

Research Article

Efficacy of Different Priming Methods on Germination and Growth of French Bean (*Phaseolus vulgaris* L.) Under Field Conditions in Mugu, Nepal

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Article Information Abstract Received: 31 July 2024 Low seed germination and poor growth have been major problems in French Revised version received: 2 September 2024 bean production in the hilly region of Nepal. An effective solution may lie in Accepted: 5 September 2024 seed priming, which enhances rapid and uniform seed emergence, thereby Published: 30 September 2024 improving yield. A field experiment was conducted to find out the effect of different seed priming methods on various varieties of French beans under field Cite this article as: conditions in Chhaayanath Rara-4, Mugu, Nepal. The experiment was S. Shrestha et al. (2024) Int. J. Appl. Sci. Biotechnol. Vol conducted in two factorial RCBD with three replications. Factor A was three 12(3): 137-144. DOI: 10.3126/ijasbt.v12i3.68405 French bean varieties; black, ghoratte, and red beans, and Factor B included two methods of priming; hydro and halo along with control. The results *Corresponding author revealed that the germination parameter was significantly influenced by the Saroj Shrestha, method of priming and it was found non-significant in the case of varieties. Agriculture and Forestry University, Rampur, Chitwan, Maximum germination percentage and higher speed of germination were found Nepal. in seeds treated with hydro-priming. Similarly, the growth parameter was found Email: saroojsth02@gmail.com significant for both varieties as well as priming methods. Vegetative growth parameters such as plant height, number of leaves, and number of primary and Peer reviewed under authority of IJASBT secondary branches were found significantly higher in hydro-primed seeds and ©2024 International Journal of Applied Sciences and ghoratte variety. Likewise, the yield parameters such as days to first flowering, days to 50% flowering, and yield per hectare were found significantly higher in



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Keywords: Seed Priming; French bean; Germination; Growth; Yield.

Introduction

French Bean, a summer crop of the family Fabaceae, is widely grown for vegetable and pulse crops. Planted for its green pods and dry seeds, the French Bean is significant and is planted from terai to high hills (Neupane *et al.*, 2005). It is the second-ranking legume after soybean in the world. There are two types of French bean i.e., Pole type or climbing type and Dwarf type or bush type. The French bean is rich in nutritious elements like protein, calcium, phosphorus, and so on. It is reported that French bean contains the different amounts of nutrients required for the body; protein 1.7 g, calcium 50 mg, phosphorus 28 mg, iron

1.7 mg, carotene 132 mg, thiamine 0.08 mg, riboflavin 0.06 mg, vitamin C 24 mg per 100 g of edible pods (Chadha, 2001). So, the French bean is beneficial from an economic as well as a health point of view. Grain legumes rank fourth in terms of area and fifth in terms of agricultural productivity, and they play a significant role in Nepalese agriculture, contributing to food and nutritional security, economy in nitrogen, crop intensification, diversification, and sustainable farming methods (Gharti *et al.*, 2014). The area and production of bush-type French beans in the fiscal year 2021/22 are 1,461 ha and 12,738 mt respectively

(MOALD, 2023). The French bean is widely planted as an

hydro-primed ghoratte beans. Hence, hydro-priming can be used as a simple and cost-effective technique to improve germination and growth of French

beans along with suitable variety under field conditions in Mugu district,

Karnali province, Nepal.

intercrop in apple orchards in Karnali, where it is cultivated from April to October under conditions of soil moisture and nutrient stress (Aryal et al., 2020). The cultivated French bean is a bush type rather than a pole type. It is cultivated in 370 ha of land with a production of 320 mt and productivity of 0.86 mt/ha in the Mugu district (ADO Mugu, 2021). People traditionally grow beans without using a specific method of cultivation. The seeds are randomly broadcasted into the field without any treatment. This practice leads to low production due to poor germination. The absence of pre-sowing treatment can lead to poor germination of crops. So, treatment like seed priming can be done before sowing. In addition to ensuring uniformity and improved establishment, seed priming increases yield in a variety of environmental conditions, increases resistance to environmental stress, and aids in overcoming dormancy (Dhal et al., 2022). So, it is essential to make the farmers well-known for seed priming for good production.

