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Evaluating the Efficacy of Organic and Inorganic Seed Priming Methods in Promoting Cucumber Germination and Growth

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ABSTRACT

Seed priming is a promising pre-sowing physiological treatment that utilizes a high osmotic potential solution and stores reserves in seed material to enhance germination. This study is aimed to investigate the influence of different seed priming methods in the germination and early seedling stages of cucumber. A completely random design (CRD) was used for the experiment, including six priming treatments, i.e., T1 (control), T2 (hydropriming), T3 (halopriming 0.5% NaCl), T4 (osmopriming 0.5% PEG), T5 (buffalo milk), and lastly T6 (cow urine). There was a significant effect of seed priming; the highest water imbibition was observed on halopriming (53.71%) and buffalo milk (53.53%); however, the highest germination percentage was observed in cow urine (93.75%), with the least mean germination time (3.65). The highest seedling length, root lengths, and shoot length were also observed on cow urine priming. The dry weight and moisture content were also observed to be highest in cow urine priming. Different priming techniques significantly impact cucumber seed germination and growth. Cow urine priming is the most effective, cost-effective, and environmentally friendly pre-sowing method for cucumber seedlings, benefiting farmers and promoting better germination without harming the environment.

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Introduction

Cucumber (*Cucumis sativus* L.) belongs to Cucurbitaceae Family which chromosome number is 2n=14 (J et al., 2013a). It is a summer vegetable crop. The worldwide production of cucumber is increased by 2.3% from 2021"List of Countries by Cucumber Production". But fulfilling the demands of the increasing population is quite challenging. It is might due to low germination percentage or low quality of seed. Quality seeds are essential for achieving successful germination and crop growth. Germination is a crucial process during this period, various physiological and biochemical activities occur, and softening of the seed coat which influences the emergence of radicles and growth of the embryonic axis (Suksa-ard et al., 2024). . Numerous biotic and abiotic variables affect the germination of seeds. It could differ from seed to seed, therefore germination might not happen all at once or consistently. To overcome this type of problems different alternative methods are use and one of the most commonly used method is seed priming (Abdullahi et al., 2021). Seed invigoration is a powerful and widely used alternative strategy to enhance seed vigor which is economically viable, environment friendly, and cost effective (Budhathoki et al., 2024).

Seed priming is one of the most promising pre-sowing physiological treatment where high osmotic potential solution is use and seed material utilizes store reserve that can enhance seed germination and improve tolerance to environmental stresses (Dar & Amir, 2024). Different types of priming are used such as hydropriming, halopriming, osmopriming, hormonal priming, biopriming and nutrient priming. At present, different priming techniques are use so they are categorized as traditional and advanced methods. Traditional priming includes hydropriming, halopriming, chemical priming, osmopriming and biopriming. Using any physical agents like microwaves, magnetic fields ultraviolent and other laser radiation falls under advanced seed priming (Lazim & Ramadhan, 2023).

Hydropriming is one of the most widely used and cost effective among others. Seeds are soaked in tap water for the different time periods and redrying seeds it also activates the metabolic activities (Adhikari et al., 2021a). Due to high water potential water enters the seed ad is absorbed (J et al., 2013b). Hydropriming application of different duration caused positive effects such as 18-h period increased corn germination and seedling growth

