J. Sher-e-Bangla Agric. Univ., 14(1&2): 31-41, January & July 2023 ISSN 1997-6038

POTENTIAL OF MORINGA LEAF EXTRACT ON SEED PRIMING AND GROWTH PROMOTION OF CAULIFLOWER

J. Nesa[1], M. N. Touhid[2], S. J. Tripty[3], M. Z. A. Shishir[4] and K. Hossain[5*]

ABSTRACT

Moringa (*Moringa oleifera* Lam) leaf extract (MLE) enhances the germination percentage and seedling growth of cauliflower through its rich nutrient profile, essential compounds like vitamins, minerals, and amino acids. The study explores the potential of MLE as a novel agent for seed priming as well as growth promotion in cauliflower cultivation. This study provides insights into sustainable agricultural practices by using MLE for improving crop productivity. Experiment was conducted in controlled laboratory condition. Four treatments (T_0: Tap water as control, T_1: MLE prepared from 10 minutes boiling, T_2: MLE prepared from 20 minutes boiling, and T_3: Fresh MLE) were used in this experiment by following Completely Randomized Design (CRD) with four replications. In second stage of screening, the best selected treatment in the first stage of screening (T_1) was further evaluated (M_0: control, M_1, M_2, M_3) on growth promotion of cauliflower plant. Application of MLE prepared from 10 minutes boiling (T_1 treatment) showed the significantly highest germination percentage (100%), and mean daily germination (97.5%). Besides, treatment T_1 produced larger root (1.8 cm), accumulated higher fresh (0.244 g) and dry weight (0.043 g) of cauliflower seedlings compared to other treatments. In pot study, treatment M_3 (15 ml water:1 ml MLE−T_1) produced the highest plant height (30.9 cm), leaf number (15.0), leaf width (11.1cm), petiole length (8.7 cm) and dry weight (9.7 g) at harvest. Thus, MLE creates a conducive environment for optimal cauliflower seed germination and robust early seedling growth.

Keywords: *Brassica oleracea,* dry matter accumulation, moringa leaf extract, phytohormone, pre-sowing technique

INTRODUCTION

Cauliflower is an important winter vegetable in Bangladesh which has many health benefits such as aiding blood circulation and digestion. Cauliflower scientifically known as *Brassica oleracea* Lam under Brassicaceae family. Cauliflower is the 24th powerhouse of fruits and vegetables which is a cruciferous vegetable that is naturally high in fiber, vitamin B and water (Ware, 2023). In general, cauliflower is not difficult to grow, but it is sensitive to extreme temperatures. Different climatic hazards such as, heavy rainfall, drought, flood etc. cause crop failure (BBS, 2014). To survive in such situation, vigorous and healthy seedlings are needed. Vigorous seedlings possess robust physiological mechanisms that enable them to better withstand adverse environmental conditions such as extreme temperatures, drought, salinity, and various other stressors. Vigorous and healthy seedlings can be obtained by adopting seed priming technique (Biswas *et al.,* 2023*;* Devika *et al.,* 2021).

Seed priming is a pre-sowing treatment improves seed performance which change metabolic concentration and physiological process by application of synthetic and natural compounds. Seed priming shows the tremendous success in seed germination and seedling establishment thus adapting in abiotic stresses. Priming seeds supports germination even in challenging conditions, improves crop performance, and ultimately increases crop yield (Marthandan *et al.,* 2020). Seed priming plays role to initiate pre-germination activities without allowing the radicle to emerge, followed by the restoration of seeds to their initial moisture content through drying. These hydration- dehydration process influence metabolic activities within the seed, preparing it for germination without the actual sprouting of the root (Yadav *et al.*, 2011). According to Espanany *et al.* (2016) and Tu *et al.* (2022) cauliflower seed priming reduce germination time, increasing of seed vigour, and promote more uniform germination.