Due to its climatic suitability, Bean is grown commercially for cash income across the east-west length along high hills in Nepal. Farmers in Mugu are struggling with low production due to inadequate knowledge and inappropriate agricultural technology. Inadequate knowledge of quality seeds for planting purposes and insufficient technical knowledge of plant protection measures are the major constraints at the input supplier level. The production of French beans in Mugu differs significantly from that of other places. This disparity may result from poor seed germination, which lowers seed vigor and yield. Seed priming is one way to address the issue of poor germination, which will improve crop stand and yield (Khan, 2010). Most of the farmers are unaware of the seed priming technique of French beans and hence face the problem of delayed seed emergence, poor germination percentage, and

minimum speed of germination (Project Implementation Unit, PMAMP, 2022). The other significant issue linked to French bean productivity in the hilly areas is inadequate vegetative and yield-related traits, which include fewer nodes, branches, leaves, flowers, pods, seeds per pod, and the lowest plant biomass (Noor *et al.*, 2017).

Only a few studies have been carried out related to the effect of priming on the germination and growth of French beans in Nepal. Priming can favor good germination and good seed vigor. "Seed priming is an effective technology to enhance rapid and uniform emergence and to achieve high vigor, leading to better stand establishment and yield (Singh *et al.*, 2015)." It has been effectively shown that seed priming increases the germination and emergence of seeds from a wide variety of crops, especially vegetables and small-seeded grasses (Donaldson *et al.*, 2001). This research might help farmers to know about effective priming methods and suitable varieties for better growth of French beans in Mugu. Besides, the findings could be useful for research stations, future researchers, and farmers.

Materials and Methodology

Experimental Site Details

The experiment was conducted in Chhayanath Rara Municipality-4, Mugu. Mugu, a district of Karnali province lies on the geographical coordinates of $22^{\circ}46'40''$ to $29^{\circ}56'30''$ N and $81^{\circ}46'40''$ to $82^{\circ}49'57''$ E. Its area is about 3,535 km² with a population of 66,658 (CBS, 2021). It lies at an altitude of 1,201 to 6,616 m asl. The area covered by the bean zone includes ward no. 4,7,8,9,10,11 and 12 of Chhayanath Rara municipality and ward no. 4,5,6,7,8, and 9 of Mugam Karmarong rural municipality. The average annual rainfall is 706 ml and the mean annual temperature ranges between -4° to 30°C (Fig.1).



Fig. 1: Map of the study area

Experimental Details

The experiment was carried out in two factorial RCBD (Randomized Complete Block Design) with 9 treatments consisting of 3 varieties and 3 priming methods with three replications. The bush-type Frech bean is used in the experiment. The plot size is $3m^2$ (2m x 1.5m) having three rows and ten plants (2 plants per hill) in each row (Fig. 2). The spacing for bush type variety as proposed by (MOAD, 2078) is 70 cm X 30 cm. The spacing between replications is 1m and between treatments is 0.5m. As Karnali province has promoted organic farming, the application of a recommended dose of N:P:K is avoided in this proposed research.

Replication 1	Replication 2	Replication 3
T1	T5	T3
T4	T2	T7
T6	T3	T1
Т8	T7	Т9
T2	Т9	T5
Т9	T4	Τ8
Τ7	Т8	T6
Т3	T1	T4
T5	T6	T2

Fig. 2: Layout of the experiment

SN	Treatments with symbol	Treatment combination
1.	T1	Black bean with hydro- priming
2.	T2	Black bean with halo- priming
3.	Т3	Black bean with control
4.	T4	Ghoratte bean with hydro- priming
5.	Т5	Ghoratte bean with halo- priming
6.	Т6	Ghoratte bean with control
7.	Τ7	Red bean with hydro- priming
8.	Т8	Red bean with halo- priming
9.	Т9	Red bean with control

The experiment comprised 9 treatments consisting of three varieties and three priming methods with three replications.

A. Varieties

i. Black bean

ii. Ghoratte bean

iii. Red bean

B. Method of priming

i. Hydro-priming (Priming on normal water for 24 hours)

ii. Halo-priming (Priming with common salt; NaCl for 24 hours)

iii. No priming (Control)

Detail of the Operation

Seeds were collected from the farmers' fields which were preserved by the farmers. The field was plowed twice followed by planking to attain good tilth. The seeds were soaked in normal water and salt (NaCl) water for 24 hours for each treatment. The seeds were planted in the third week of Chaitra (April). The primed seeds were planted in the spacing of 0.7m (row to row) X 0.3m (plant to plant). The nutrient was supplied by FYM. Since Karnali focuses on organic farming; NPK was not used. Surface irrigation was carried out as per the plant's requirements. Weeding was carried out at regular intervals as per plant requirements to control weeds and improve soil structure. Depending upon the disease and pest occurring in the plant appropriate management and control measures were applied.