(Dawadi et al., 2023). It helps to faster germination and stronger seedling growth of crops. In halopriming seeds are soaked in inorganic salts such as sodium chloride (NaCl), potassium nitrate (KNO_3) and calcium chloride (CaCl) solution before sowing. Initially, halopriming lowers the electrical conductivity (EC) of leachates from seeds, decreasing the amount of salt released. This decrease in salt content lessens the detrimental effects of salinity and enhances the seed germination and seedling growth even in saline soil condition (Pandey & Bhanuprakash & Umesha, 2017). Osmopriming means high water potential that rapidly enters water into the seed and leads to seed imbibition. For this, seeds are soaked in an osmotic solution like; sugar, polyethylene glycol (PEG), glycerol and mannitol (Jarrar et al., 2024). Osmosis prevents the intake of excess water into seeds during imbibition, which reduces the accumulation of reactive oxygen species and protects the cell from oxidative damage (Y. Wang et al., 2023). When seeds are osmotically primed, their solute concentration can be raised, which lowers their water potential and increases the rate of germination uniformly. PEG is widely using as an osmotic agent as compare to other and it boosts the antioxidant enzyme activities, helps to stabilize the cell members, increases compatible solutes like proline and enhance the germination without triggering radicle emergence (Ekeruo et al., 2024). Nutrient priming is a technique where seeds are soaked in different nutrient containing solutions like vit, potassium, zinc and other micronutrient and enhances the nutrient content and its efficiency. In biopriming, seeds are soaked in different microbial solution which enable to entry of beneficial microorganisms like *Bacillus spp, Trichoderma spp and Pseudomonas spp* which help to nutrient uptake and fasten the germination of seed. Bio priming not only enhance the germination also protect from biotic and abiotic stresses. It makes resilience to different adverse environmental factors (Quality, 2024) It also helps to production of plant hormone (Phooi et al., 2023). Hormonal priming is also another technique in which different plant growth regulator (PGR) use like GA3, abscisic acid, auxin and salicylic acid (Tan, 2024). It helps to improve the harmful effect of abiotic stress and also maintains the male and female flower ratio that makes the increase in germination and crop production (G. Wang et al., 2024). Cow urine and milk priming may revolutionary method in the current situation and a great substitute for other nutritional and biopriming. Cow urine contains approximately 1.0% nitrogen, residues of $P_2O_5$and roughly 1.0% K_2O, ammonia, vitamin, enzymes and other nutrients (Raj Joshi & Adhikari, 2019). Moreover, cow milk is easily available, accessible and fresh milk consistency helps to break the physiological dormancy of seed and enhance the seed germination (Shreevastav et al., 2023). Cow urine is a universal cure-all for ailments and cows are mobile pharmacies. The inhibitory response to seed emergence, shoot growth and seedling vigor index all are influence by the presence of Fe, urea, estrogen and progesterone in cow urine (Choudhary et al., 2023).

There are difficulties in timely seed germination which makes late production of vegetable crops eventually reduction in yield. Poor seed quality, susceptibility to insect pest and diseases, and lack of synchronized seed germination at sub-optimal condition obstructs germination and growth of plants (Anwar et al., 2020). There is a presence of high male and female flower ratio that makes less production. There is a huge understanding gap about benefit of priming in farmer's fields. There is lack of sustainable and easily accessible priming method. There is always debate in which one is the ideal priming method. From this study cucumber producers, retailer and other marketing aspects get benefited. Priming is one of the promising methods of pre germination treatment. This study was aimed to assess the effect of different priming methods on germination and early seedling growth stage of cucumber. Also, to find the most effective, economical and accessible method of priming to the farmers.

Materials and Methods

Experimental Setup

A completely randomized design (CRD) was used to carry out the experiment. Six treatments in all, each replicated four times, were randomized and allocated to twenty-four Petri plates. For the purpose of guaranteeing randomization and removing bias, each Petri plate served as a single experimental unit and was handled independently of the others. There were four replications and a total of six treatments (Table 1) in the completely randomized block design experiment. Treatments were; T1 (control), T2 (hydropriming), T3 (halopriming), T4 (osmopriming), T5 (buffalo milk) and last T6 (cow urine). The experiment employed a total of 24 Petri plates, each of which contained 20 seeds and was considered a separate treatment that was replicated four times.The study was carried out in the IAAS, Gokuleshwor Agriculture and Animal Science Campus laboratory Situated in the mid-hill region.

Seed Materials

Cucumis sativus L. seeds of a local variety called Bhaktapur local were used. The seeds had a minimum germination rate of 70%, and 97 % physical purity.

Sterilization

In order to prevent contamination, Petri-plates, wash bottles, experimental trays, and all other necessary equipment were surface sterilized using ethanol and formaldehyde solution to avoid the contamination.

Preparation of Priming Solution

A 1000 mL measuring cylinder was used to measure 100 mL of tap water, which was then added to each beaker for hydropriming. Before usage, 0.5 g of sodium chloride (NaCl) was thoroughly dissolved in 100 mL of tap water to create the 0.5% NaCl solution. Likewise, 100 mL of tap water was thoroughly combined with 1.5 g of polyethylene glycol (PEG 6000) to create the 0.5% PEG 6000 solution. Each beaker was filled with 100 mL of pure, undiluted cow pee for the purpose of priming. Similarly, 100 milliliters of pure buffalo milk were used in each beaker for buffalo milk priming. For a full 24-hour period, the seeds were immersed in their corresponding priming solutions to ensure proper treatment and absorption. To preserve uniformity throughout the experimental setting, care was taken to guarantee that all treatments received the same priming conditions.

