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Research Article

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Unlocking the Green Potential: Enhancing Maize (*Zea mays* L.) Germination, Growth, and Yield through Innovative Seed Priming in Taplejung, Nepal

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ABSTRACT
A cheap and ecologically friendly pre-sowing method that increases germination rates and seedling vigor, which in turn enhances growth and productivity, is considered seed priming. A field experiment was conducted in 2022 in farmer's field to evaluate the effective priming method suitable for the balanced germination, growth, and yield of maize. An experiment consists of a single-factor randomized complete block design with 3 replications and 8 treatments. The analysis of the data was conducted with R Studio software at the $p < 0.05$ level of significance, and a mean comparison was done using DMRT (Duncan's Multiple Range Test). According to this study, seed priming increased the germination, growth, and yield of crop growth parameters such as germination percentage, germination index, days to 100% germination, stem diameter at 35 days, days to 100% tasseling, days to 100% silking, plant population/ha, number of cobs per plant, ear length, ear girth, kernels per cob, 1000 kernel weight, fresh grain yield, adjusted grain yield, fresh Stover yield, and fresh biomass yield. The highest germination percentage (98.61%) and index (11.89 days), the earliest days to 100% germination (12.00 days), early tassel emergence (63.00 days), early silking emergence (66.00 days), the highest number of kernels per cob (529.37), 1000 kernel weight (337.10 g), harvest index (0.62), ear length (22.38 mm), and ear girth (49.72mm) were recorded on the treatment primed with cow urine for 18 hours. However, the highest fresh Stover yield (2954.07 kg/ha) and fresh biomass yield (6336.29 kg/ha) are recorded on stoves primed with DAP for 18 hours. Cow-urine priming for 18 hours improved the germination and economic yield of maize. Efficient cow-urine priming is a cost-effective, eco-friendly, and finest alternative approach to increasing the maize yield.
Keywords: Hydro-Priming, Cow urine Priming, Effect, Germination, growth, yield.

INTRODUCTION

Maize is a significant agronomic crop that is cultivated globally and is cross-pollinated long-day plant belonging to Poaceae. Maize is referred as the "queen of cereals." It is used for food, feed, fodder, and a source of industrial raw materials in Nepal, where it is recognized as the second most important cereal crop after rice (Ghimire, et al., 2018). Per capita, maize consumption in Nepal was 98g per person in one day which was reported to be the highest in South Asia (Ranum, et al., 2014). The demand for maize has been steadily increasing by about 5% annually in the past few decades (Sapkota & Pokhrel, 2010). Currently, maize covers 9,56,447 ha of land with a total yield of 2,71,3635 in Nepal (Krishi Diary, 2078) whereas in Taplejung district maize is cultivated in 22,052ha of land, 54,413Mt production and yield of 2.47Mt/ha were recorded (MOAD, 2014). In the winter and spring, maize is farmed in the terai, valleys, and low-lying river basin areas using irrigation whereas during the summer (April-August), maize is grown under the rainfed condition as sole crop or relayed with millet at the end of season (Sapkota & Pokhrel, 2010). The potential yield of maize in Nepal is 5.7 t/ha, which is higher than the farm-level production of 2.55 t/ha (MOAD, 2017; Karki et al., 2015). In Nepal, maize being impacted by various technological and socio-economic factors, there is an enormous gap between the demand and production. It is mostly unknown in Nepalese context how the pretreated seed would affect the entire growing season. Seed is considered as primary component that has an impact on crop's production and productivity (Shrestha & Shrestha, 2017).

Seed priming is considered a low-cost and eco-friendly pre-sowing technique that can improve the germination rate and vigor of seedlings which reflects enhanced crop growth and productivity. (Sudozai, et al., 2013) found maize seeds hydro-primed for 18 hours at a soil moisture level of 55-65% enhances germination characters like vigor index, greatest emergence and increase in growth parameters like greatest leaf area, shoot as well as root dry weights. Additionally, it also promotes yield parameters like greatest number of cobs per plant, grains per cob and improved seed index along with increasement in seed yield by 35%. Maize is one of the important cereal crops of Bangladesh which can be produced successfully by priming (Ahammad, et al., 2014).

