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Research Article

Impact of Various Priming Treatments on Rato Basmati Rice Variety

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Abstract

An important crop, used as stable food worldwide is rice. The germination stage is crucial for rice crop and is mainly affected by drought stress. A laboratory experiment was conducted in GAASC laboratory, Gokuleshwor, Baitadi to study the impact of various priming treatments on Rato basmati rice variety. The experiment was done in a completely randomized design using 9 treatments replicated thrice. The treatments were Hydro priming 24hrs, Hydro priming 12hrs, Gibberellin 5mg/liter, Gibberellin 10mg/liter, Gibberellin 15mg/liter, NaNO3 0.5%, $NaNO_3$ 5%, $NaNO_3$ 15%, and control where seeds were washed three times followed by 5 hours drying at room temperature. For 10 days, each Petri plate was observed every 12 hours. The priming treatment with Gibberellin 10mg/L showed the most promising results for germination percentage (100%) within 120 hours, Germination index (11.06) and germination energy (0.97), while Gibberellin 15 mg/L showed higher vigor index (1522), plumule length (40.02 cm), radicle length (15.73 cm). However, the germination energy (0.93) was found similar in Gibberellin 15 mg/L and 24hrs hydropriming. Regarding Plumule to radicle length ratio, highest result was obtained from 24hrs hydropriming (0.55 cm). Thus, this study suggests that using Gibberellin of both 10 and 15 mg/L for priming in rato basmati variety of rice showed the best results compared to other treatments.

Keywords: Priming; Gibberellin; germination; imbibition; hydropriming; seedling growth.

Introduction

Rice (*Oryza sativa* L.) is the most significant staple food for people worldwide; rice accounts for about 40% of food calories consumed, nearly 20% of the agricultural gross domestic product (AGDP), and nearly 7% of the country's overall gross domestic product (GDP) (Gadal *et al*., 2019). According to S. Subedi *et al*., (2020), rice is farmed in three major agroecological zones: the Terai and inner Terai (60 - 900 masl), the Mid hills (900 -1,500 masl), and the Mountains/High hills (1,500 - 3,050 masl). It is grown on an area of 1552469 ha, yielding 5230327 mt with an average productivity of 3.37 t/ha, taking up 58% of the nation's total arable land and producing 55% of the nation's food grain output (Gairhe and Yadaw, 2020). Rice is a source of revenue for farmers. 73% of rice is produced in the Terai region, with the hills producing 24% and the high hills 4% of the total (Joshi *et al*., 2020). The world produces 178 million tons of paddy rice, with Asia producing 90.6 percent of it, America producing 5.2 percent, and Africa producing 3.5 percent (Ekeruo, 2023).

Humans prioritize the agriculture subsector, and agricultural operations strongly rely on natural resources, especially water, soils, and forests. It is especially susceptible to extreme weather conditions, which eventually reduce agricultural productivity, such as extreme warmth, intense rainfall, droughts, floods, cold waves, etc. (Pokhrel *et al*., 2021). In areas that are prone to dryness, germination is more likely to be irregular and to last for extended periods (Bourgne *et al*., 2000). Due to the subsequent poor crop germination, there are gaps in the canopy that, when the brief rainy season begins, are quickly filled in by weeds that are developing quickly and competing with the crop plants for nutrients, light, and water (Kropff *et al*., 1993). Poor seed germination behavior in field crops can lead to crop failure and lower agricultural yield (Ghiyasi *et al*., 2008). This can be prevented by priming treatments, which improve germination and establishment in all crops (Basra *et al*., 2005; Ghiyasi *et al*., 2008). Abiotic stress tolerance in plants has been induced using various techniques in recent years. Under many field crops, seed priming is an effective, useful, and simple approach to promote quick and uniform emergence, high seedling vigor, and higher yields, especially under adverse environmental circumstances (Hussain *et al*., 2016).

