Original Research Article

Impact of Seed Priming and Growing Media on Bitter Gourd Germination and Seedling Growth

ABSTRACT

Aims: The aim of this study was to determine the suitable growing media and priming treatments for seed germination and seedlings growth of bitter gourd (cv. BARI hybrideKarolla 2).

Study Design: The experiment was set up in a completelyrandomized design (CRD) with sixteen treatments and three replications.

Place and Duration of the Study: The experiment was conducted on Horticulture farm at Sher-e-Bangla Agricultural University; Dhaka, Bangladesh from March to April 2021.

Methodology: Four seed priming treatments were used in the experiments: T_0: Seed soak in distilled water for 12 h (control), T_1:Hot water treatment (45^0 C 5 min), T_2: Poly ethylene glycol (PEG) 6000 (5%), T_3: Sodium chloride solution (NaCl) (2%) soak 12h and four growing media viz.,M_0: soil + cowdung (1:1), M_1: soil + cowdung + vermicompost (1:1:1), M_2: soil + cowdung + sawdust (1:1:1), M_3: soil + cowdung + cocopeat (1:1:1).

Results: The maximum germination percentage (100%), highest shoot (21.00 cm) and root length (12.83 cm), seed vigor index (3383.3) was recorded in T_2M_3 treatment. However, the maximum and photosynthetic pigment (30.31 mg/g), relative water content (96.99%) and shoot (3.53g) and root fresh weight (0.47 g) was found in T_2M_1 treatment.

Conclusion: It can be concluded that the most suitable treatment for seed germination and vigorindex of bitter gourd is soil + cowdung + cocopeat (1:1:1) with PEG 6000 (5%).In contrast, soil + cowdung + vermicompost (1:1:1) with PEG 6000 (5%) are favorable for healthy seedling.

Keywords: Germination percentage; photosynthetic pigments; bitter gourd; fresh weight

1. INTRODUCTION

In Bangladesh, bitter gourd (*Momordicacharantia* L.) is a popular summer vegetable. Its immature fruit is heavy in nutritional fibers, minerals, and vitamins (C and A), and it also functions as a blood purifier, which is extremely useful to diabetics [1].There is an urgent need to boost production of bitter gourd due to rising demand for medicinal and culinary uses. Even though bitter gourd seeds have a high germinability, field emergence is always an issue because of the thick seed coat, which causes the seed to progressively consume water and result in delayed germination [2].

In many crops, seed priming is an effective, useful, and straightforward method to promote quick and consistent emergence, high seedling vigor, and yield in adverse environmental circumstances [3].It has been demonstrated to help a variety of horticultural and agricultural crops in terms of seed germination, seedling establishment, and eventually yield [4].Hot water treatment was shown to be effective due to its greater penetrative potential[5]. By enhancing water uptake and nutrient availability, seed priming with PEG is an effective treatment for boosting canola crop performance in terms of seedling growth, chlorophyll content, and yield [6].It has been shown that halopriming with NaCl improves germination and seedling establishment in milk thistle [7].Seed germination, development, and the effectiveness of the roots system are all directly impacted by the choice of growing media or substrates [8]. An ideal growing medium would give the plant enough anchoring or support, act as a reservoir for nutrients and water, diffuse oxygen to the roots, and facilitate gaseous exchange between the roots and the surrounding environment [9].Vermicompost has a variety of phenolic and humic active ingredients, each with a unique dosage and genotype-dependent effects on seed germination and the early phases of seedling growth [10]. Organic-based media encourages superior root development when compared to soil-based media [11]. There has been little research published on the use of various growing media and priming treatments in bitter gourd production. Consequently, the goal of the study was to ascertain how different growing media and priming treatments affected the germination of bitter gourd seeds and the growth of seedlings.