[1, 2 &4] M.S. Student, [5*]Corresponding author & Professor, Dept. of Agroforestry and Environmental Science, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207 [3]MS Student, College of Agricultural Sciences, International University of Business, Agriculture and Technology, Dhaka-1230, Bangladesh

There are different types of seed priming such as hydro-priming, chemical priming, osmo-priming, hormonal priming and redox priming which play an important role to promote plant in different adverse climatic conditions. Moringa Leaf Extract (MLE) as phytohormone may be used for seed priming. Moringa leaves contain a variety of active compounds including cytokinin, antioxidants, amino acids, flavonoids, carotenoids, and vitamins (El Sheikha *et al.,* 2022). MLE is rich in hormones and nutrients and perhaps a cost effective priming agent for cauliflower seed. Besides, MLE is being used as plant growth enhancer due to its affordability, safety, ease of preparation, and environmentally friendly nature. MLE promotes plant growth, increases leaf area, bolsters plant resistance to pests and diseases, and produce larger and more abundant fruits (Fuglie, 2001). Seed priming with MLE helps to regulate hydration process which enables seed to initiate germination more effectively and exhibits improved resistance to different stresses (Jisha, 2013). However, to best of our knowledge, no work has been done on cauliflower seed priming as well as plant growth promotion by using MLE. Moringa leaf extract as a seed priming agent perhaps enhance the germination rate, seedling vigor, and overall growth parameters of cauliflower plants (Ahmed and El-Mahdy, 2022). Therefore, this study had been undertaken to assess the bio-efficacy of Moringa leaf extract on seed priming of cauliflower seeds, establishment and growth promotion of cauliflower plants.

MATERIALS AND METHODS

Moringa (*M. oleifera*) leaf extract (MLE) were evaluated on cauliflower seed germination and seedling development at Agroforestry Field Laboratory, Department of Agroforestry and Environmental Science, Sher-e-Bangla Agricultural University, Dhaka (23° 46′N, 90° 22′E) from 1st October, 2023 to 18th January, 2024.

Seed materials: Seeds of cauliflower (*Brassica oleracea*) cv. BARI Fulkopi-2 were collected from Bangladesh Agricultural Research Institute, Gazipur. Before starting of experiment, seeds were surface sterilized in 1% sodium hypochlorite solution for 3 min, then rinsed with sterilized water and air-dried.

Extract preparation:

Fresh young Moringa leaf was collected from Agroforestry Field Laboratory, SAU, Dhaka. In this experiment, three types of extract were used.

Moringa Leaf Extract (MLE) prepared from 10 minutes boiling: MLE was prepared by following the method of Han *et al.* (2022) with some modification. Fresh leaf was chopped into small pieces of approximately 2 cm. Chopped leaf was thoroughly washed with tap water to remove dirt, sand, and other debris. In short, 100 g of chopped leaf was added into 500 mL of distilled water, and boiled for 10 min. Then the decoction was cooled at room temperature. Two fold muslin cloth was used to separate the Moringa leaf extract from the solid leaves, and were kept as crude or stock solution at 4 ^{0}C until required for further studies.

MLE prepared from 20 minutes boiling: Same procedure was followed as described previously except boiling time. Here, it was 20 minutes.

MLE prepared without boiling: MLE was prepared by following the method of Hoque *et al.* (2020) with some modification. Here, 200 gm fresh, cleaned, and chopped moringa leaves was added with 1000 ml water. The mixture was grinded for 3 minutes by using a grinding machine (HAVELLS Power Hunk, 800W Motor, 2100 RPM). The mixture was strained out four times by using cheese cloth to separate Moringa extract from solid particles. The extract was stored in a clean, airtight container at 4^{0}C for further use.

Evaluation of MLE on seed priming of cauliflower

Seed priming. Four treatments namely T_0= Tap water as control, T_1= MLE prepared from 10 minutes boiling, T_2= MLE prepared from 20 minutes boiling, and T_3= Fresh MLE were used in this experiment. A total of 100 cauliflower seeds were soaked into each extract according to the treatments for 6 hours at room temperature and drained the primed seed on paper towels for 24 hours and re-dried near to original weight (Sundstrom *et al.,* 1987).

Germination test. Four replicates of 20 seeds each were placed in 9 cm diameter petri dishes at room temperature. Five mL of MLE (treatment wise) was applied in each Petri dish, while distilled water was applied for normal conditions. A seed was scored germinated when coleoptile and root lengths reached 2 - 3 mm. Counts of germinating seeds were made every 6 h, starting on the first day of imbibition, and terminated when maximum germination was achieved.

Measurements and definitions

Several traits were evaluated to determine the role of different MLE on cauliflower seed priming.