Furthermore, research conducted by (Soleimanzadeh, 2013) hydro-primed maize seeds for duration of 18 hours enhances seed germination and yield factors. Koirala (2017) reported seed priming treatment reduces silking and maturity days, enhancing increasement of grain yield by 11.6% and greatest increasement of yield by 27.8% in maize (Manakamana-1 variety) at Palpa, Gulmi, and Myagdi districts of Nepal. The significant numbers of farmers (75.1 to 88.9%) who took part in the Mother-Baby trial in western Nepal expressed keen interest in seed priming technology in maize (Koirala, 2017). For solving the issue of low seedling establishment problem, farmers require priming reagents that are not only affordable but also user-friendly and readily available in Nepal. Hence, this experiment was undertaken in the farmers' field in Mid hills of Nepal with the motive of identification of priming treatments in seed germination, better establishment, vegetative growth, and Yield of maize in Mid hills of Nepal. If Germination, growth, and yield performance are unaffected by the use of different treatments, it will be considered as a null hypothesis.

Experimental site

From February 2022 to June 2022, the experiment was carried out in a farmer's field in Phungling municipality-04, Simle, Taplejung. The site is situated at an elevation of 1140 meters above sea level, with coordinates of 27.3487973 N, 87.6398160 E..

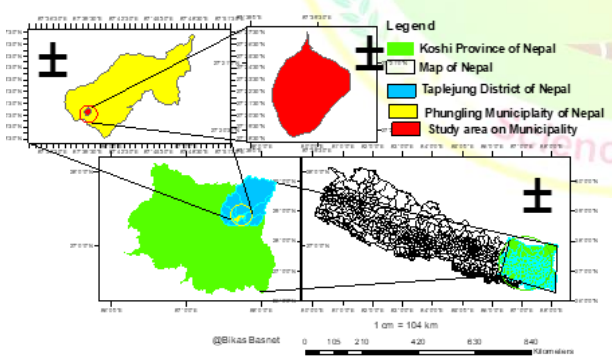

Figure 1. Geographic Information System (GIS) Map of the Study Area in Taplejung, Nepal. This study marks the inaugural investigation conducted in this specific location. Map created by the author

Experimental design

The experiment was set up in a single factor Randomized complete block design involving 3 replications and 8 different treatments. Maize (var. Manakamana-04) seed was obtained from Prime Minister Agriculture Modernization Project, Taplejung, and was placed in a bowl and soaked in water (Hydropriming (12 hours)-T1 & Hydropriming (18 hours)-T2), cow urine(Cow- Urine priming (12 hours)- T3, @ 2.5 times dilution & Cow-Urine priming (18 hours) @ 2.5 times dilution-T4), DAP(DAP priming (12 hours) @1%-T5 & DAP priming (18 hours) @1%-T6), and the salt solution(Salt priming (12 hours) @0.5%-T7) and Control-T8 respectively. Then the primed seed was surface dried under the shade for 2 hours.

In an area of 155m^2 of a well-prepared plot, seeds were shown at a depth of 5-8 cm. The distance between rows and plants of the maize was 75cm×25cm. Every replication had 1 m gap, and each plot had 0.5 m gap. Altogether there were 24 plots with an Individual plot area 2m×2.25m (4.5m2), each plot had 3 rows with 8 plants in each row (*Figure 2*). Six of the plants were chosen at random from each plot by a lottery procedure to provide the necessary data for the experiment. The experimental field was well-ploughed, weeds were cleared from the field and was given a fine tilth. After, the field layout was completed, the recommended doses of well-decomposed FYM (15 tons), Urea: DAP: MOP@120:60: 40 kg/ha were applied. FYM, DAP, MOP, and half dose of urea were applied as basal dose. While, half dose of urea was top-dressed during crucial stages i.e. knee-high stage and earthing up.