2. MATERIALS AND METHODS

2.1 Plant materials and growing conditions

The experiment was conducted at the Horticulture Farm of Sher-e-Bangla Agricultural University, located in Sher-e-Bangla Nagar, Dhaka, Bangladeshis located at 23°42′37″N (Latitude), 90°24′26″ E (Longitude) and has an average elevation of 4 meters, according to the National Mapping Organization of Bangladesh.The experiment took place from March to April of 2021. Bitter gourd seed cv. BARI hybrid karolla 2 was collected from Bangladesh Agricultural

Research Institute, Gazipur, Bangladesh andplanted under poly net house in plastic polybag (6 inch × 5 inch). Using sixteen treatments and three replications, the experiment was set up in acompletelyrandomized design (CRD).During the trial period of March to April 2021, the average minimum and maximum temperatures were 30.5 °C and 33.2 °C, respectively, with an average relative humidity of 60%.Throughout the study, all critical cultural practices and plant protection measures were applied to all plants in the same way. For morphological and physiological observations, three plants were chosen at random in each replication.

2.2 Treatments

The four priming treatments: T_0: Seed soak in distilled water for 12 h (control), T_1: Hot water treatment (45^0 C 5 min), T_2: Poly ethylene glycol (PEG) 6000 (5%), T_3: Sodium chloride solution (NaCl) (2%) soak 12h. Except for the hot water treatment, seeds were washed in the solution for 12 hours at room temperature in the dark. After priming, seeds were placed in plastic polybags containingvarious types of growth material, including M_0:soil+cowdung (1:1), M_1:soil+cowdung+vermicompost (1:1:1), M_2: soil + cow dung + sawdust (1:1:1), M_3: soil + cow dung + cocopeat (1:1:1).The nutrient level of the soil used in this experiment was measured at the soil research and development institution in Dhaka and the results are displayed in table 1 and 2

Table 1: Physical and chemical characteristics of the experimental soil

Characteristics	**Value**
Particle size analysis % Sand	27
% Silt	43
% Clay	30
Textural class	Silty Clay Loam (ISSS)
pH	5.6
Organic carbon (%)	0.45
Organic matter (%)	0.78
Total N (%)	0.03
Available P (ppm)	20
Exchangeable K (me/100 g soil)	0.1
Available S (ppm)	45

Table 2. Initial nutrient composition of the following substrates

Properties	Nutrients		
	N%	P%	K%
Cowdung	0.42	0.18	0.30
Sawdust	0.45	0.009	0.018
Cocodust	0.46	0.80	1.30

Vermicompost	1.23	1.13	1.18

2.3 Germination percentage and vigor index of bitter gourd seedling

The number of days to germination was determined from the beginning of germination to the end of germination. The following formula was used to calculate seedling vigor and germination percentage:

Germination percentage = total number of seeds germination/total number of seeds sown × 100[12]

Vigor index = germination percentage × total length of seedling[13].

2.4 Length of shoot and root (cm)

Shoot length was measured from the collar region to the apical bud of the shoot 11 days after seeding. At 11 days old seedling day after sowing, root length was measured using a meter scale from the spot where the first root started up to the end tip of the main root. The average length of the shoot and root was measured in centimeters.

2.5 Fresh and dry weight of shoot and root (g)

The seedling was cleaned and chopped into the collar region after it had been uprooted. Then, at 11 days old seedling DAS, fresh shoot and root weight were measured using an electric digital scale, and the mean value was computed.After drying the shoot and root in an electric oven drier at 65℃ for 72 hours, the dry weight was measured and the mean value was computed.

2.6 Leaf area (cm^2) and number of leaves per plants

Leaf area was estimated by multiplying the leaf's length and width. At 11 days old seedling DAS, the total number of leaves and leaf area were counted. The average leaf area and leaf number were computed, as well as the mean value.

2.7 SPAD chlorophyll meter reading

A SPAD-502 chlorophyll meter (Minolta, Tokyo, Japan) was used to determine the amount of chlorophyll present in the first fully opened leaves. Measurements were taken from the middle of the leaf lamina of each treated and control plant [14].