Germination percentage is one of the important traits for harvesting optimal yield. Due to the use of poor-quality genetic material, the germination potential of seed is low leading to poor grain yield (quality and quantity), a significant problem among farmers in developing countries. A successful crop's establishment depends on rapid emergence and germination, which is why seed priming may be crucial (Yadav *et al*., 2023). A regulated hydration method called "seed priming" stimulates the regular metabolic processes in the early stages of germination before germination is visible (Koirala *et al*., 2019). Seed priming is the pre-sowing treatment that helps to improve the germination percentage (Subedi *et al*., 2015a). Priming is important in agriculture for producing better-quality seedlings. It has the potential to enhance seed quality and enable the release of dormancy, which will increase the ultimate germination speed and uniformity. Pre-germination modifications are induced by a variety of priming techniques, including hormone priming, hydropriming, Osmo priming, and halo priming (Goswami *et al*., 2013). Different priming techniques had a significant role in promoting plant performance. It is an inexpensive and low-risk method (Pawar and Laware, 2018). Recently, commercial seed priming has been employed to increase seed vigor in terms of prospective stress tolerance and germination (Anwar *et al*., 2020). Hormonal priming has been reported by several authors to promote germination under stress, shorten the time it takes for germination to occur, and impart high seed vigor (Gnawali and Subedi, 2021; Khan *et al*., 2020; Sukifto *et al*., 2020). Priming can be done easily on the farm with less effort and does not require expensive materials. Primed seeds develop a stronger, more reliable crop stand and have a high emergence capacity (Marthandan *et al*., 2020). They also emerge faster. They are still working under less-than-ideal field conditions. Primed seeds sprouted more readily than unprimed seeds, which led to an earlier flowering period and a higher yield of crops(Yadav *et al*., 2023). Due to limited adverse environmental exposure, primed seeds germinate more quickly at any temperature and produce uniformly more seedlings than unprimed seeds, which is the main advantage of priming (Khan *et al*., 2024).

Hence, the objective of this experiment is to study the effect of different priming treatments on seedlings growth traits of rice and identify the most effective priming treatment that can be acquired in farming practices.

Materials and Methods

Materials and Chemicals Used

Petri plates, cotton, forceps, wash bottles, distilled water, blotting paper, gibberellin, sodium nitrate, ethanol, etc. were used for the study.

Experiment Site

The experiment was conducted in the Agronomy laboratory of Gokuleshwor Agriculture and Animal Science College (GAASC), Gokuleshwor, Baitadi, which is located in the far-west region of Nepal from 13 July to 23 July, 2023.

Treatment Details

Rato basmati variety of rice was used in the experiment which was carried out in a completely randomized design (CRD). There were 9 treatments and each treatment was replicated three times. The treatments consist of priming using different priming agents (Table 1).

Table 1: List of 9 treatments which are used as priming agents for the study

S.N.	Treatments	Priming agent
1.	T_1	Hydro priming 24hrs
2.	T_2	Hydro priming 12hrs
3.	T_3	Gibberellin 5mg/L
4.	T_4	Gibberellin 10mg/L
5.	T_5	Gibberellin 15mg/L
6.	T_6	$NaNO_3$ 0.5%
7.	T_7	$NaNO_3$ 5%
8.	T_8	$NaNO_3$ 15%
9.	T_9	Control

Preparation of Priming Solution

All the priming solutions were prepared in 1000 ml solution. The priming treatment calculation was done by using a formula given by (Harvey, 2000).

$$Weight - to - volume\ \%\left(\%\frac{w}{v}\right) = \frac{gm\ solute}{1000\ ml\ solution}$$

For the preparation of $NaNO_3$ 0.5% solution, 0.059gm of $NaNO_3$ was added to 1000ml of water. For $NaNO_3$ 1% solution, 0.118gm of $NaNO_3$ was added to 1000ml of water.

For the preparation of $NaNO_3$ 1.5% solution, 0.236gm of $NaNO_3$ was added to 1000ml of water. Similarly, For the preparation of gibberellin 5mg/L, 0.005gm of gibberellin was added to 1000mL of water. For gibberellin 10mg/L, 0.01 gm of gibberellin was added to 1000mL of water. For gibberellin 15mg/L, 0.015 gm of gibberellin was added to 1000mL of water.

Post-Priming Operation

The primed seeds were removed and thoroughly rinsed with distilled water three times and air dried in newspaper at room temperature for 5 hrs.

Germination Medium

The Whatman filter paper was used as a substrate that covers the base of Petri plates. Seeds were distributed uniformly in sterilized petri plates for the germination tests.