Germination Medium

The substrate that covers the Petri-plate bases is Lokta paper. In sterile Petri dishes, seeds were evenly distributed in a linear fashion to conduct germination tests.

Data collection

Water imbibition (WI)

After weighing the seeds, each treatment was primed with 100ml of room temperature tap water in a separate beaker. In order to determine the proportion of water uptake (imbibition) after priming, the seed weight was once more measured after the surface water was removed (Adhikari et al., 2021a).

$$WI\% = \frac{(WSAP - WSBP)}{WSBP} \times 100\% \quad (1)$$

WSAP: Weight of seed after priming
WSBP: Weight of seed before priming

Germination Percentage (GP)

A population of seeds' viability is estimated by the germination percentage. At least 2 mm of radicle length was required for a seed to be deemed germination. Regular observations were made on Petri plates at 12-hour intervals, and the total number of seeds that germinated was noted. The formula used to determine GP was,

$$GP\% = \frac{\text{Total no.of seed germinated}}{\text{Total no.of seed sown}} \times 100 \quad (2)$$

Mean germination time (MGT)

MGT is a reliable indicator of how long it takes for a lot to germinate, however it does not provide a good correlation with the uniformity or time spread of germination. A population of seeds germinates faster when the MGT is lower (Orchard, 1977). The MGT as embraced by,

$$MGT = \frac{\Sigma(n \times d)}{\Sigma N} \quad (3)$$

Where n= number of seeds germinated on each day, d= number of days from the beginning of the research and N= total number of seeds germinated.

Measurement of shoot length

Using a ruler scale (in centimeters), five samples were randomly chosen from each Petri-plate to determine the total length of the shoot length. After 21 days following seed germination, measurements of radicle were made.

Measurement of root length

Using a ruler scale (in centimeters), five samples were randomly chosen from each Petri- dishes to determine the length of root length. After 21 days of following seed germination, measurements of radicle length are made.

Measurement of seedling length

Five randomly chosen seedlings had their plumule and radical length with great care detached from their radical and measured in centimeters with a ruler.

Fresh weight (FW)

When they are uprooted the seedlings were washed with clear water, let to dry out in the shade, and then put on a weighing balance to be weighed.

Dry Weight (DW)

Following the measurement of the length of the roots and shoots, the seedlings from each dish were placed inside an envelope, labelled, and left for a 12-hour oven dry at 90°C in a hot air oven Subsequently, the dry weight of each envelope's seedlings was determined by weighing them.

Moisture content

After an hour at 130± 20 C in a hot air oven, two grams of seeds were placed in a petri plate and cooled down in room temperature. Using this formula, the seed moisture content was stated in percentage on wet weight basis (Bereded Sheferie, 2023),

$$MC(\%) = (W2-W3)/ (W2-W1) \times 100 \quad (4)$$

Where,
W1 = weight of the empty aluminum container
W2 = weight of the empty aluminum container +seeds before drying
W3 = weight of the empty container + seeds after drying

Seed vigor index (SV)

The following formula is used to calculate the Seed vigor index (Parvin et al., 2024);

$$SV = (SL \times \text{Germination percentage})/100 \quad (4)$$

Where,
SL: Seedling length=Root length + Shoot length

Statistical Analysis

The collected data were subjected to Microsoft Excel and analyzed by using R-studio version 4.3.2. The analysis of variance (ANOVA) was used to examine the significant difference for each parameter. Fisher's least significant difference (LSD) was used to establish the comparisons of mean values. The mean values were considered at a 5% significant level.

Results and Discussion

Water Imbibition (WI)

This study revealed that, the effect of different priming treatments was found significant difference ($p \leq 0.001$) on water imbibition. Halopriming had the highest response among all treatments with (53.71%) which is statistically similar to buffalo milk priming (53.53%), followed by hydro priming showed moderately effective in water imbibition with (49.95%) which is statistically at par with osmopriming. Without priming (control) showed no any effect however treatment with cow urine (46.69%) is effective but statistically lower than both halopriming and buffalo milk priming which indicates a less potent effect but notable (Table 1). This showed that the treatments like halopriming and buffalo milk were most effective also markedly improved performance in comparison to the control and cow urine.