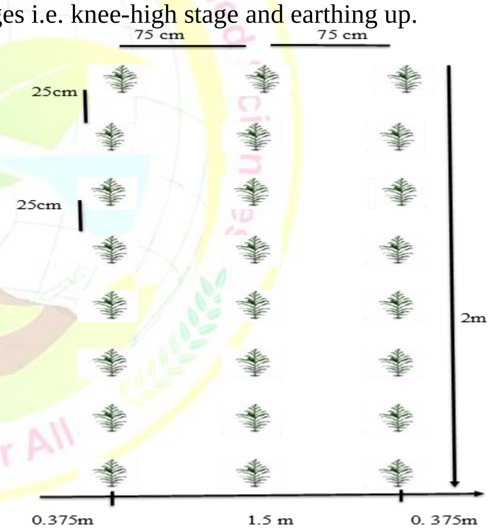

Figure 2. Experimental design of the Single Plot with the spacing of 75*25cm^2

Data collection and analysis

Six plants were randomly chosen as sample plants from each plot. Each plot included data collected on seedling character, Vegetative character, phenological observations, and yield attributing measurements. Seedling characters included seed germination percentage, germination index, and Days to 100% germination. Vegetative characters included Plant height (cm), Stem diameter (mm), and Number of leaves. Days to 100% Tasseling, Days to 100% silking were recorded as phenological observation. Yield attributing characters included Plant population per hectare, Number of cobs per plant, ear length(cm), ear girth(mm), kernels per cob, 1000 kernel weight(gm), Fresh Stover yield(gm), Fresh Biomass yield(gm), Harvest index. At a 5% level of significance, the data were examined using Duncan's Multiple Range Test (DMRT) and information gathered from field was put into MS-Excel 2019. The data were further analyzed with R studio version 4.2.1.

Seed germination percentage

The seedlings were observed daily until complete emergence and readings were taken.

$$\% \text{ Germination} = \frac{Number\ of\ seeds\ germinated}{Number\ of\ seeds\ sown} \times 100$$

Germination index

The germination index (GI) was calculated by the following formula:

$$\frac{Number\ of\ germinated\ seeds}{Days\ of\ first\ count} + \cdots + \frac{Number\ of\ germinated\ seeds}{Days\ of\ final\ count}$$

Days to 100% germination

Days to full germination were calculated when nearly 100% of the germination of seeds took place.

Plant height

Plant height were measured by using measuring tape from the randomly selected 6 plants from the middle row of each plot. Plant height were measured from the soil surface to the highest point of the arch of the uppermost leaf whose tip was pointing down.

Stem diameter

Stem diameter were measured through Vernier caliper from the randomly selected 6 plants from the middle row of each plot.

Number of leaves

Emerged number of leaves were counted from the randomly selected plants

Days to 100% tasseling and silking

After the emergence of 100% tassel in the plot, this data was recorded.

Plant population per hectare

Plant population per hectare was calculated by using the formula:

$$\frac{Total\ number\ of\ plants\ in\ given\ area\ of\ the\ plot\,(3m^2)}{Total\ area\ of\ the\ plot\ (3m^2)} \times 10000$$

Yield parameters

The total number of ears harvested from the net harvestable area were recorded. The length of the ear was measured from the base to the tip of the ear and were recorded in centimeters at the time of harvest. Ear girth was measured i.e., from the middle of the ears using a Vernier caliper, and was recorded in millimeters. Fresh cob weight along with husk was harvested from the net harvestable area and weighed using a weighing machine. De-husking of the cob was performed and was weighted using a weighing machine. Kernel per row, Kernel per column of the sample plant cob was calculated and was multiplied for calculation of kernels per cob

So,

(Number of Kernels per cob= kernel per row × kernel per column).

10000 kernels were counted and weighed in a weighing machine for calculation of 1000 grain weight. For Stover weight, Stover was cut down from the net harvestable area and was weighted using a weighing machine and converted in kg/ha Biological yield was calculated using the formula (Biological yield= Grain yield + Stover yield)

The Harvest index was calculated using the formula:

$$(HI = \frac{\text{Economic yield(Grain yield)}}{\text{Biological yield (Grain yield+Stover yield)}}).$$

Grain moisture content (%): Ears from random plants were selected. Kernels were shelled out and was placed in a moisture meter for calculating moisture content.

RESULTS AND DISCUSSIONS

Germination percentage

The data analysis performed showed that there was significant effect ($p<0.05$) of priming in germination percentage (Table 1). In comparison to other treatments, T4 primed with cow urine for 18 hours had the greatest germination rate (98.61) whereas T8 no priming, had the lowest recorded germination percentage (73.61).

Germination index

The data analysis performed showed that there was significant effect ($p<0.05$) of priming in Germination index (Table **1**) & *Figure* **3**. In comparison to other treatments, T4 primed with cow urine for 18 hours had the greatest germination rate (11.89) whereas T8 no priming was performed had the lowest germination index (4.22).