2.8 Relative water content

Smart and Bingham [15] estimated the relative water content (RWC). Three leaves were pooled for each replication and their fresh weights (FW) were computed. The turgid tissue was swiftly blotted to remove excess water after soaking the leaves in room temperature water for twelve hours to recover turgidity, and their turgid weights (TW) were determined. The samples were subsequently dried in an oven set at 65°C for 24 hours in order to calculate their dry weights (DW). The following formula was used:

RWC % = ((FW−DW)/(TW−DW))*100.

2.9 Photosynthetic pigments

Moran and Porath[16] approach was used to detect photosynthetic pigments. Liquid nitrogen was used to grind 0.2 g of leaf tissue into a powder, which was then homogenized with 1 ml of 100% N, N-dimethylformamide (DMF). To collect the supernatant, homogenized materials were centrifuged at 10,000 g for 10 minutes. The samples were centrifuged after another 1 ml of DMF was added. After removing the supernatant, 1 ml DMF was added. A spectrophotometer was used to measure absorbance at 663 and 645 nm. Calibration was performed with a 100% DMF blank. The following formulas were used to determine chlorophyll a, b, and total chlorophyll:

Chlorophyll *a* (mg g-1 tissue) = $\frac{[12.7(OD663)-2.69(OD645)]\times V}{1000}\times W$

Chlorophyll *b* (mg g^{-1} tissue) = $\frac{[22.9(OD645)-4.68(OD663)]\times V}{1000}\times W$

Total Chlorophyll (mg g^{-1} tissue) = $\frac{[8.02(OD663)+20.20(OD645)]\times V}{1000}\times W$

Where OD denotes the optical density at each nm, V is the final volume of chlorophyll extract, and W denotes the fresh weight of the extracted tissue.

2.10 Statistical analysis

Statistics 10 (IBM Corp, Armonk, NY, USA) was used for all statistical analyses. The mean value across treatments was refereed statistically significant when $p<0.05$.

3. RESULTS AND DISCUSSION

3.1 Germination percentage and vigor index

Statistically significant variation was recorded for germination percentage due to combined effect of priming treatment and growing media (Fig. 1A).Results of the experiment indicated that highest germination percentage (100%) was recorded from T_2M_3 treatment whereas the lowest germination percentage (76.67%) was recorded from M_0T_0 treatment.Osmo primed seeds have a higher germination rate and improved uniformity in seedling emergence, which help to crop establishment and thus yield [17]. PEG is non-damaging to proteins and has no negative effects on seed embryos, resulting in increased germination [18].The physical and chemical features, structure, texture, pH, and nutrients of coco peat and peat moss are constrictive [19]. Organic matter like coco dust and sawdust increased the germination rate, which might be due to contain of higher amounts of essential nutrients (N, P, K)[20].

The effect of several priming treatments and growth media on vigor index varied dramatically (Fig.1B).The results revealed that highest vigor index (3383.3) was recorded from T_2M_3 treatment while the lowest vigor index (1631) was recorded from T_0M_0 treatment.PEG may help with seed vigor index by seed priming since this treatment is suitable for the metabolic response, improving seed germination efficiency, seedling establishment, and boosting seedling growth in soybean[21]. Islam *et al*.[22] stated that the vigor index reflects the health of the seedling and, ultimately, the plant's output. The use of coco peat with soil as a growing media, increased germination percentage and reduced the incidence of damping off seedling, plant height of tomato seedlings will enhance the final production of healthy and vigorous tomato seedlings which provide better yields [23].