Germination percentage (%) = (Number of Germinated Seeds/Total Number of Seeds) ×100.

Germination Initial Time (GIT, day): **is the period** it takes for a seed to begin the process of germination after being sown under specific environmental conditions, Number of days of first germinated seed.

Maximum Germination Time (MGT, day): MGT is the longest period within which a seed is expected to germinate under ideal conditions. It is the duration beyond which a seed is considered non-viable or unable to germinate, Number of days until the highest germination seeds.

Germination Duration Time (GDT, day): GDT = MGT – GIT, The interval from GIT to MGT.

Mean Daily Germination (MDG, %): It is calculated by dividing the total number of germinated seeds by the number of days it took for germination to occur.

MDG (%) = GP/MGT.

Mean Germination Time (MGT, day): It is calculated by recording the number of germinated seeds at regular intervals and then determining the time it takes for half of the seeds to germinate, the count days until reached to 50% of germinated seeds.

Speed of germination rate: Speed of Germination Rate (SGR) =1/Germination Time, Where, SGR is the Speed of Germination Rate, typically measured in units of per day (e.g., seeds per day).

Seedling Vigour Index (SVI): The vigour level of each treated seed lot was calculated according to Kim *et al.* (1994) as percentage normal emergence multiplied by seedling shoot height and divided by 100 (Adebisi, 2004).

Shoot length (cm) and root length (cm): Measured by measuring scale.

Fresh and dry weight (gm): Measured by weighting balance.

Evaluation of MLE on growth promotion of cauliflower: The best selected MLE (T_1= MLE prepared from 10 minutes boiling) was further evaluated to investigate its potentials on growth promotion of cauliflower.

Crop husbandry

Planting trays (42 × 21 × 19 cm) with drainage holes were surface sterilized in 2.5% (w/v) Sodium hypochlorite (NaOCl), rinsed with distilled water, and used in seedling establishment tests. Each tray was filled with 12.5 kg of soil mixture which was prepared by mixing soil and cowdung (3: 1, w/w). Thirty cauliflower seeds were sown in soil mixture at a depth of 1 cm, placed in three circles, with 10 seeds in each circle. Basal doses of N, P and K fertilizers were applied at 0.84g, 0.84g and 0.68g/tray in the form of urea, triple super phosphate (TSP) and muriate of potash (MoP) according to the treatments tested (Table 1). Soil moisture was maintained at field capacity. Each treatment consists of one planting tray. Data on plant growth promotion were recorded at intervals of ten days and were continued up to 50 days. Two additional splits of total 0.84g and 0.68g/tray urea and muriate of potash (MoP) were top dressed at 15 and 35 days after seeding (DAS). Soil moisture was maintained at field capacity by daily watering.

Fig.1. Evaluation of Moringa leaf extract (MLE) on cauliflower seedling growth promotion

Table1. Treatments for evaluating the effectiveness of MLE−T_1 on cauliflower growth promotion

Treatments	Details
M_0	Control, basal doses of fertilizers
M_1	Basal doses of fertilizers plus MLE−T_1 (5 ml water:1 ml MLE; v/v)
M_2	Basal doses of fertilizers plus MLE−T_1 (10 ml water:1 ml MLE; v/v)
M_3	Basal doses of fertilizers plus MLE−T_1 (15 ml water:1 ml MLE; v/v)

Measurements and definitions

Plant height (PH), distance from ground level to the tip of the longest leaf, were measured in cm from 5 randomly selected plants at 10 (PH10), 20 (PH20), 30 (PH30), 40 (PH40), 50 (PH50) days after seeding (DAS).

Leaf number (LN) were counted at different growth stages, and designated as 10 (LN10), 20 (LN 20), 30 (LN 30), 40 (LN 40), 50 (LN 50) days after seeding (DAS), respectively.

Petiole length (PL), distance from ground level to the tip of the longest leaf, distance from leaf base up to the base of leaf blade, were measured in cm from 5 randomly selected plants at, 40 (PL40), 50 (PL50) days after seeding (DAS).

Leaf breadth (LB), using a ruler measurement is taken from the outer edge of one side of the leaf to the outer edge of the other side, were measured in cm from 5 randomly selected plants at 40 (LB40), 50 (LB50) days after seeding (DAS).