Days to 100% germination

The data analysis performed showed that there was significant effect ($p<0.05$) of priming in Days to 100% germination. In comparison to other treatments, T4 primed with cow urine for 18 hours had the greatest days to 100% germination (12.00) whereas T8, no priming performed had the lowest (13.00) (Table **1**) & *Figure* **3**.

Table 1. Effect of priming on germination parameter of Manakamana-4 variety of maize

Treatment	Germination percentage	Germination Index	Days to 100% germination
T1	93.06^{c}	9.06^{bc}	12.67^{ab}
T2	94.30^{b}	10.21^{b}	12.67^{ab}
T3	89.06^{c}	7.11^{c}	12.67^{ab}
T4	98.61^{a}	11.89^{a}	12.00^{b}
T5	84.72^{d}	5.60^{c}	13.00^{a}
T6	82.56^{d}	5.50^{c}	13.00^{a}
T7	90.06^{bc}	9.01^{bc}	13.00^{a}
T8	73.61^{e}	4.22^{d}	13.00^{a}
LSD (0.05)	3.58	1.21	0.40
S. E_m (±)	2.17	0.46	0.20
F-probability	<0.001	<0.001	<0.05
CV%	4.22	8.66	2.772968
Grand Mean	88.24	7.82	12.75

Note: The common letter(s) within the column indicate non-significant difference based on Duncan Multiple Range Test (DMRT) at 0.05 level of significance, ** significant at 1% level of significance, *** significant at 0.1% level of significance. (S. Em – Standard Error of mean, CV – Coefficient of Variation, LSD – Least Significance Difference)

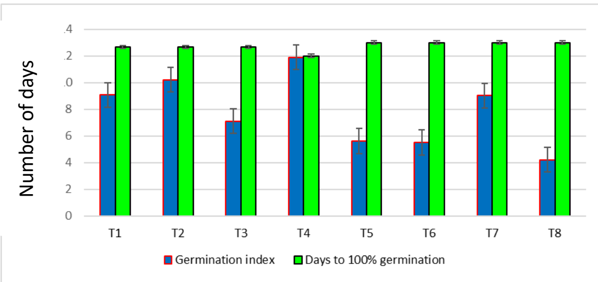

Figure 3. Effect of priming on germination index and days to 100% germination

Plant height

The analyzed data revealed that the plant heights 35, 50, 65, 80, and 105 days is not significantly ($p<0.05$) influenced (*Figure 4*).

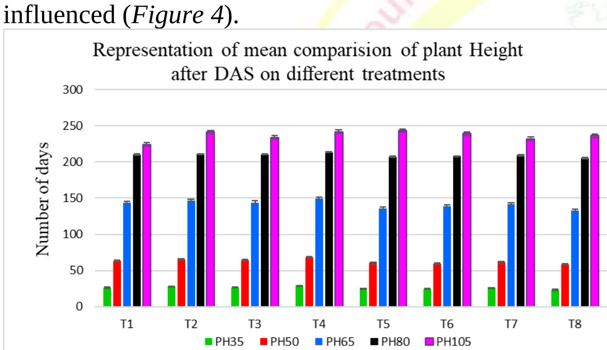

Figure 4. Effect of priming on plant height on different days after sowing

Leaves number

The analyzed data revealed that the Leaf number 35, 50, 65, 80, and 105 days is statistically not significantly ($p<0.05$) influenced (Figure 5).

Stem diameter

The analyzed data revealed that the stem diameter 35, 50, 65, 80, and 105 days is not significantly ($p<0.05$) influenced by the treatments (Figure 6).

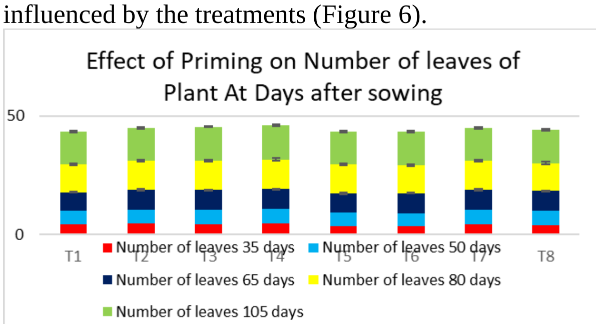

Figure 5. Effect of Seed priming on the number of leaves on different days of sowing

Days to 100% tasseling

The analyzed data revealed that the days to tasseling are significantly ($p<0.05$) influenced. However, the early tassel emergence was recorded in treatment T4, primed with cow-urine for 18 hours (63.00), and the late tassel emergence was recorded in T8, control no priming performed (69.67).