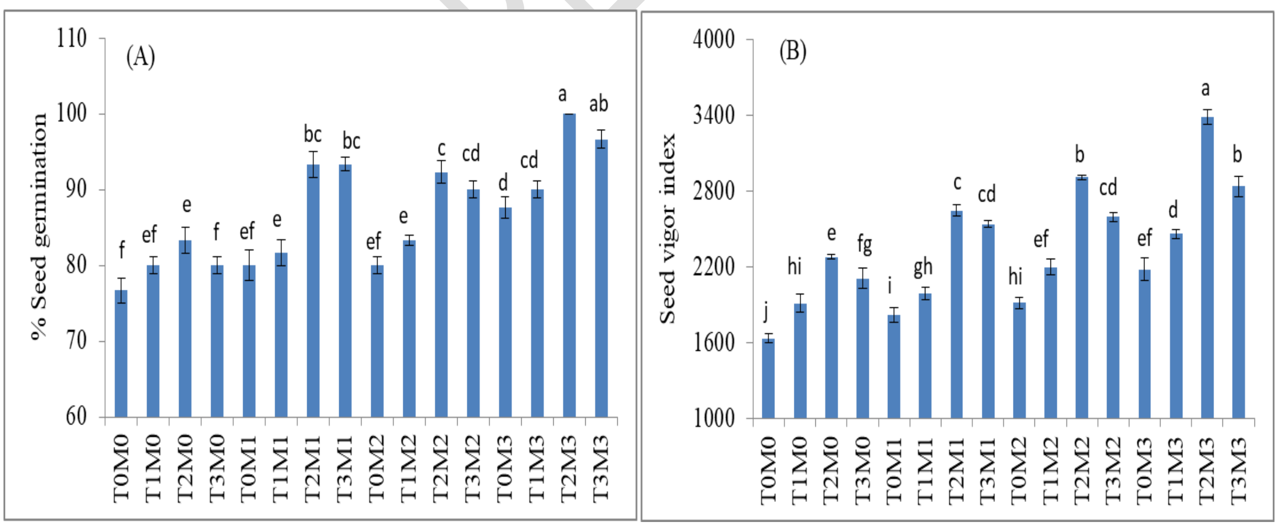

Fig.1. Effect of seed priming treatments and growing media on seed germination (%) (A) and seed vigor index (B) of bitter gourd seedling

T_0=Distilled water, T_1= Hot water, T_2= Polyethelene glycol (PEG), T_3=Sodium Chloride (NaCl), M_0=soil+cow dung, M_1=soil + cow dung + vermicompost, M_2= soil + cow dung + sawdust, M_3=

soil + cow dung + cocopeat. Means in a column that are followed by the same letter (s) do not differ significantly at the 5% level of LSD.

3.2 Shoot and root length (cm)

Combined effect of differentpriming and growing media showed significant variation for shoot and root length of bitter gourd (Table 1). Results revealed that the tallest shoot (21.00cm) was recorded from T_2M_3 treatment whereas the shortest shoot (14.29cm) was recorded from $T_0M_{0.}$ The longest root length (12.83cm) was found from T_2M_3 treatment while the shortest root length (6.83 cm) was found from T_0M_1 treatment. Osmo priming with PEG primed wheat seeds resulted in a faster and longer elongation of shoot and root of wheat seedling than non-primed seeds [24].Themaximum seedling length of bitter gourd occurs in Poly Ethylene Glycol over control treatment[25].Organic manure like cocodust contain macro and micro nutrients in available forms during mineralization and improving physico-chemical properties of soils which led to taller seedling[26].

3.3 Leaf area and Number of leaf / plant

Different seed priming and growing media showed significant variation for leaf area and leaf number of bitter gourd plant^{-1} (Table 3). The maximum leaf area plant^{-1} (100.83 cm^2) was recorded in T_2M_1 and the minimum leaf area plant^{-1} (50.33cm^2) was observed in T_0M_0 treatment. The application of organic manure such as vermicompost increased the leaf area of watermelon plant because it improves the soil physical, chemical and biological properties and creates optimum conditions for vigorous plant growth and development[27]. Seeds treated with PEG400 for 24 h produced maximum leaf area of okra than other treatment [28].

In most of the treatments, there was no significant difference on leaf number plant^{-1} of bitter gourd (Table 3). However, the highest leaf number plant^{-1} was observed (5.00) fromT_1M_1, T_2M_1 and T_1M_3 treatment and the lowest leaf number plant^{-1}(3.50) was observed from T_0M_0 treatment. Osmo priming with PEG significantly increased leaf number of rape seed as compared to un-primed seeds[29]. Plants treated with vermicompost developed a large leaf area. It could be because of increased nutritional availability, which results in increased production of photo synthetically effective leaves.