Table 1. Effect of seed priming on germination and early seedling parameters of cucumber seed (*Cucumis sativus)*.

Treatments	WI %	G %	MGT (Days)	SVI	SDL (cm)	RL (cm)	SL (cm)	FW(g)	DW(g)	MC
Control	0.00^{c}	70.50^{c}	6.84^{a}	914.20^{a}	12.54^{ab}	7.74^{ab}	4.77^{ab}	831.95^{b}	92.60^{ab}	88.76^{ab}
Hydropriming	49.95^{ab}	80.50^{b}	4.97^{b}	762.76^{a}	11.40^{b}	6.72^{b}	4.69^{ab}	752.77^{b}	91.00^{ab}	87.94^{b}
Halopriming	53.71^{a}	79.75^{b}	5.32^{b}	839.94^{a}	12.00^{ab}	7.52^{ab}	4.48^{b}	853.17^{b}	86.75^{b}	89.76^{ab}
Osmopriming	48.43^{ab}	85.50^{b}	5.18^{b}	940.98^{a}	13.54^{a}	8.56^{a}	5.04^{ab}	841.42^{b}	96.65^{ab}	88.39^{ab}
Buffalo Milk	53.53^{a}	71.00^{c}	4.83^{bc}	757.68^{a}	11.14^{b}	6.73^{b}	4.40^{b}	852.50^{b}	94.52^{ab}	88.74^{ab}
Cow Urine	46.69^{b}	93.75^{a}	3.65^{c}	959.24^{a}	13.93^{a}	8.76^{a}	5.22^{a}	1063.15^{a}	103.50^{a}	90.21^{a}
Mean	42.06	80.16	5.13	862.46	12.42	7.67	4.76	865.82	94.17	88.97
Sem (±)	13.3	27.47	0.73	26118	1.74	1.04	0.19	12021	91.52	1.65
CV	8.66	6.53	16.68	18.73	10.63	13.29	9.31	12.66	10.15	1.44
LSD	5.41	7.78	1.27	240.08	1.96	1.51	0.65	162.87	14.21	1.90
F-Test	***	***	**	NS	*	*	NS	*	NS	NS

***significant at $p \leq 0.05$, **Significant at $p \leq 0.01$ level of significant, ***Significant at $p \leq 0.001$ level of significant, NS Non-significant, Water imbibition percentage (WI%) Germination percentage (G%), Mean germination time (MGT), Seed vigor index (SVI), Shoot length (SL), Root length (RL), Seedling's length (SDL), Fresh weight (FW), Dry weight (DW), and Moisture content (MC), LSD: Least significant difference and CV: Coefficient of variation, and Standard error mean (SEM). Means with the same letter denotes the treatment are not significantly different.

Through the activation of β-1, 3-glucanase, chitinase, and thaumatin-like protein genes, hydropriming and osmotic priming provide resistance against Aspergillus niger in wheat (Triticum aestivum L.), hydropriming and osmopriming also performed well but were slightly less effective (Gul et al., 2022). Research shows that seed priming treatments, like hydropriming, can significantly enhance seed performance by triggering metabolic pathways and promoting water absorption. Halopriming optimizes moisture content and nutrient availability for better seedling establishment, while buffalo milk priming may enhance seed vigor through organic additives in seed treatment (Adhikari et al., 2021b).

Germination Percentage (GP)

During this study, ANOVA demonstrated significant results ($p \leq 0.001$) in germination percentage among different priming methods. Priming with cow urine had the highest germination percentage with (93%) outperformed all other treatments followed by priming with osmotic solutions (85.50%) which significantly improves seed germination. The lowest GP was recorded in control (70.50%) which resulted in poor seed germination. The effects of Hydropriming (80.50%) and halopriming (79.75%) were statistically similar which means both had improved germination rates compared to control which revealed that water and salt-based priming can enhance the seed viability. However, priming with buffalo milk (71.00%) showed no substantial improvement over control. Studies showed that, cow urine priming aids in breaking down macromolecules, enhances enzymatic action and makes the availability of nitrogen, K_2O, P_2O_5, Iron, Urea, uric acid and other minerals makes faster in seed germination and growth of seedling. This result was supported by (Deeksha et al., 2023; Damalas et al., 2019). Hydropriming and halopriming showed positive results, but buffalo milk showed minimal improvement, suggesting different organic ingredients' effectiveness in seed germination, necessitating further research.