Observation and Measurement

For the observation, five plants were tagged randomly in each plot. The data were collected from the tagged plants at different times according to the requirement of plant growth. The following data were collected.

Germination Percentage:

The germination percentage was calculated as

Germination (%) = $\frac{No.of \ seeds \ germinated}{Total \ no.of \ seeds \ sown} x \ 100$ (Eqn. 1)

Speed of Germination/Germination Index:

The number of seedlings emerging was counted from the day of sowing till germination was ceased. The days to germination of each replication were noted. Then the seed of germination (X) was computed using the formula given by (Maguire, 1962)

X =

Growth Parameters:

The plant height was measured at 15-day intervals after 30 DAS with the help of a meter scale. The plant was measured from the soil surface to the topmost growing point of the plant. The number of leaves per plant of each sample plant

was counted at 45 and 60 days of sowing and their average was calculated. The number of primary and secondary branches per plant of each sample plant was counted at 45 and 60 days of sowing and their average was calculated.

Yield Parameters:

The days to first flowering in each sample plant were recorded when the first flower started blooming. The days to 50% flowering in each sample plant were recorded when 50% of the flowers started blooming. The yield of each sample plant was taken after harvesting and the average was calculated.

Statistical Analysis

The data obtained from experimental plots on various parameters were statistically analyzed to find out the significant difference among treatments according to the principles of experimental design. Data entry and processing were carried out using Microsoft Excel software. Analysis of variance was calculated using R-studio. The hypothesis was tested using an f-test at a 5 percent level of significance and the means were compared using Duncan's Multiple Range Test (DMRT).

Results and Discussions

Germination Percentage and Speed of Germination

The results revealed no significant effect on germination percentage and speed of germination among the bean varieties (Table 2). However, the germination percentage was statistically significant with the method of priming. The highest germination percentage (92.45) was observed in hydro-priming followed by halo-priming (87.67). The lowest germination percentage was observed in control (86.76). Similarly, the speed of germination was statistically significant with the method of priming. The highest speed of germination (4.28) was observed in hydropriming followed by halo-priming (3.65). The lowest germination percentage was observed in control (3.6).

In the present investigation, germination percentage and speed of germination were significantly influenced by the priming methods (Table 2). No significant effect was seen among the bean varieties on germination percentage and speed of germination. When the bean seeds were treated with water, the maximum percentage and speed of germination were observed. Water intake, which starts the early germination processes up to the radicle germination point, may be the cause of this phenomena. This result conforms with the findings of (Dhal *et al.*, 2020) who reported maximum germination percentage and high speed of germination in seeds treated with hydro-priming.

Growth Parameters

Plant Height:

At 30 DAS, the maximum plant height (11.58 cm) was observed in the ghoratte bean followed by the red bean (10.41 cm). The minimum plant height (9.39 cm) was observed in black beans. At 45 DAS, the maximum plant height (39.96 cm) was observed in the ghoratte bean, and the minimum plant height (32.68 cm) was observed in the black bean. Similarly, at 60 DAS, the maximum plant height (57.51 cm) was observed in the ghoratte bean, and the minimum plant height (49.88 cm) was observed in the black bean.

Table 2: Effect of different priming techniques on
germination and the speed of germination of
different varieties of French bean at Mugu, Nepal,
2022

Treatments	Germination (%)	Speed of germination
Variety		
Black bean	89.62 ^a	3.9 ^a
Ghoratte Bean	88.31 ^a	3.69 ^a
Red bean	88.95 ^a	3.94 ^a
Sem (±)	1.5	0.15
LSD(a=0.05)	4.5	0.46
CV	5.06%	12.1%
F test	NS	NS
(a=0.05)		
Priming		
Hydro	92.45 ^a	4.28 ^a
Halo	87.67 ^b	3.65 ^b
Control	86.76 ^b	3.6 ^b
Sem (±)	1.5	0.15
LSD(a=0.05)	4.5	0.46
CV	5.06%	12.1%
F test	*	*
(a=0.05)		
Grand Mean	88.96	3.84