Table 3.Effect of seed priming and different growing media on shoot and root length of bitter gourdplant^{-1}

Treatment	Shoot length (cm)	Root length(cm)	Leaf area (cm^2)	Leaf number
T_0M_0	14.29^{f}	7.00^{ij}	50.33^{j}	3.50^{d}
T_1M_0	16.02^{e}	7.83^{g-i}	61.50^{h}	4.67^{ab}
T_2M_0	18.33^{c}	9.00^{ef}	76.67^{e}	4.67^{ab}
T_3M_0	18.00^{c}	8.333^{f-h}	69.58^{f}	4.33^{bc}
T_0M_1	15.50^{ef}	6.83^{j}	72.08^{f}	4.33^{bc}
T_1M_1	16.00^{e}	8.33^{f-h}	81.75^{cd}	5.00^{a}
T_2M_1	19.00^{bc}	9.333^{de}	100.83^{a}	5.00^{a}
T_3M_1	18.50^{c}	8.67^{e-g}	92.25^{b}	4.33^{bc}
T_0M_2	16.27^{e}	7.67^{h-j}	54.50^{i}	4.00^{cd}
T_1M_2	17.67^{cd}	8.67^{e-g}	68.58^{fg}	4.67^{ab}
T_2M_2	20.17^{ab}	11.33^{b}	84.08^{c}	4.67^{ab}
T_3M_2	18.83^{bc}	10.00^{cd}	78.75^{de}	4.67^{ab}
T_0M_3	16.50^{ed}	8.33^{f-h}	65.33^{g}	4.33^{bc}
T_1M_3	18.33^{c}	9.00^{ef}	71.50^{f}	5.00^{a}
T_2M_3	21.00^{a}	12.83^{a}	94.41^{b}	4.67^{ab}
T_3M_3	19.00^{bc}	10.33^{c}	82.83^{c}	4.67^{ab}
LSD (0.05)	1.39	0.99	3.71	0.65
CV (%)	4.73	6.66	2.96	8.64

T_0=Distilled water, T_1= Hot water, T_2= Polyethelene glycol (PEG), T_3=Sodium Chloride (NaCl), M_0=soil+cow dung, M_1=soil + cow dung + vermicompost, M_2= soil + cow dung + sawdust, M_3= soil + cow dung + cocopeat. Means in a column that are followed by the same letter (s) do not differ significantly at the 5% level of LSD.

3.4 Fresh weight of shoot and root

Growing media and seed priming caused a considerable variation in the fresh weight of the shoot and root (g) at bitter gourd seedlings (Table 4). The bitter gourd treatment T_2M_1 produced the maximum fresh weight of the shoot (3.53 g) and root (0.47 g), while the treatment T_0M_0 produced the lowest fresh weight of the shoot (2.07 g) and root (0.16 g) (Table 4).Poly Ethylene Glycol 5% for 12 hrs showed a substantially higher fresh weight of shoot of bitter gourd [30]. When compared to unprimed seeds, PEG priming increased the biomass of shoots and roots because primed seeds had faster metabolisms, which facilitated faster imbibitions[31].Organic manure, such as vermicompost, improves the physico-chemical properties of soil, which promotes nutrient availability and, as a result, increases the fresh weight of shoots and roots of cucumber seedlings [32].