Days to 100% silking

The analyzed data revealed that the days to silking are significantly ($p<0.05$) influenced. However, the early silking emergence was recorded in treatment T4, primed with cow-urine for 18 hours (66.00) and the late tassel emergence was recorded in T8, with control no priming performed (72.66) (Table 2).

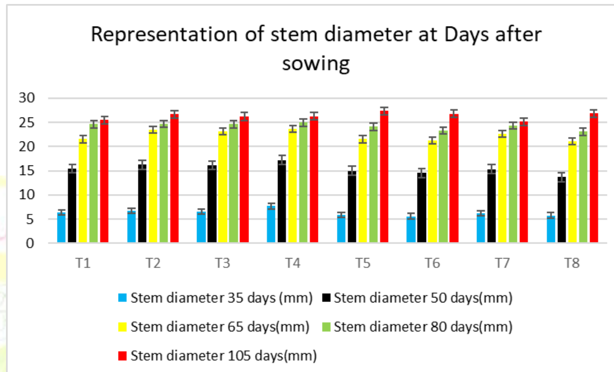

Figure 6. Effect of Seed priming on Stem diameter of maize at different days of sowing

Table 2. Effect of seed priming on days to tasseling, days to silking of maize

Treatment	Days to 100% tasseling	Days to 100% silking
T1	64.67^{ab}	68.33^{ab}
T2	64.67^{ab}	68.00^{ab}
T3	64.67^{ab}	68.00^{ab}
T4	63.00^{c}	66.00^{c}
T5	68.00^{b}	70.66^{b}
T6	68.67^{b}	71.66^{b}
T7	67.00^{b}	70.66^{b}
T8	69.67^{a}	72.66^{a}
LSD (0.05)	0.82	1.05
S. E_m (±)	0.27	0.34
F-probability	<0.001	<0.001
CV%	0.70	0.86
Grand Mean	66.29	69.49

Note: The common letter(s) within the column indicate non-significant difference based on Duncan Multiple Range Test (DMRT) at 0.05 level of significance, ** significant at 1% level of significance, *** significant at 0.1% level of significance. (S. Em – Standard Error of mean, CV – Coefficient of Variation, LSD – Least Significance Difference)

Plant population per hectare

The analyzed data revealed that the plant population per hectare is significantly ($p<0.05$) influenced (Table 3). However, the highest plant population per hectare was recorded on the treatment T4, primed with cow-urine for 18 hours (75555.56), and the lowest plant population per hectare was recorded in T8, control no priming performed (53333.33) but statistically similar (at par) with T5, T6.

Number of cobs per plant

The analyzed data revealed that the number of cobs per plant is significantly (p<0.05) influenced (Table 3). However, the highest number of cobs per plant was recorded on the treatment T3, primed with cow-urine for 12 hours (2.01) and the lowest number of cobs per plant was recorded in T8, with control no priming performed (1.00).

Ear length

The analyzed data revealed that the ear length is significantly (p<0.05) influenced. However, the highest ear length was recorded in treatment T4, primed with cow-urine for 18 hours (22.38) and the lowest ear length was recorded in T8, with control no priming performed (16.85).

Ear girth

The analyzed data revealed that the ear girth is significantly (p<0.05) influenced. However, the highest ear girth was recorded in treatment T4, primed with cow-urine for 18 hours (49.72) and the lowest ear girth was recorded in T8, control no priming performed (42.44) (*Table 3*).

Table 3. Effect of seed priming on plant population per hectare, number of cobs per plant, ear length, ear girth

Treatment	Plant population per hectare	Number of cobs per plant	Ear length (cm)	Ear girth (mm)
T1	65777.78^{bc}	2.00^{ab}	18.15^{c}	46.61^{b}
T2	70057.00^{b}	1.42^{b}	19.65^{bc}	49.34^{a}
T3	69777.78^{b}	2.01^{a}	20.74^{b}	46.28^{ab}
T4	75555.56^{a}	1.68^{b}	22.38^{a}	49.72^{a}
T5	55555.56^{d}	1.05^{c}	17.05^{cd}	43.44^{c}
T6	55555.56^{d}	1.05^{c}	17.02^{cd}	43.02^{c}
T7	66888.89^{bc}	1.21^{bc}	18.04^{c}	45.67^{b}
T8	53333.33^{e}	1.00^{c}	16.85^{d}	42.44^{d}
LSD (0.05)	4000.55	0.18	1.42	2.46
S. E$_m$ (±)	1599.16	0.06	0.81	1.14
F-probability	<0.001	<0.05	<0.05	<0.05
CV%	4.30	9.21	7.16	4.30
Grand mean	64062.68	1.42	18.73	45.81