Experimental Details

The germination test was conducted using the petri-plate method. Ten seeds from each priming treatment were placed in each petri plate containing a double layer of filter paper and allowed to germinate for 10 days inside the controlled environment of 27.5℃ and relative humidity of 98%. Each petri plate was observed every 12 hours for 10 days and germinated seeds were counted in each observation.

Data Collection Parameters

Germination Percentage (GP):

Germination Percentage is an estimate of the viability of a population of seeds. Seeds with a radicle length of at least 2 mm were considered germinated. Observations on Petri plates were done regularly at 12 hrs intervals and the total number of seeds germinated was recorded. The formula is given in the study of Basnet *et al*., (2018) was used to calculate the germination percentage.

$$Germination\ Percentage = \frac{\text{Total number of normal seedlings germinated}}{Total\ number\ of\ seeds\ sown} \times 100\%$$

Germination Index (GI):

The formula of (R. Subedi *et al*., 2015b) study was used to calculate the germination index.

$$GRI = (G1/1) + (G2/2) + (G3/3) + \cdots + (G12/12) + \cdots + (G_n/n)$$

It means the sum of no. of seeds germinated on day 1 divided by 1, no. of seeds germinated on day 2 divided by 2 likewise no. of seeds germinated over day 12 divided by 12 up to 'n' days.

Germination Energy (GE):

The percentage of seeds germinated in 3 days i.e., 72hrs is known as germination energy (GE) (Bam *et al*., 2006). By changing the formula of (Li, 2008), germination energy—which is the rate at which germination occurs—was calculated.

$$GE = \frac{\text{No. of total germinated seeds in different priming solutions in 72hrs}}{\text{Total no. of seeds used for germination}}$$

Measurement of Radicle Length (RL), Plumule Length (PL):

5 samples from each petri-plate were selected randomly to measure the length of the overall seedling, root, and plumule length by using a measuring scale (in centimeters). Radical and plumule length was measured and the data was noted.

Seedling Vigor Index (SVI):

The seedling vigor index (SVI) was calculated by using the modified formula given by Abdul-Baki and Anderson, (1973).

$$\text{Seedling Vigor Index (SVI)} = \text{Germination percentage (\%)} \times \text{Seedling length(cm)}$$

Statistical Analysis

The data was entered and data tabulation was done through MS-excel whereas statistical analysis was done using RStudio software version 4.1.1. The least significant digit (LSD) was used to separate means and compare treatment mean at a 5% level of significance.

Result and Discussion

Germination Percentage

The analysis of variance revealed that priming treatments had a significant influence on rice seed germination percentage. Gibberellin (10mg/L), Gibberellin (15mg/L), Gibberellin (5mg/L), and $NaNO_3$ (15%) showed higher germination than 12hrs hydro priming and control, during the entire experimental period. The highest total germination was found in treatment 5 i.e. Gibberellin 10 mg/L (100%) which is statically at par with Gibberellin 15mg/L (96.67%), Gibberellin 5mg/L (96.67%), $NaNO_3$ 15% (96.67%), and the lowest was found in treatment 9 i.e. control (76.67%) which is statically at par with 12hrs hydro priming (86.67%). Germination percentages at 36hrs, 48hrs, 60hrs, 72 hrs, 84hrs and 96 hrs have shown non-significant results whereas at 24hrs, 108hrs, and 120hrs have shown significant results. The current study found that following seed priming with reagents, the rate of hydration increased significantly compared to the control group. This means that seed priming may boost rice seed germination by speeding up imbibition, which could help to accelerate the emergence phase of rice. Similarly, priming was found to promote maize cultivar germination by accelerating imbibition (Tian *et al*., 2014). Additionally, the exogenous application of Gibberellin has been reported to be effective in increasing seed germination (Pipinis and Milios, 2015). Significant improvement in seed germination might be due to the enhanced breakdown of reserve metabolites present in the seed (Ullah *et al*., 2020). GA-treated seed was closely associated with their rapid utilization in the synthesis of

various amino acids and amides, which could be the reason for the increased germination percentage (Gupta and Mukherjee, 1982) (Table 2)