Fresh and dry weights of plants, the growth performance of cauliflower was assayed on the basis of fresh and dry weights of plants determined after 50 days of seeding. The plants were carefully uprooted from the pots and thoroughly washed without damaging the roots to remove all adhering soil mixture. After recording the fresh weight, the plants were placed in an oven at 65 ^{0}C for 72 hours and dry weight was recorded.

Experimental design and data analysis

All experiments were conducted using completely randomized design (CRD) with four replications. The data were subjected to analysis of variance (ANOVA) and tested for significance using Least Significant Difference (LSD) using R-3.5.1 software (R Core Team, 2017).

RESULTS AND DISCUSSIONS

In this study, MLE was evaluated on cauliflower seed germination and growth promotion in two stages of screening where MLE performed significantly better over control treatments.

Germination percentage (GP)

Germination percentage is linked to seed vigor, which refers to overall health and robustness of the seeds. Significant differences of germination percentage of cauliflower seeds among the different treatments were recorded (Table 2). Germination percentages were 95, 100, 95 and 83.3% at T_0 (Control), T_1, T_2 and T_3 respectively at day 3. The highest germination percentage was found in T_1 treatment (100%) and the lowest germination percentage was found in T_3 treatment (83.3%). The second highest germination percentage was found in both T_0 and T_2 treatments which was 95.0%. Higher germination percentages post priming are an indicator of enhanced seed vigor.

Table 2. Effect of MLE on germination percentage of cauliflower seed priming

Treatment	Germination percentage (%)
T_0	95.0 b
T_1	100.0 a
T_2	95.0 b
T_3	83.3 c
LSD	2.718

Means within columns with the same letters are not significantly different at 5% level of probability

Remarks: T_0: Tap water as control; T_1: MLE prepared from 10 minutes boiling; T_2: MLE prepared from 20 minutes boiling; T_3: Fresh MLE

MLE contains vitamins, minerals, phytochemicals and natural antioxidants may enhance the overall physiological process involved in cauliflower seed germination. Our results were supported by the results of Goel *et al.* (2003) who reported that seed priming improved germination percentage and reduced deterioration of seeds under accelerated aging of cotton.

Germination initial time and maximum germination time

Monitoring germination initial time and maximum germination time in seed priming is essential for assessing the effectiveness of the priming treatment in terms of accelerating germination initiation, achieving uniform germination and optimizing the overall germination period. There were no significant differences of germination initial time among the treatments, but there were significant differences for maximum germination time of cauliflower seeds among the treatments (Table 3). Rapid GIT is beneficial in the field as it can lead to quicker and more uniform seedling emergence. In all treatments GIT was 1. Maximum germination time provides information about the overall germination period and the extent of synchronization in seed germination. The significantly lowest MGT was found in T_0 treatment (1 day), followed by T_1 and T_2 treatment with the values of 1.7 and 2 respectively. The highest MGT was found in T_3 treatment which was 2.7 day. Seed priming results in a shorter GIT and a more condensed MGT, indicating higher percentage of seeds germinate more quickly and synchronously. In this regards, treatment T_0 appeared as the best treatment closely followed by T_1 treatment in priming of cauliflower seeds.

Table 3. Effect MLE on Germination Initial Time (GIT) and Maximum germination time (MGT) of cauliflower seed priming

Treatment	Germination Initial Time (GIT, day)	Maximum germination time (MGT, day)
T_0	1.0	1.0 c
T_1	1.0	1.7 bc
T_2	1.0	2.0 ab
T_3	1.0	2.7 a
LSD	NS	0.769

Means within columns with the same letters are not significantly different at 5% level of probability

Remarks: T_0: Tap water as control; T_1: MLE prepared from 10 minutes boiling; T_2: MLE prepared from 20 minutes boiling; T_3: Fresh MLE

Germination Duration Time (GDT) and Mean Daily Germination (MDG)

Germination Duration Time (GDT) and Mean Daily Germination (MDG) were used to assess the efficiency of the priming treatment. A successful priming treatment might lead to a shorter GDT and a higher MDG, indicating quicker and more synchronized germination. There were significant differences of GDT and MDG on cauliflower seed among the treatments (Table 4). The lowest value of GDT was found in T_0 treatment where cauliflower seed were treated with water and the second lowest value of GDT was found in T_1 treatment which was 0.7 day. The highest value of GDT was found in T_3 treatment which was 1.7 day. As expectation, the highest MDG was found in T_1 treatment (97.5%) and