Note: Sem±, Standard error of the mean; CV, Coefficient of Variation; LSD, Least Significant Difference. Means followed by common letter(s) within column are non-significantly different based on LSD at P=0.05, * significant at 0.05 P level, ** significant at 0.01 P level, *** significant at 0.001 P level, NS= non-Significant

At 30 DAS, the maximum plant height (11.59 cm) was observed in hydro-priming. The minimum plant height (9.64 cm) was observed in the control treatment which was statistically at par with halo-priming (10.14 cm). At 45 DAS, the maximum plant height (40.29 cm) was observed in hydro-priming and the minimum plant height (31.79 cm) was observed in control treatment. Similarly, at 60 DAS, the maximum plant height (58.8 cm) was observed in hydropriming and the minimum plant height (47.94 cm) was observed in control treatment.

The results revealed that both the varieties as well as priming methods had a significant effect on the plant height of French beans (Table 3). Among the three varieties, the Ghoratte bean had the maximum plant height. The difference in plant height among the varieties might be due to their genetic makeup. In the case of methods of priming, maximum height was observed in hydro-priming (Table 3). This might be a result of the case that hydro-priming promotes improved root development, which raises plant height and increases dry matter accumulation. This result conforms with the findings of (Damalas *et al.*, 2019) who reported taller plant heights as a result of hydro-priming.

Table 3: Effect of different priming techniques on plantheight of different varieties of French bean atMugu, Nepal, 2022

Treatments	Plant height (cm)		
	30 DAS	45DAS	60 DAS
Variety			
Black bean	9.39°	32.68 ^c	49.88 ^c
Ghoratte Bean	11.58 ^a	39.96 ^a	57.51ª
Red bean	10.41 ^b	35.34 ^b	54.02 ^b
Sem (±)	0.27	0.53	1.13
$LSD(\alpha=0.05)$	0.81	1.6	3.39
CV	7.75%	4.46%	6.3%
F test (α=0.05)	***	***	***
Priming			
Hydro	11.59ª	40.29 ^a	58.8ª
Halo	10.14 ^b	35.9 ^b	54.67 ^b
Control	9.64 ^b	31.79 ^c	47.94°
Sem (±)	0.27	0.53	1.13
$LSD(\alpha=0.05)$	0.81	1.6	3.39
CV	7.75%	4.46%	6.3%
F test (α=0.05)	***	***	***
Grand Mean	10.46	35.99	53.8

Note: Sem±, Standard error of the mean; CV, Coefficient of Variation; LSD, Least Significant Difference. Means followed by common letter(s) within column are non-significantly different based on LSD at P=0.05, * significant at 0.05 P level, ** significant at 0.01 P level, *** significant at 0.001 P level, NS= non-Significant

Number of Leaves:

At 45 DAS, the ghoratte bean variety showed the maximum number of leaves (22.11) and the black bean variety showed a minimum number of leaves (17.44). At 60 DAS, the maximum number of leaves (39.11) was observed in the ghoratte bean, and the minimum number of leaves (27.44) was observed in the black bean. Similarly, at 45 DAS, the maximum number of leaves (20.33) was observed in hydropriming which was statistically at par with halo-priming (19.56). The minimum number of leaves (19) was observed in the control treatment. At 60 DAS, the maximum number of leaves (34) was observed in hydro-priming and the minimum number of leaves (32.67) was observed in the control treatment. This was statistically similar with halopriming (33).

In this study, both the varieties as well as priming methods had a significant effect on the number of leaves per plant French bean (Table 4). A maximum number of leaves were observed in the Ghoratte bean among the three varieties. This can be due to the genetic makeup or environmental conditions. Similarly, in the case of methods of priming, a maximum number of leaves per plant was observed in hydro-primed seeds (Table 4). Hydro priming increases plant height and growth, which results in more nodes and internodes and hence increased leaf production. This result conforms with the (Harris *et al.*, 1999) which reported a greater number of leaves per plant due to hydro-priming.