Table 4.Effect of different seed priming treatments and growing media on shoot and root fresh and dry weight of bitter gourdplant^{-1}

Treatment	Shoot fresh weight(g)	Root fresh weight(g)	Shoot dry weight(g)	Root dry weight(g)
T_0M_0	2.07^{g}	0.16^{h}	0.18^{g}	0.019^{h}
T_1M_0	2.37^{fg}	0.21^{f}	0.29^{a-d}	0.022^{f-h}
T_2M_0	2.80^{b-e}	0.30^{d}	0.26^{de}	0.027^{b-d}
T_3M_0	2.84^{b-d}	0.23^{ef}	0.28^{b-d}	0.023^{d-g}
T_0M_1	2.56^{c-f}	0.29^{d}	0.21f^{g}	0.026^{c-f}
T_1M_1	2.46^{d-f}	0.35^{c}	0.22^{ef}	0.028^{bc}
T_2M_1	3.53^{a}	0.47^{a}	0.32^{a}	0.035^{a}
T_3M_1	2.62^{c-f}	0.41^{b}	0.26^{de}	0.034^{a}
T_0M_2	2.33^{fg}	0.18g^{h}	0.19^{fg}	0.020^{gh}
T_1M_2	2.64^{b-f}	0.25^{e}	0.30^{ab}	0.022^{e-h}
T_2M_2	2.66^{b-f}	0.37^{c}	0.26^{d}	0.029^{bc}
T_3M_2	2.89^{bc}	0.30^{d}	0.30^{ab}	0.026^{c-e}
T_0M_3	2.43^{e-g}	0.21^{fg}	0.21^{fg}	0.023^{e-g}
T_1M_3	2.62^{c-f}	0.30^{d}	0.29^{a-d}	0.026^{c-f}
T_2M_3	3.01^{b}	0.42^{b}	0.29^{a-d}	0.031^{ab}
T_3M_3	2.94^{bc}	0.36^{c}	0.27^{cd}	0.029^{bc}
LSD (0.05)	0.39	0.02	0.03	4.05
CV (%)	8.64	5.98	7.98	9.16

T_0=Distilled water, T_1= Hot water, T_2= Polyethelene glycol (PEG), T_3=Sodium Chloride (NaCl), M_0=soil+cow dung, M_1=soil + cow dung + vermicompost, M_2= soil + cow dung + sawdust, M_3= soil + cow dung + cocopeat.Means in a column that are followed by the same letter (s) do not differ significantly at the 5% level of LSD.

3.5 Dry weight of shoot and root

The highest dry weight of bitter gourd seedling (0.32 g) and root (0.035 g) was observed from T_2M_1, while the minimum dry weight of bitter gourd seedling (0.18 g) and root (0.019 g) was observed from T_0M_0(Table 4).Seed priming with PEG significantly increased dry biomass of rice seedling [33].According to Lenin *et al.*[34],using vermicompost as growing media resulted in the greatest increase in plant dry weight of groundnut.Vermicompost significantly improves photosynthetic rate, dry matter production, and fresh and dry weight of tomato seedling [35].Vermicompost increased water and nutrient mobilization, which may have resulted in better photosynthetic product production and transport to different plant sections, resulting in higher seedling growth of papaya and thus more fresh and dry weight[36].

3.6 Photosynthetic pigment

The highest content of chlorophyll a, chlorophyll b and total chlorophyll were found in T_2M_1 treatment i.e. 18.94 mg/g, 11.37 mg/g and 30.31 mg/g respectively and the lowest was found in T_0M_0 treatment i.e. 13.79 mg/g, 6.94 mg/g and 20.73 mg/g respectively) (Table 5). Seed priming protects chlorophyll breakdown and increases pigment concentrations in photosynthetic pigments [37]. The application of PEG markedly elevated the amounts of carotenoids and chlorophylls a, b in date palm plantlets [38].Organic manure like vermicompost acts as nutrient reservoir and these nutrients are released slowly during entire growth period leading to accumulate more photosynthates accumulation as well as yield of crop [39].

3.7Relative water content and SPAD value

There was a significant difference in relative water content was observed in different seed priming and growing media (Table 5). The maximum RWC was recorded in T_2M_1treatment (96.99%) and the minimumRWC was recorded T_0M_0treatment (69.32%). When compared to non-primed seeds, primed seeds with PEG solution significantly improved RWC in wheat plants [40].Vermicompost increased the relative water content of guava seedlings [41].