Table 4. Effect of MLE on Germination Duration Time (GDT) and Mean Daily Germination (MDG) of cauliflower seed priming

Treatment	Germination Duration time (GDT, day)	Mean daily Germination (MDG) (%)
T_0	0.0 c	95.0 ab
T_1	0.7 bc	97.5 a
T_2	1.0 ab	92.0 b
T_3	1.7 a	81.1 c
LSD	0.769	2.879

Means within columns with the same letters are not significantly different at 5% level of probability

Remarks: T_0: Tap water as control; T_1: MLE prepared from 10 minutes boiling; T_2: MLE prepared from 20 minutes boiling; T_3: Fresh MLE

the least MDG was found in T_3 treatment which was 81.1%. Hydropriming is a technique for initiating germination that involves soaking of seeds in water followed by drying. Seed priming of Rice with

MLE recorded earlier and uniform crop stand, improved yield and quality attributes than hydropriming (Kamran, 2011).

Mean Germination Time (MGT) and Speed of Germination Rate (SGR)

Table 5 showed that there was significant differences of Mean Germination Time (MGT) and Speed of Germination Rate (SGR) of cauliflower seed among treatments. The highest MGT value was found in T_3 treatment which was 1.9 day and the second highest value of MGT was found in T_2 treatment which was 1.5 day. The lowest value of MGT was found in T_0 treatment (control) which was 1 day. In seed priming, a shorter MGT is generally desirable as it indicates quicker and more synchronized germination which may lead to a more uniform stand of seedlings in the field. The highest values of SGR was found in T_0 treatment (control) which was 1 seed/day but the second highest values of SGR was found in T_1 treatment which was 0.7 seed/day. The lowest values of SGR was found in T_3 treatment which was 0.4 seed/day. An increased SGR implies that priming treatment has accelerated the initiation and progress of germination. In our study, T_0 appeared the best treatment in terms of MGT and SGR followed by T_1 treatment. Application of MLE often results in a shorter MGT and a higher SGR, demonstrating that seeds are germinating more quickly and uniformly (Ahmed and El-Mahdy. 2022).

Table 5. Effect of MLE on Mean Germination Time (MGT) and Speed of Germination Rate (SGR) of cauliflower seed Priming

Treatment	Mean Germination Time (MGT, day)	Speed of Germination Rate (SGR, seed/day)
T_0	1.0 c	1.0 a
T_1	1.3 bc	0.7 b
T_2	1.5 ab	0.5 bc
T_3	1.9 a	0.4 c
LSD	0.425	0.293

Means within columns with the same letters are not significantly different at 5% level of probability

Remarks: T_0: Tap water as control; T_1: MLE prepared from 10 minutes boiling; T_2: MLE prepared from 20 minutes boiling; T_3: Fresh MLE

Shoot length (cm) and root length (cm)

There were no significant differences of shoot length (cm) and root length (cm) of cauliflower seedling among treatments (Fig. 2). The highest shoot length was recorded in the seedling belong to T_3 treatment with the value of 3.2 cm and the lowest shoot length was found in the seedlings of T_0 with the value of 3.0 cm. Treatments T_1 and T_2 produced intermediate type of shoot (3.1 cm).

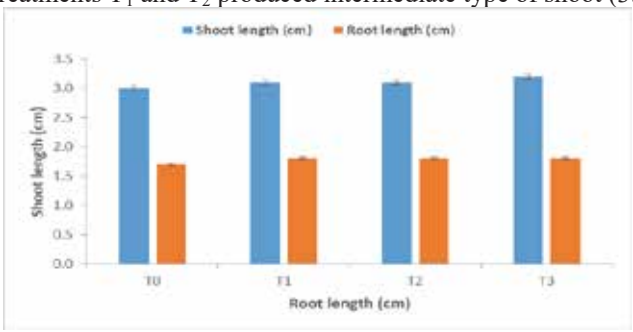

Fig. 2. Effect of MLE on Shoot length (cm) and Root length (cm) of cauliflower seedling