Note: The common letter(s) within the column indicate non-significant difference based on Duncan Multiple Range Test (DMRT) at 0.05 level of significance, ** significant at 1% level of significance, *** significant at 0.1% level of significance. (S. Em- Standard Error of mean, CV – Coefficient of Variation, LSD – Least Significance Difference)

Fresh cob weight

The analyzed data revealed that the fresh cob weight with husk is not significantly(p<0.05) influenced. fresh cob weight without husk is not significantly (p<0.05) influenced Table 4

Number of Kernels per cob

The analyzed data revealed that the number of kernels per cob is significantly (p<0.05) influenced. However, the highest kernels per cob were recorded on treatment T4, primed with cow-urine for 18 hours (529.37), and the lowest kernels per cob were recorded in T8, with control no priming performed (376.00).

1000 kernel weight

The analyzed data revealed that the 1000 kernel weight is significantly (p<0.05) influenced (Table 4). However, the highest fresh 1000 kernel weight was recorded on the treatment T4, primed with cow-urine for 18 hours (337.10), and the lowest fresh 1000 kernel weight was recorded in T8, with control no priming performed (306.00) (*Table 4*).

Table 4. Effect of seed priming on fresh cob weight with husk, fresh cob weight without husk, kernels per cob, 1000 kernel weight

Treatment	Fresh cob weight with husk (gm)	Fresh cob weight without husk (gm)	Number of Kernels per cob	1000 kernel weight (gm)
T1	357.50	294.20	436.03^{bc}	324.30^{b}
T2	385.60	321.70	474.93^{ab}	334.00^{a}
T3	365.00	297.80	469.37^{ab}	335.00^{a}
T4	395.90	337.10	529.37^{a}	337.10^{a}
T5	370.00	285.60	417.02^{bc}	320.70^{bc}
T6	364.20	285.60	393.55^{c}	314.30^{c}
T7	348.30	293.60	442.02^{bc}	322.80^{bc}
T8	317.10	265.90	376.00^{c}	306.00^{d}
LSD (0.05)	59.90	49.43	63.68	8.30
S. E$_m$ (±)	19.75	16.29	20.99	2.73
F-probability	ns	ns	<0.01	<0.001
CV%	9.42	9.48	8.22	1.46
Grand mean	362.94	297.66	442.28	324.27

Note: The common letter(s) within the column indicate non-significant difference based on Duncan Multiple Range Test (DMRT) at 0.05 level of significance, ** significant at 1% level of significance, *** significant at 0.1% level of significance. (S. Em – Standard Error of mean, CV – Coefficient of Variation, LSD – Least Significance Difference)

Fresh stover yield

The analyzed data revealed that the fresh Stover yield is significantly (p<0.05) influenced. However, the highest fresh Stover yield was recorded on the treatment T6, primed with DAP for 18 hours (2954.07) and the lowest fresh stover yield was recorded in T7, priming with salt (1880.741).

Fresh Biomass yield

The analyzed data revealed that the fresh biomass yield is significantly (p<0.05) influenced. However, the highest fresh biomass yield was recorded in treatment T6, primed with DAP for 18 hours (6336.29) and the lowest Fresh biomass yield was recorded in T7, priming with salt for 12 hours performed (4085.18).

Harvest index

The analyzed data revealed that the harvest index is significantly (p<0.05) influenced. However, the highest harvest index was recorded on the treatment T4, primed with cow-urine for 18 hours (0.62) but statistically similar (at par) with T7, and the lowest harvest index was recorded in T8, control no priming performed (0.49) (*Table* **5**).