Fable	4:	Effect	of	different	priming	techniques	on	the
	r	number	of l	eaves per	plant of d	lifferent vari	ietie	s of
	ł	French b	bear	n at Mugu	Nepal, 2	022		

Treatments	Number of leaves		
	45 DAS	60 DAS	
Variety			
Black bean	17.44 ^c	27.44 ^c	
Ghoratte Bean	22.11 ^a	39.11ª	
Red bean	19.33 ^b	33.11 ^b	
Sem (±)	0.27	0.31	
LSD(a=0.05)	0.8	0.93	
CV	4.06%	2.82%	
F test (α=0.05)	***	***	
Priming			
Hydro	20.33ª	34 ^a	
Halo	19.56 ^{ab}	33 ^b	
Control	19 ^b	32.67 ^b	
Sem (±)	0.27	0.31	
$LSD(\alpha=0.05)$	0.8	0.93	
CV	4.06%	2.82%	
F test (α=0.05)	**	*	
Grand Mean	19.63	33.22	

Note: Sem±, Standard error of the mean; CV, Coefficient of Variation; LSD, Least Significant Difference. Means followed by common letter(s) within column are non-significantly different based on LSD at P=0.05, * significant at 0.05 P level, ** significant at 0.01 P level, *** significant at 0.001 P level, NS= non-Significant

Number of Primary and Secondary Branches per Plant:

At 45 and 60 DAS, the maximum number of primary branches (2.89) and (3.67) was observed in the ghoratte bean, and the minimum number of primary branches (1.67) and (2.44) was observed in the black bean. Similarly, the maximum number of secondary branches at 45 DAS (5.78) and 60 DAS (7.89) was observed in the ghoratte bean, and the minimum number at 45 DAS (4.67) and 60 DAS (6.78) was observed in the black bean.

Likewise, at 45 DAS and 60 DAS, the maximum number of primary branches (3.11) and (3.89) was observed in hydropriming, and the minimum number of primary branches (1.33) and (2.33) was observed in the control treatment. Similarly, the maximum number of secondary branches at 45 DAS (5.89) and 60 DAS (8.11) was observed in the hydro-priming, and the minimum number at 45 DAS (4.56) and 60 DAS (6.44) was observed in the control treatment.

In this investigation, both the varieties as well as priming methods had a significant effect on the number of primary and secondary branches per plant (Table 5). A maximum number of primary and secondary branches per plant were observed in the Ghoratte bean among the three varieties. This can be due to the genetic makeup of the plant such as vigorous growth. Similarly, in the case of methods of priming, maximum number of primary and secondary branches per plant was observed in hydro-primed seeds (Table 5). It may be concluded that increased plant growth and plant height result in a greater number of primary and secondary branches. A similar result is reported by (Harris *et al.*, 1999).

Treatments	Number of primary branches		Number of secondary branches	
	45 DAS	60 DAS	45 DAS	60 DAS
Variety				
Black bean	1.67 ^c	2.44 ^c	4.67 ^c	6.78 ^b
Ghoratte Bean	2.89 ^a	3.67 ^a	5.78ª	7.89 ^a
Red bean	2.11 ^b	3 ^b	5.22 ^b	7.11 ^b
Sem (±)	0.12	0.14	0.15	0.2
LSD(a=0.05)	0.35	0.44	0.46	0.6
CV	15.9%	14.34%	8.74%	8.23%
F test (α=0.05)	***	***	***	**
Priming				
Hydro	3.11 ^a	3.89 ^a	5.89ª	8.11 ^a
Halo	2.22 ^b	2.89 ^b	5.22 ^b	7.22 ^b
Control	1.33°	2.33°	4.56 ^c	6.44 ^c
Sem (±)	0.12	0.15	0.15	0.2
LSD(a=0.05)	0.35	0.44	0.46	0.6
CV	15.9%	14.34%	8.74%	8.23%
F test (α=0.05)	***	***	***	***
Grand Mean	2.22	3.03	5.22	7.26

Table 5: Effect of different priming techniques on the number of primary and secondary branches per plant of different varieties of French bean at Mugu, Nepal, 2022

Note: Sem±, Standard error of the mean; CV, Coefficient of Variation; LSD, Least Significant Difference. Means followed by common letter(s) within column are non-significantly different based on LSD at P=0.05, * significant at 0.05 P level, ** significant at 0.01 P level, *** significant at 0.001 P level, NS= non-Significant

Yield Parameters

Days To First Flowering and 50% Flowering:

The maximum days to first flowering (32.22) and 50% flowering (43.89) were observed in the black bean variety. The minimum days to first flowering (30.67) and 50% flowering (41.11) were observed in the ghoratte bean variety. Early flowering (28.22) was observed in hydro-priming followed by control treatment (31.44). The delay in flowering (34.22) was observed in halo-priming. The maximum days to 50% flowering (45.11) were observed in halo-priming followed by control treatment (42.11). The minimum days to 50% flowering (39.44) were observed in hydro-priming.