Germination Index

The analysis of variance revealed that priming treatments had a significant influence on rice seed germination index. All the priming methods Gibberellin (10 mg/L), Hydro priming 24hrs, Gibberellin (15 mg/L) and $NaNO_3$ 0.5% showed higher germination index than control and 12hrs hydro priming. The highest average value was observed in Gibberellin 10mg/L (11.06) which is statistically at par with Hydro priming 24hrs (10.64), Gibberellin 15 mg/L (10.46) whereas the lowest was observed in control (6.98) which is followed by 12hrs hydro priming (8.68). During the entire time highest value for germination index was observed for Gibberellin 10 mg/L. Germination index at 24hrs and 120hrs shown significant result, however, at 48hrs,72hrs, 96hrs it was non-significant. In this work, we investigated the effects of several seed priming agents on germination index under standard setting. The use of various seed priming methods significantly increased the germination index, which is consistent with the findings of the study by (Tabatabaei and Ansari, 2020). Similar to our result, overall, all priming agents produced GI, with averages higher than those of controls (Fourati and Ahmed, 2024) (Table 3).

Table 2: Effect of different priming treatments on germination percentage (12-hour interval) of rice

Treatments	24hrs	36hrs	48hrs	60hrs	72hrs	84hrs	96hrs	108hrs	120hrs
Hydro priming 24hrs	10^{c}	23.33^{a}	46.67^{b}	66.67^{ab}	93.33^{b}	93.33^{b}	93.33^{bc}	93.33^{bc}	93.33^{bc}
Hydro priming 12hrs	10^{c}	20^{a}	30^{a}	43.33^{a}	73.33^{ab}	80^{ab}	80^{ab}	83.33^{ab}	86.67^{ab}
Gibberellin 5mg/L	0^{a}	20^{a}	40^{ab}	66.67^{ab}	83.33^{ab}	90^{b}	96.67^{bc}	96.67^{c}	96.67^{bc}
Gibberellin 10mg/L	10^{c}	23.33^{a}	46.67^{b}	83.33^{b}	96.67^{b}	96.67^{b}	100^{c}	100^{c}	100^{c}
Gibberellin 15mg/L	10^{c}	26.67^{a}	40^{ab}	70.00^{ab}	93.33^{b}	96.67^{b}	96.67^{bc}	96.67^{c}	96.67^{bc}
$NaNO_3$ 0.5%	10^{c}	26.67^{a}	43.33^{ab}	70.00^{ab}	90.00^{b}	90^{ab}	90^{abc}	90.00^{bc}	90^{bc}
$NaNO_3$ 5%	0^{ab}	23.33^{a}	36.67^{ab}	60.00^{ab}	83.33^{ab}	90^{ab}	90^{abc}	93.33^{bc}	93.33^{bc}
$NaNO_3$ 15%	10^{c}	26.67^{a}	40^{ab}	56.67^{ab}	83.33^{ab}	86.67^{ab}	90^{abc}	96.67^{c}	96.67^{bc}
Control	3.33^{a}	20^{a}	30^{a}	43.33^{a}	53.33^{a}	66.67^{a}	73.33^{a}	76.67^{a}	76.67^{a}
CV	27.3	20.8	20.5	27.8	21.6	14.1	10.8	6.7	6.5
LSD	3.33	8.41	13.94	29.91	31.14	21.39	16.82	10.73	10.4
Grand mean	7.04	23.33	39.3	62.2	83.3	87.8	90	91.9	92.2
SEM	1.11	2.8	4.65	9.98	10.39	7.14	5.61	3.58	3.47
F test	<.001	0.436	0.157	0.174	0.188	0.163	0.075	0.006	0.007

NOTE: Treatment's mean is separated at 5% probability level using LSD (Least significant difference) test. Means followed by the same letter(s) within a column are non-significantly different among each other. NS, ".", *, and ** represent non-significance, significance at confidence level greater than 5%, 5% and 10% respectively.

Table 3: Effect of different priming treatments on germination index (24-hour interval) of rice