Means within columns with the same letters are not significantly different at 5% level of probability

Remarks: T_0: Tap water as control; T_1: MLE prepared from 10 minutes boiling; T_2: MLE prepared from 20 minutes boiling; T_3: Fresh MLE

In case of root, the least length of root was recorded in the seedlings of T_0 treatment where other treatments (T_1, T_2 and T_3) produced root of 1.8 cm length. All the treatments contain MLE produced longer shoot and root length compared to hydropriming. Our research results were in line with the

findings of Afzal *et al.*(2006) reported that shoot length improved due to seed priming in wheat. MLE is rich in zeatin, ascorbates, phenolic and minerals like Ca, K and Fe which increase crop growth (Anjorin *et al.,* 2010). An increase of 94% in radish and 65% in bean because of MLE application (Foidle, 1999)

Germination Vigor Index (GVI)

Germination Vigor Index (GVI) reflects the quality and speed of seed germination. Higher values indicate better seed vigor in terms of both the rate and uniformity of germination. The significantly highest GVI were observed in the seeds treated with MLE compared to hydro-priming seeds (Table 6). Results showed that the significantly highest GVI was recorded in T_3 treatment (168.5mg) where T_2 (142.5mg) and T_1 treatments were statistically same. The least GVI was recorded in T_0 treatment (95.0mg). MLE, when used in seed priming, plays a crucial role in enhancing seed germination and early seedling growth. The extract contains bioactive compounds, cytokinins and antioxidants, which stimulate enzymatic activity and protects seed from oxidative stress, promoting overall seed vigor. Additionally, the presence of nutrients like vitamins and minerals in MLE contributes to improve seedling establishment and resilience during the early stages of plant development.

Table 6. Effect of MLE on Germination Vigor Index (GVI) of cauliflower seed priming

Treatment	Germination Vigor Index (GVI) (mg)
T_0	95.0 b
T_1	133.3 ab
T_2	142.5 a
T_3	168.5 a
LSD	43.081

Means within columns with the same letters are not significantly different at 5% level of probability

Remarks: T_0: Tap water as control; T_1: MLE prepared from 10 minutes boiling; T_2: MLE prepared from 20 minutes boiling; T_3: Fresh MLE

Fresh weight (g) and dry weight (g)

Table 8 represents fresh and dry weight (g) of cauliflower seedling and showed that there were significant differences in fresh and dry weight in between the treatments. The data clearly demonstrates that when cauliflower seed was primed by MLE, there were a noteworthy increment in fresh and dry weight of cauliflower seedling. The significantly highest fresh weight (0.244g) was recorded in T_1 treatment (Figure 3) and T_0 recorded the significantly lowest (0.222g) fresh weight.

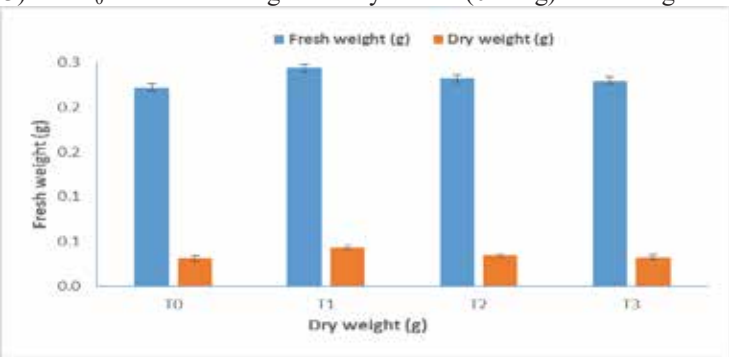

Fig. 3. Effect of seed priming by different MLE on cauliflower seedling on fresh weight (gm) and dry weight (gm) of cauliflower seedling

Means within columns with the same letters are not significantly different at 5% level of probability

Remarks: T_0: Tap water as control; T_1: MLE prepared from 10 minutes boiling; T_2: MLE prepared from 20 minutes boiling; T_3: Fresh MLE

As expectation, similar trends were also recorded in dry weight of cauliflower seedlings. Results showed that maximum dry weight (0.043g) was recorded in T_1 treatment followed by, T_2 and T_3 treatments and the significantly lowest dry weight (0.031g) was found in the seedlings of T_0 treatment.