Mean Germination Time (MGT)

Analysis of variance showed that, seed priming technique has a considerable impact ($p \leq 0.01$) on the mean germination time (MGT) of cucumber seeds. The highest mean germination time resulted with no priming (6.84 days) which means cucumber seeds took the longest time without priming. The germination period was greatly shortened by hydropriming (4.97 days), halopriming (5.32 days) and osmopriming (5.18 days) when compared to the control nevertheless, there were no significant differences across the treatments. Priming with buffalo milk also reduced the MGT, performing slightly better than above mentioned treatments but is not statistically different from hydro, halo and osmopriming. The shortest mean germination time (3.65days) was obtained in priming with cow urine compared to all other treatments due to its unique chemical properties, and showing the most effective method followed by buffalo milk which also performed well (Mekonnen et al., 2024). This study highlights the importance of seed priming techniques in boosting cucumber seed germination rates and reducing mean germination time. Cow urine priming showed a significant reduction in MGT, suggesting its nutrient-rich composition accelerates metabolic processes. Other methods, like hydropriming, halopriming, and osmopriming, shortened MGT but did not offer distinct advantages (Cifuentes et al., 2023).

Seed Vigor Index (SVI)

The statistical analysis findings showed that non-significant ($p > 0.05$) influence on the seed vigor index of different priming treatments on cucumber seed which means statistically, there is no significant difference between the treatments at the level of confidence used. The highest SVI was found in priming with cow urine (959.24) and osmopriming (940.98). This means these treatments might improve cucumber seed vigor and the least SVI was found in hydro priming (762.76). The treatment with water, osmotic agent and buffalo milk had less or no positive impact on seed vigor. High SVI in treatments like cow urine and buffalo milk may enhance seedling development, while hydropriming's lower SVI suggests limited benefits. Impact of Purification Methods Using Cow's Milk and Urine on Kupeelu's Strychnine and Brucine Content (*Strychnos nuxvomica Linn.*) Seeds, Buffalo milk's lack of impact highlights different organic materials' seed performance benefits (Mitra et al., 2011).

Seedling length (SL)

Analysis of the result showed that there was a significant effect ($p \leq 0.05$) of priming on the seedling length of cucumber. The result depicted the longest seedling length in cow urine pre-treatment (13.93cm), which is statistically at par with osmopriming (13.54cm). Followed by control (12.54 cm), halopriming (12.00 cm) and hydropriming (11.40cm). Priming with buffalo milk showed the shortest seedling length (11.14cm) among all the treatments. Compared to the control, while hydropriming, halopriming and buffalo milk did not show a significant benefit. Cow urine and osmopriming are effective priming agents in sustainable agriculture, improving crop performance. Hydropriming, halopriming, and buffalo milk may not provide sufficient advantages for cucumber seedlings, suggesting that nutrient-rich treatments are more effective (Debta et al., 2023).

Root Length (RL)

Data analysis of ANOVA showed significant variation ($p \leq 0.05$) in root length among different seed priming treatments. The highest root length (8.76cm) was observed in cow urine priming which was statistically at par with osmopriming (8.56cm), followed by control (7.74 cm) and halopriming (7.52cm). The shortest root length (6.72 cm) was found in Hydropriming followed by priming with buffalo milk (6.73 cm) but these were not statistically different. Cow urine when compared to other treatments and they promote the root development of cucumber seeds this finding was supported by (R. K. Kumar et al., 2022). These treatments provide better growth conditions and physiological responses, highlighting their potential as sustainable agricultural inputs (SHARMA et al., 2021).

Shoot Length (SL)

This study revealed that no significant difference ($p>0.05$) was found in the shoot length of cucumber seed under different priming treatments. The longest shoot length (5.22cm) found treatment with cow urine followed by osmopriming (5.04cm), and control (4.77cm) which was statistically similar with hydropriming (4.69 cm). The shortest shoot length was found in priming with buffalo milk (4.40cm) however halopriming resulted in slightly longer shoot length (4.48cm) than buffalo milk treatment. Nevertheless, cow urine had a significantly positive effect on cucumber shoot growth compared to halopriming and buffalo milk treatments (Krishnaveni & Mamatha, 2021). Osmopriming positively impacts cucumber shoot length, but environmental conditions or seed genetics may influence growth outcomes (Tarchoun et al., 2024). Hydropriming, control, and halopriming methods may not significantly improve shoot length.