Treatments	**24hrs**	**48hrs**	**72hrs**	**96hrs**	**120hrs**	**GI**
Hydro priming 24hrs	1^{c}	2.333^{b}	3.111^{b}	2.33^{bc}	1.87^{bc}	10.64^{bc}
Hydro priming 12hrs	1^{c}	1.500^{a}	2.444^{ab}	2^{ab}	1.73^{ab}	8.68^{ab}
Gibberellin 5mg/L	0^{a}	2.000^{ab}	2.778^{ab}	2.42^{bc}	1.93^{bc}	9.13^{abc}
Gibberellin 10mg/L	1^{c}	2.333^{b}	3.222^{b}	2.50^{c}	2^{c}	11.06^{c}
Gibberellin 15mg/L	1^{c}	2.000^{ab}	3.111^{b}	2.42^{bc}	1.93^{bc}	10.46^{bc}
$NaNO_3$ 0.5%	1^{c}	2.167^{ab}	3.000^{b}	2.25^{abc}	1.80^{bc}	10.22^{bc}
$NaNO_3$ 5%	0^{ab}	1.833^{ab}	2.778^{ab}	2.25^{abc}	1.87^{bc}	8.73^{abc}
$NaNO_3$ 15%	1^{c}	2.000^{ab}	2.778^{ab}	2.25^{abc}	1.93^{bc}	9.96^{bc}
Control	0.33^{a}	1.500^{a}	1.778^{a}	1.833^{a}	1.53^{a}	6.98^{a}
CV	27.3	20.5	21.6	10.8	6.5	12.6
LSD	0.33	0.7	1.04	0.42	0.21	2.08
Grand mean	0.704	1.96	2.78	2.25	1.84	9.54
SEM	0.11	0.23	0.35	0.14	0.07	0.69
F test	<.001	0.157	0.188	0.075	0.007	0.017

NOTE*:* Fisher's LSD (Least Significant Difference) test is used to differentiate treatment means at the 5% probability level. Means followed by the same letter(s) within a column are non-significantly different. NS, *, and ** represent non-significance, and significance at confidence levels of 5%, and 1% respectively. CV: Coefficient of Difference, LSD: Least significant difference, SEM: Standard error of the mean.

Germination Energy and Vigor Index

For germination energy and vigor index, the highest value was observed in the seed primed with Gibberellin 10 mg/L, Gibberellin 15 mg/L and 24hrs hydro priming whereas the lowest was for control, followed by 12hrs hydro priming. The result depicted highest germination energy in Gibberellin 10 mg/L (0.97) which is statically at par with Gibberellin 15 mg/L (0.93), 24hrs hydro priming (0.93), $NaNO_3$ 0.5% (0.90), $NaNO_3$ 5% (0.83), $NaNO_3$ 15% (0.83), Gibberellin 5mg/L (0.83), while control (0.53) and 12hrs hydro priming (0.73) showed lowest germination energy. In stark contrast to this, no priming compounds used caused substantial changes to GE when applied at different levels (Subedi *et al*., 2015c) .

The treatment Gibberellin 15mg/L primed seed displayed the highest vigor index (1522) which is followed by Gibberellin 5mg/L (1385) and Gibberellin 10mg/L(1088) but the lowest value was observed for control (583), and $NaNO_3$ 15% (847). Additionally, VI has shown significant result. The higher seed vigor index in primed seeds could be attributed to elevated dehydrogenase and amylase enzymatic activity (Reichheld *et al*., 1999). Seed priming boosts α amylase activity, leading to increased soluble sugar and protein levels (Bajwa *et al*., 2018). During seed germination, GA stimulates the seed cells to produce mRNA molecules that comprise code for hydrolytic enzymes (Balchhaudi, 2023) for the elimination of germination inhibitors, the counteraction of ABA (Miransari and Smith, 2014) (Table 4).

Table 4: Effect of different priming treatments on Germination energy and Vigor index of rice

Treatments	GE (72hrs)	VI
12hrs hydro priming	0.73^{b}	876^{ab}
24hrs hydro priming	0.93^{ab}	892^{ab}
Gibberellin 5mg/L	0.83^{ab}	1385^{cd}
Gibberellin 10mg/L	0.97^{b}	1088^{bcd}
Gibberellin 15mg/L	0.93^{b}	1522^{d}
$NaNO_3$ 0.5%	0.90^{b}	988^{abc}
$NaNO_3$ 5%	0.83^{ab}	978^{abc}
$NaNO_3$ 15%	0.83^{ab}	847^{ab}
Control	0.53^{a}	583^{a}
CV	21.6	24
LSD	0.31	422.2
Grand Mean	0.83	1018
SEM	0.1	140.8
F test	0.188	0.008

NOTE*:* Fisher's LSD (Least Significant Difference) test is used to differentiate treatment means at the 5% probability level. Means followed by the same letter(s) within a column are non-significantly different among each other. **'*'** represent significance at confidence level 5%.