The research study shows that both the varieties as well as priming methods had significant effects on days to first flowering and days to 50% flowering (Table 6). Early flowering and early 50% flowering were observed in the ghoratte bean among the three varieties. This difference in flowering days among the varieties might be due to their genotypes, day length, and temperature. Similarly, in the case of priming methods, early flowering and early 50% flowering were observed in hydro-primed seeds (Table 6). Hydro-priming enhances a better plant root system leading to faster growth, improved nutrient absorption, and early flowering. Similar results are reported by (Maiti *et al.*, 2013) and (Singh *et al.*, 2014).

Yield Per Hectare:

The analyzed data revealed that the yield per hectare was significantly influenced among the bean varieties (Table 6). The maximum yield (2.51 t/ha) was recorded in the ghoratte bean variety followed by the red bean variety (2.34 t/ha). The minimum yield (1.8 t/ha) was recorded in the black bean variety. Ghoratte bean due to its rapid growing habit and genotype resulted in maximum yield per hectare. The difference in yield among the varieties might also be due to day length and prevailing temperature.

Similarly, the priming methods showed a significant effect on yield per hectare (Table 6). Maximum yield (2.46 t/ha) was observed in hydro-priming which is followed by halopriming (2.19 t/ha). The minimum yield (2.01) was observed in the control treatment. This may occur because priming seeds encourage rapid sprouting, which speeds up germination and results in early emergence, improved crop stand, quicker growth, a longer vegetative growth period, and eventually high yield as mentioned by (Awasthi *et al.*, 2022).

Treatments	Days to first flowering	Days to 50% flowering	Yield (t/ha)
Variety			
Black bean	32.22ª	43.89 ^a	1.8 ^c
Ghoratte Bean	30.67 ^b	41.11 ^b	2.51ª
Red bean	31 ^b	41.67 ^b	2.34 ^b
Sem (±)	0.33	0.4	0.02
LSD (a=0.05)	0.98	1.2	0.07
CV	3.12%	2.85%	3.22%
F test (α=0.05)	**	***	***
Priming			
Hydro	28.22°	39.44°	2.46 ^a
Halo	34.22ª	45.11 ^a	2.19 ^b
Control	31.44 ^b	42.11 ^b	2.01°
Sem (±)	0.33	0.4	0.02
LSD(a=0.05)	0.98	1.2	0.07
CV	3.12%	2.85%	3.22%
F test (α=0.05)	***	***	***
Grand Mean	31.3	42.22	2.22

Table 6: Effect of different priming techniques on days to first flowering, 50% flowering, and yield or bectare of different varieties of Erench bean at Mugu Nepal 2022

Note: Sem±, Standard error of the mean; CV, Coefficient of Variation; LSD, Least Significant Difference. Means followed by common letter(s) within column are non-significantly different based on LSD at P=0.05, * significant at 0.05 P level, ** significant at 0.01 P level, *** significant at 0.001 P level, NS= non-Significant

Conclusion

The result of this study revealed that the germination and growth parameters of different French bean varieties were significantly influenced by priming. Seed treatment with hydro-priming resulted in higher germination percentage and speed of germination. Likewise, the vegetative growth parameters were found significantly higher in hydro-primed seeds and ghoratte variety. Similarly, the yield parameters were found significantly higher in hydro-primed ghoratte beans. Hence, hydro-priming can be used as a simple and cost-effective technique to improve the germination and growth of French beans, along with a suitable variety under field conditions in Mugu district, Karnali province, Nepal.

Authors' Contribution

S. Shrestha designed the research plan; S. Shrestha and S. Gyawali performed the experimental works and collected the required data. S. Shrestha and D. Regmi analyzed the data; S. Shrestha and S. Chaulagain prepared the manuscript. S. Gyawali and D. Regmi finalized the manuscript. The final form of the manuscript was approved by all authors.

Conflict of Interest

The author declares that there is no conflict of interest with the present publication.

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