methods

2.1. Materials

Seeds of Local cultivar of Bitter gourd were purchased from the local market of Burewala. Glass petri plates of 9cm diameter, Glass conical flasks of 500ml, Tissue papers as growing medium, Forceps, Ethanol for sterilization of hands and tools, Distilled water for irrigation all were acquired from Olericulture Section of Horticulture Laboratory at UAF Sub Campus Burewala.

2.2. Methods

Techniques: Bitter gourd seeds from a nearby market of Burewala were used in the study at UAF Burewala Campus. Tissue papers were trimmed to fit sterile Petri plates after the seeds were sterilized with ethanol. Seven treatments were administered in triplicate on a total of twenty-one plates. Seeds were soaked for 24 or 48 hours in solutions of aloe vera extract (AVE) at varying concentrations (0.5% and 2.5%). As controls, unprimed and hydro primed seeds were used. Following priming, seeds were placed on damp tissue in Petri plates, cleaned with distilled water, and allowed to air dry. The study used a completely randomized design (CRD) and was carried out in a sterile environment. Energy of germination, final germination %, germination rate index, mean germination duration, root and shoot lengths, root-shoot ratio, fresh and dry weights, vigor index, and dry matter contents were among the parameters for which observations were made every day. The data for energy of germination (EG) was calculated using standard formulas, which are as follows:

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

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

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

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

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

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

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

Fresh Weight and dry weight are measured by digital weighing balance, and dry matter contents were derived from the following formula.

$$\text{DMC (\%)} = \frac{\text{Fresh weight-Dry weight}}{\text{Freshweight}} \times 100$$

A plastic ruler was used to estimate root length and shoot length, and they are expressed in centimeters.

$$\text{Root Shoot Ratio} = \frac{\text{Average Root Length}}{\text{Average Shoot Length}}$$

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

$$VI = (Avg.Root\ length + Avg.Shoot\ length) \times FG(\%)$$

3. Results

Significant differences were observed across all seed quality attributes due to the applied priming treatments (Table 1). One-day priming generally yielded superior outcomes compared to two-day priming, indicating that extended soaking may not be beneficial for bitter gourd seeds. Among the one-day priming treatments, hydropriming achieved a high energy of germination (EG) at 85%. However, the best EG (90%) was recorded in seeds primed with 2.5% Aloe vera extract (AvE) for two days. Final germination percentage (FGP) was highest (100%) in both one-day hydropriming and the control, but the results were statistically at par with one-day AvE at 0.5% and two-day AvE at both 0.5% and 2.5%. Similar trends were observed for germination rate index (GRI) and mean germination time (MGT), where Aloe vera treatments, particularly 2.5% AvE for two days, demonstrated enhanced performance.

The enhancement in germination traits through Aloe vera extract may be attributed to its naturally occurring plant hormones like gibberellins, auxins, and cytokinins, as well as vitamins, amino acids, and polysaccharides, which stimulate seed metabolism and cell division (Imran *et al.*, 2014; René *et al.*, 2024). These bioactive compounds likely facilitated early metabolic activation and uniform emergence. Hydropriming also contributed positively by initiating pre-germinative processes and improving seed water uptake without causing oxidative damage (Adhikari *et al.*, 2021).

Average shoot length was statistically non-significant, whereas vigor index, average root length, and seedling root-shoot ratio showed significant differences across treatments (Table 2). One-day priming generally resulted in better performance compared to two-day priming, reaffirming that prolonged exposure to water or extracts may not always enhance seedling quality in bitter gourd. Among one-day priming treatments, hydropriming resulted in the highest vigor index (946.67), followed closely by two-day priming with Aloe vera extract at 0.5% (857.77) and 2.5% (845.67).

Similarly, the longest average root length (5.87 cm) was recorded in hydropriming for one day, which was statistically

Table 1. Priming treatment effects on bitter gourd seed germination energy (EG), final germination percentage (FGP), germination rate index (GRI), and mean germination time (MGT)

Treatments	EG (%)	FGP (%)	GRI	MGT (Days)
T_1=2.5% AVE 1day	36.67c	70b	6.54a	0.78b
T_2=0.5% AVE 1day	73.33b	95a	10.13b	1.06a
T_3=Hydropriming 1day	85ab	100a	12.62a	1.11a
T_4=2.5% AVE 2day	90a	93.33a	12.59a	1.04a
T_5=0.5% AVE 2day	80ab	96.67a	11.47a	1.07a
T_6=Hydropriming 2day	40c	70b	8.46c	0.72b
T_7=Control (Un-primed)	40c	100a	8.36c	1.11a
S.E.	6.49	5.7	0.57	0.08
LSD Value	13.91	12.24	1.22	0.17

Table 2. Effect of priming treatments on bitter gourd seedling Root Shoot Ratio, Vigor Index, Seedling Root Length (RL), and Seedling Shoot Length (SL)