Plumule, Radical Length, and Radicle to Plumule Length

The analysis of variance showed that Plumule length, Radicle length, and ratio of both have depicted significant results. Regarding the Plumule length, seed primed with Gibberellin 15mg/L (40.02 cm) portrayed the highest value which is statically at par with Gibberellin 5mg/L (33.16 cm), Gibberellin 10 mg/L (28.99 cm), and the lowest length was obtained from the control (14.43 cm) and $NaNO_3$ 15% (16.46 cm). The longer plumule length resulting from the priming treatments aligns with the results reported by (Tian *et al*., 2014) on maize. The notable increase in Plumule length in the primed seeds may be due to meristematic growth, cell division, or its role in cell elongation. The longer seedling length in GA_3 priming was attributed to α-amylase activity, which produces more reducing sugars (Fallah *et al*., 2018)

Similarly, the highest radical length was observed in seed primed with GA 15 mg/L which is followed by GA 5mg/L, while the lowest was in control which is statically at par with $NaNO_3$ 15% and 24hrs hydro priming. In stark contrast to this, the highest radical to Plumule length was portrayed in seed-primed water for 24hrs (0.55) which is statically at par with seed primed in $NaNO_3$ 15% (0.54) and control (0.53) but the lowest was the value for seed primed in GA 10 mg/L (0.37). Statistically significant results for root length may be linked to how priming therapies modify hormones or signaling pathways, which may affect root growth and development (Table 5).

Table 5: Effect of different priming treatments on Radicle length, Plumule length, and Radicle to Plumule of rice seeds

Treatments	PL	RL	RL:PL
12hrs hydro priming	19.99^{ab}	10.29^{ab}	0.51^{bc}
24hrs hydro priming	17.14^{a}	9.45^{a}	0.55^{c}
Gibberellin 5mg/L	33.16^{c}	14.31^{bc}	0.43^{abc}
Gibberellin 10mg/L	28.99^{bc}	10.88^{ab}	0.37^{a}
Gibberellin 15mg/L	40.02^{c}	15.73^{c}	0.41^{ab}
$NaNO_3$ 0.5%	21.62^{ab}	10.98^{ab}	0.52^{bc}
$NaNO_3$ 5%	20.97^{ab}	10.40^{ab}	0.52^{bc}
$NaNO_3$ 15%	16.46^{a}	8.75^{a}	0.54^{c}
Control	14.43^{a}	7.60^{a}	0.53^{bc}
CV	26.2	21.4	14
LSD	10.73	4.06	0.12
Grand Mean	23.6	10.93	0.49
SEM	3.58	1.35	0.04
F test	0.001	0.014	0.043

NOTE*:* Fisher's LSD (Least Significant Difference) test is used to differentiate treatment means at the 5% probability level. Mean followed by the same letter(s) within a column are non-significantly different among each other.". ", ** represent significance at confidence level greater than 5%, and 1% respectively.

Conclusion

From the results of the present study, it may be stated that seed priming through various treatments can significantly boost seed germination of rice. This enhancement is likely due to the acceleration of biochemical processes and enzymatic activities facilitated by these chemicals. For germination percentage, germination index and germination energy, best results were obtained from Gibberellin 10

mg/L, while for vigor index, root length, and shoot length best result was obtained from Gibberellin 15 mg/L. The result showed that Gibberellin 10 mg/L, Gibberellin 15 mg/L, and $NaNO_3$ (15%) were comparatively effective and produced positive outcomes for measured variables compared with those of control and 12hrs of hydro priming treatment. Gibberellin priming is recommended as a successful approach for enhancing and hastening seed germination and improving crop stand in the Rato basmati variety. More experiments are needed, however, to gain a better understanding of priming-induced mechanisms and corroborate these findings.

Authors' Contribution

M. Karki and K. Kshetri conceptualized the research plan. The experimental work was carried out by A. Dhami, A.K. Limbu, M. Karki, K. Kshetri, and K. Subedi, with data collection managed by M. Karki, K. Kshetri, and K. Subedi. A. Dhami performed the data analysis, and the manuscript was drafted collaboratively by A. Dhami and A.K. Limbu. M. Karki and A. Gaire reviewed and finalized the manuscript. All authors approved the final version.

Conflict of Interest

The authors declare that they have no conflict of interest regarding the publication of this paper.

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