Table 5. Effect of seed priming on fresh kernel yield, adjusted kernel yield, Stover yield, biomass yield, harvest index

Treatment	Stover yield (kg/ha)	Biomass yield (kg/ha)	Harvest index
T1	2564.44^{ab}	5551.55^{b}	0.53^{bc}
T2	2671.29^{a}	5783.14^{ab}	0.52^{c}
T3	2599.36^{ab}	5618.25^{b}	0.52^{bc}
T4	2007.41^{c}	4425.18^{c}	0.62^{a}
T5	2076.94^{bc}	4472.50^{c}	0.53^{bc}
T6	2954.07^{a}	6336.29^{a}	0.49^{c}
T7	1880.74^{c}	4085.18^{c}	0.58^{ab}
T8	2920.37^{a}	5882.22^{ab}	0.49^{c}
LSD (0.05)	506.85	666.40	0.06
S. E_m (±)	167.10	219.70	0.01
F-probability	<0.01	<0.001	<0.01
CV%	11.76	7.22	6.36
Grand Mean	2459.33	5269.29	0.53

Note: The common letter(s) within the column indicate non-significant difference based on Duncan Multiple Range Test (DMRT) at 0.05 level of significance, ** significant at 1% level of significance, *** significant at 0.1% level of significance. (S. Em – Standard Error of mean, CV – Coefficient of Variation, LSD – Least Significance Difference)

According to (S, K, & K, 2014) reported that seeds treated with cow urine (5%) resulted in in increasing the germination rate and a higher germination percentage in paddy. Cow urine which consists of iron, urea, uric acid, estrogen, and progesterone has been observed to influence the inhibitory effects on seed germination, seedling vigor and growth of shoot (Dilrukshi, H.N.N, Perera, A.N.F, 2009). The study of (Shrestha, Pradhan, Shrestha, & Subedi, 2019) showed that the germination index was highest in 24-hour urine-based priming in maize. Urea and Urine priming helped in improving the germinat ion and growth parameters of maize when compared to MOP, hydro-primed, salt and un-primed seeds (Shrestha, Pradhan, Shrestha, & Subedi, 2019). The result was found consistent to (Tian, et al., 2014) where he reported that there were no significant differences in plant height observed between the priming and controlled groups. However, this result was not found before (Shivamurthy & Patil, 2010) seed treated with cow urine priming recorded significantly greater plant height and greater number of green leaves. Cow urine treated seeds increases growth parameters due to the proper maintenance of high-water content in the cell, increased cell division and elongation of cell (Shivamurthy & Patil, 2010).

The results are not in line with (Wolie, Zewudie, & Feleke, 2017) as their research indicated that hydro-primed seeds were earlier in emergence and heading as compared to the cow urine-primed seeds in wheat. Seeds treated with urine showed improved yield due to the presence of plant nutrients including N, P, K, and micronutrients (Schouw, S, Mosbaek , & Tjell, 2002). As mentioned by (D, Tripathi, & Joshi, 2002) and (Harris, Tripathi, & Joshi, 2002) pre-soaking followed by surface drying in field crops is considered to be more advantageous. However, as (Giri & Schillinger, 2003) the result is not in line with their research as there was no significant impact of priming on the grain yield of wheat cultivars. (D, Tripathi, & Joshi, 2002) reported that similar results were obtained in Pakistan and Zimbabwe where seed priming helped to boost the maize yield of grain by 17-76% and 14% when compared with non-primed ones.

CONCLUSIONS

Better germination, early tasseling, silking, maturity, and crop production are all enhanced by seed priming. It is evident that using various priming treatments has been shown to increase the yield of crops. The best germination qualities for seedlings were obtained with an 18-hour cow urine solution priming. However, cow urine solution primed for 18 hours leads to earlier tasseling and silking as well as having a positive effect on yield. Also, as compared to no priming, it resulted in the least preferable germination characteristics of seedlings. All other priming treatments performed better than control or no priming. This study suggests, seed priming with cow urine solution as an easy and cost-effective technique for improving the germination and yield of maize crop.

CONFLICT OF INTEREST

The author here declares that there is no conflict of interest in the publication of this article.

AUTHOR CONTRIBUTIONS

Prashna Budhathoki designed the experiments, collection & analyzed the data, wrote the paper. Shreejan Kumar Pandey designed the experiments, collection of data. Shiva Shankar Bhattarai & Prakash Ghimire provided guidance to undertake the work. All authors provided feedback on the manuscript with significant input from all co-authors.

DATA AVAILABILITY STATEMENT

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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