Treatment No.	Root Shoot Ratio	Vigor Index	RL (cm)	SL (cm)
T_1=2.5% AVE 1day	1.2abc	349.78b	2.75bc	2.35b
T_2=0.5% AVE 1day	1.86a	667.1ab	4.53ab	2.35b
T_3=Hydropriming 1day	1.64ab	946.67a	5.87a	3.59a
T_4=2.5% AVE 2day	1.73ab	845.67a	5.74a	3.31ab
T_5=0.5% AVE 2day	1.7ab	857.77a	5.56ab	3.28ab
T_6=Hydropriming cc2day	1.15bc	424.27b	3.63abc	2.95ab
T_7=Control (Un-primed)	0.59c	372b	1.38c	2.34b
S.E.	**0.31**	**154.38**	**1.32**	**0.5**
LSD Value	**0.67**	**331.11**	**2.82**	***ns***

similar to Aloe vera extract treatments for two days. In contrast, the control (unprimed seeds) showed significantly lower root length (1.38 cm) and vigor index (372), indicating the effectiveness of priming. The seedling root-to-shoot ratio was highest (1.86) in one-day priming with 0.5% Aloe vera extract, which suggests a more balanced partitioning of biomass and improved early root development. This can be crucial for crop establishment under field conditions. The lowest root-shoot ratio (0.59) was recorded in the unprimed control, reflecting weak seedling development.

The bioactive components present in the aloe vera extract, including amino acids, gibberellins, salicylic acid, and antioxidants, initiate enzymatic activity in seedling development and support early root elongation (Rauf *et al.*, 2022). In addition, hydropriming will probably be responsible for enhanced enzymatic activation and oxygen levels and, therefore, enhanced root vigor and growth reducing the danger of phytotoxicity (Rahman *et al.*, 2021). The fresh weight (FW), dry weight (DW), dry matter content (DMC), and moisture content (MC) of the seedlings were significantly different under the different seed priming treatments (Table 3). In terms of seedling biomass improvement, one-day priming treatments were significantly better than the two-day treatments. Among the treatments tested, one-day hydropriming (T3) produced the maximum fresh weight of 3.94 g, showing enhanced water uptake and metabolic processes. Shreevastav *et al.* (2023) observed that hydropriming renders bitter gourd seedlings more robust and biomassful, and this is in line with their findings. The maximum dry weight (0.58 g) achieved through two days of hydropriming was recorded (T6), and this suggests that extended hydropriming durations may enhance dry matter assimilation. But if priming is prolonged, it may at times lead to seed deterioration, and thus, timing is crucial

Table 3. Effect of priming treatments on Fresh Weight (FW), Dry Weight (DW), Moisture Content (MC), and Dry Matter Content (DMC) of Bitter Gourd Seedlings

Treatment No.	FW (g)	DW (g)	DMC (%)	MC (%)
T_1=2.5% AVE 1day	2.56c	0.47b	15.91b	84.09a
T_2=0.5% AVE 1day	3.2bc	0.57a	16.55b	83.45a
T_3=Hydropriming 1day	3.94a	0.53ab	20.25ab	79.75ab
T_4=2.5% AVE 2day	3.62ab	0.52ab	11.85b	88.15a
T_5=0.5% AVE 2day	3.15bc	0.49b	19.06ab	80.94ab
T_6=Hydropriming 2day	3.83ab	0.58a	17.23b	82.77a
T_7=Control (Un-primed)	3.15bc	0.46b	27.16a	72.84b
S.E.	**0.33**	**0.03**	**2.74**	**2.74**
LSD Value	**0.72**	**0.07**	**9.37**	**9.37**

(Madhusudhanreddy *et al.*, 2021). Interestingly, control treatment T7 had the highest dry matter content (DMC) of 27.16% because of reduced fresh biomass as a result of reduced water content and reduced metabolic processes. On the other hand, primed seeds had lower DMC readings, indicating more hydration and metabolic processes.

The 2.5% Aloe vera extract for two days (T4) contained the maximum water content of 88.15%, indicating greater water retention. Polysaccharides and gibberellins present in aloe vera, which are bioactive molecules, have been reported to enhance water intake and water retention of seedlings (Jahnavi *et al.*, 2020). Seed priming was reported by previous research to promote bitter gourd germination and seedling growth traits (Shreevastav *et al.*, 2023; Madhusudhanreddy *et al.*, 2021). The above results are in agreement with that work.

Table 3 shows that the highest fresh weight was for T3, i.e., 3.94g, followed by T6 and T4. In dry weights, the highest was for T6 and T2 at 0.58g and 0.57g, respectively. Notably, the control treatment (T7) exhibited the highest dry matter content (27.16%) and the lowest moisture content, suggesting limited water uptake. Conversely, T4 showed the highest moisture content (88.15%), highlighting the effectiveness of priming in enhancing seed hydration.

4. Conclusion

Seed priming with aloe vera extract, especially at 2.5% for 1 day, significantly improved seedling vigor, shoot/root growth, and seedling moisture content compared to the unprimed control. Hydropriming also showed positive effects, but plant-based priming (particularly short-duration aloe vera extract) yielded better seedling quality traits, suggesting its potential for enhancing bitter gourd seed performance under nursery conditions.

5. Funding

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6. Acknowledgment

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7. Conflict of Interest

The authors declare no conflict of interest.

8. Authors' Contribution

Nimra Kalsoom conducted the experimental research and data analysis. Salman Zahoor wrote major part of the manuscript and did overall formatting. Bisma Sagheer contributed to data collection and assisted in the review of relevant literature. Rana Binyamin critically reviewed the paper.

9. SDGs Addressed

This research aligns with SDG 2 (Zero Hunger) by enhancing crop productivity through improved seed germination, and SDG 12 (Responsible Consumption and Production) by utilizing natural, eco-friendly biopriming agents. It also supports SDG 15 (Life on Land) by promoting sustainable agricultural practices that safeguard the environment.

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