The treatment combination of T_2M_1 showed the highest chlorophyll (SPAD value) content of 42.00, whereas the treatment combination of T_0M_0 showed the lowest chlorophyll content of 30.67 (Table 3). Chlorophyll is essential for absorbing photon energy during the light-dependent process of photosynthesis, and seed priming with PEG 6000 protects chlorophyll degradation and enhances chlorophyll levels in chilli pepper [42].Vermicopost increased the availability of nitrogen, which may have aided in the synthesis of amino acids and chlorophyll[43].Vermicompost increased leaf chlorophyll content of tomato[44]and chiliseedlings [45].

Table 5.Effect of different seed priming and growing media on Chlorophyll a, chlorophyll b, Total chlorophyll, Relative water content and SPAD value of bitter gourdplant^{-1}

Treatment	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total chlorophyll (mg/g)	Relative water content (%)	SPAD value
T_0M_0	13.79^{g}	6.94^{h}	20.73^{h}	69.32^{g}	30.67^{f}
T_1M_0	15.57^{c-g}	8.76^{e-g}	24.34^{e-g}	76.94^{f}	34.73^{e}
T_2M_0	16.52^{b-d}	9.89^{b-d}	26.41^{b-e}	85.21^{d}	35.97^{de}
T_3M_0	14.50^{e-g}	8.26^{fg}	22.76^{gh}	80.15^{ef}	34.60^{e}
T_0M_1	15.00^{d-g}	8.85^{e-g}	23.86^{fg}	85.38^{d}	35.33^{de}
T_1M_1	17.83^{ab}	10.82^{ab}	28.65^{ab}	89.99^{bc}	40.07^{a-c}
T_2M_1	18.94^{a}	11.37^{a}	30.31^{a}	96.99^{a}	42.00^{a}
T_3M_1	16.93^{b-d}	9.36^{c-e}	26.29^{c-e}	93.50^{ab}	37.02^{b-e}
T_0M_2	14.11^{fg}	7.11^{h}	21.22^{h}	71.34^{g}	34.67^{e}
T_1M_2	16.61^{b-d}	9.43^{c-e}	26.04^{d-f}	76.49^{f}	38.00^{b-e}
T_2M_2	17.46^{a-c}	10.01^{bc}	27.47^{b-d}	86.84^{cd}	38.30^{a-e}
T_3M_2	15.86^{b-f}	8.41^{fg}	24.27^{e-g}	78.69^{f}	35.80^{de}
T_0M_3	14.27^{e-g}	8.06^{g}	22.34^{gh}	78.63^{f}	36.18^{c-e}
T_1M_3	17.44^{a-c}	9.99^{bc}	27.44^{b-d}	84.13d^{e}	38.70^{a-d}
T_2M_3	17.84^{ab}	10.74^{ab}	28.58^{a-c}	91.62^{b}	40.37^{ab}

T_3M_3	16.26^{b-e}	9.04d^{ef}	25.31^{d-f}	86.18^{cd}	37.40^{b-e}
LSD (0.05)	2.01	0.94	2.33	4.07	3.89
CV (%)	7.43	6.13	5.50	2.94	6.33

T_0=Distilled water, T_1= Hot water, T_2= Polyethelene glycol (PEG), T_3=Sodium Chloride (NaCl), M_0=soil+cow dung, M_1=soil + cow dung + vermicompost, M_2= soil + cow dung + sawdust, M_3= soil + cow dung + cocopeat.Means in a column that are followed by the same letter (s) do not differ significantly at the 5% level of LSD.

4. CONCLUSION

Compared to the control, seed priming treatments showed rapid seed germination and strong seedling growth of bitter gourd. Among the priming treatments, PEG 6000 seed priming increased seed germination percentage, vigor index, and seedling length. The results showed that the best treatment for bitter gourd seed germination is soil + cowdung + cocodust (1:1:1) with PEG 6000 (5%). However, soil + cowdung + vermicompost (1:1:1) with PEG 6000 (5%)is beneficial for vigor and healthy seedling.

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