Fresh weight (FW)

The table demonstrated that the priming techniques had a substantial impact ($p \leq 0.05$) on the fresh weight of cucumber. The maximum fresh weight was recorded by priming with cow urine (1063.15g) followed by halopriming (853.17g) which was statistically similar to buffalo milk priming (852.50 g) and osmopriming (841.42 g). Cucumber seed with no treatment was the lowest FW among all the treatments with a mean value of (831.95 g) and even lower than control that means least result was found in hydro priming with (752.77g). This indicates these methods were less effective in promoting biomass accumulation than halopriming, buffalo milk, and osmopriming and more favorable for biomass production. Among all the treatments cow urine stands out as the most effective treatment for improving the fresh weight of cucumber seedlings, showed that significant increase compared to other treatments Zhang et al. (2021) found that priming techniques significantly impact the fresh weight of cucumber seedlings, with cow urine being the most effective treatment for enhancing biomass accumulation. This is due to its rich nutrient profile, which supports plant growth. Other treatments like halopriming and buffalo milk also showed strong results in promoting fresh weight. However, osmopriming did not offer comprehensive benefits (ANWAR et al., 2020).

Dry weight (DW)

The priming strategy has no significant ($p>0.05$) impact on the dry weight of cucumber. The highest DW was found in treatment with cow urine (103g) followed by osmopriming (96.65g) statistically similar with buffalo milk priming (94.52g) whereas halopriming (86.75g) had a lowest dry weight. However, cucumber seed with no treatment (92.60 g) and treatment with water (91.00 g) had slightly higher DW than halopriming which means these treatments performed moderately in terms of DW but not best. Cow urine may provide essential nutrients that significantly enhance solid biomass production which made this most effective treatment for improving DW among all other treatments. The study found cow urine as the most effective strategy for enhancing biomass accumulation in cucumber seedlings, attributed to its nutrient-rich composition (Ambigalakshmi N et al., 2023). Other treatments like osmopriming and buffalo milk priming also showed promising results, but halopriming and hydropriming may not provide sufficient benefits for biomass accumulation (V. K. Kumar & Rajalekshmi, 2021).

Moisture Content (MC)

Non significant result ($p>0.05$) was found in the moisture content of cucumber under different priming treatments. The highest MC was exhibited by cow urine priming (90.21%) whereas hydropriming resulted in the lowest moisture content (87.94%) among all other treatments. This revealed that seedling’s ability to retain moisture may be slightly reduced by hydropriming, possibly as a result of a lower water absorption efficiency. However, cow urine primed with higher water retention may have resulted from increased nutrient availability, which improved biomass accumulation and overall growth than all other treatments (Muhammad et al., 2015). The study found that cow urine priming may improve moisture retention in cucumber seedlings, possibly due to its nutrient-rich composition, which enhances growth and biomass accumulation. However, hydropriming's lower moisture content may not be as effective in promoting water retention, potentially hindering the seedlings' growth potential (S. & Aliero, 2004).

Conclusion

This study concludes that cucumber seeds show significant impact over the unprimed for various germination and early seedlings parameters. Based on the present findings, reveal that different priming techniques have a substantial effect on germination and growth of cucumber seedlings. From this experiment, it was found that the highest water imbibition was obtained in halopriming followed by buffalo milk priming. Cow urine priming shows better results over germination percentage, seedling length, root length, and fresh weight in successive days but in the case of seedling length and root length result was statistically at par with osmopriming. Also, the mean germination time of cucumber found better in cow urine priming. This research widened the possibility of cow urine priming of seeds before sowing may result in fasten seed germination in high germination percentage and better growth and development of cucumber seedlings through organic, cost-effective, easily access and sustainable way. Hence, seed priming with cow urine seems most effective over other priming techniques. Also, it is cost effective, easily available, environment friendly, organic and sustainable techniques of pre-sowing method. From this finding, most of the farmers benefited by use of cow urine priming with better germination of seeds in lower cost without harming the environment.

Declarations

Ethics approval and consent to participate
Not applicable

Consent for publication
Not applicable.

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All author contributed equally.

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Data will be made available on request

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The authors declare no conflict of interest.

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