Unlocking the Potential of Lucerne (*Medicago sativa* L.) in Nepal: First-time Evaluation of Varietal Suitability for Herbage and Seed Production

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Abstract

Purpose: This study aimed to evaluate the adaptability and comparative production performance of different Lucerne varieties, ultimately recommending the best-suited variety for herbage and seed yield for the mid-hill regions of Nepal.
Methods: The experiment was conducted from October 2022 to July 2023 at research field of the National Pasture and Fodder Research Program (NPFRP) of Nepal Agricultural Research Council (NARC) Lalitpur, Nepal. A field trial was set up using a Randomized Complete Block Design (RCBD) design with five replications. Sowing was carried out on the 1st of October, and the three varieties tested were Stamina 5, Kaituna, and Torlesse.
Results: The results showed that plant height and leaf area index (LAI) were significant ($p < 0.001$) among the varieties, while all the yield attributes (plant height, LAI, stem number, and leaf numbers) were significant ($p < 0.01$) for cutting management. Among the evaluated varieties, Stamina 5 exhibited the highest productivity (39.98) t/ha herbage, 11.41 t/ha dry matter (DMY), and 34.8 kg/ha seed yield.
Conclusion: The preliminary findings indicated that all three Lucerne varieties adapted well to mid-hill conditions with the Stamina 5 variety showing the best suitability and performance in the mid-hill regions of Nepal.

Keywords: Alfa alfa; Dry matter; Herbage yield; Seed yield; Variety

1 Introduction

Lucerne (*Medicago sativa* L.) also known as Alfa alfa is the "Queen of Forages. It is an important forage crop extensively cultivated across temperate (Mielmann et al. 2016), tropical and sub-tropical regions (Chand et al, 2023) worldwide. It is the most admired herbage crop because of its high protein content and better palatability (Annicchiarico et al. 2015; Li et al. 2024) and digestibility for dairy animals (Colas et al. 2013). Due to its persistence and tolerance to cold, lucerne has become a suitable alternative crop within various agrosystems (Picasso et al. 2019). Its inherent tolerance towards water deficiency, with its extensive deep root system, is an added advantage against climate variability (Moot et al. 2016; Zhao et al. 2019) to achieve high yields. Along with good production and atmospheric nitrogen fixation, the plant is also popular for hay, silage or pasture (Li et al. 2024).
In Nepal, winter fosters an acute shortage of livestock feed, especially in the hills and mountains, with the loss of grazing and farmers relying on poor or cheap feeds (Sanjyal, 2022). High quality herbage crop like Lucerne are ideal for enhancing livestock nutrition; however, in Nepal, suitable varieties have yet to be identified, as no varietal trials have been conducted to determine which varieties are best adapted.
Although Lucerne is valued worldwide as the most popular herbage crop for its quality and yield, there has been very little historical evidence confirming that Lucerne was tested in Nepal around 1987-1990. During that time various cultivars of Lucern used in trials were *M falcate,*

M. littoralis, M. polymorpha, M. rigosa, M. sativa tested in different parts of Nepal particularly Pokhara, Jumla, Marpha, Jiri and other regions (Pande,1997). However, it failed to be taken to either research or a larger scale. Similarly, a native species of Lucerne (*Medicago falcata*) known as Kote is found only in the high-altitude regions of Mustang. The survivability of Kote is very low and its survivability is mainly due to perennial nature. The limited seed yield restricts its expansion. Further, it does not grow in mid hills and terai. This knowledge gap largely hindered the widespread adaptation and optimal cultivation of Lucerne as a potential herbage crop in Nepal until recently in 2022, a well-designed research work precisely started addressing those concerns at NPFRP, NARC.
This demanded a need for testing and selection of Lucerne varieties producing higher biomass and seed production under Nepalese conditions. Successful introduction of such variety would provide farmers with an opportunity for year-round forage availability, helping to bridge the winter gap, enhancing livestock productivity, strengthening livelihood and enhancing fodder security. Thus, this study was undertaken with an objective to identify the best adaptable, high yielding, and high nutrient content Lucerne varieties, as well as compare their production performance and recommend the best variety for mid-hill areas.

2 Materials and methods

2.1 Site description and cropping history

The experiment was carried out from October 2022 to July 2023 in the experimental field of NPFRP, NARC, Lalitpur, Nepal. The farm is situated in the Bagmati Province of Nepal at 27.6588° N Latitude, 85.3247° E Longitude, and at an elevation of 1340 meter above sea level. The experimental plot had no prior history of Lucerne cultivation and was previously cultivated with Naked Oat (*Avena nuda*) from 2019 until 2022, for herbage and seed production.

2.2 Soil characteristics

Soil sample was collected on August 20, 2022, from the surface layer (0–15 cm) and sent to Agricultural Technology Center (ATC), Lalitpur for analysis of physiochemical properties before the trial. The experimental site had clay loam soil, the pH was 6.03 containing about 3.04% organic matter, 0.25% N, about 32.00 kg/ha of available phosphorus, and 170 kg/ha of available potassium. Since the pH levels were on the lower limit of those recommended for Lucerne, lime at the rate of 4 ton ha1 was applied two months before the sowing date and top worked with a plough. By incorporating the lime into the soil, it is expected to rapidly modify pH levels even below 150mm, which has been shown to be the threshold depth to induce pH changes in surface liming (Moir & Moot 2014).

2.3 Meteorological conditions

The agrometeorological data were recorded from the weather station established at National Agronomy Research Center of NARC, Khumaltar, Lalitpur from October 2022 to July 2023 as presented in Figure 1. The highest mean temperature of 29.9°C was recorded during the month of June 2023, and the lowest of 3.1°C was recorded during January 2023. Total rainfall recorded during the crop growing period was 752.3 mm with the highest value of 221.1 mm during July 2023 and a total 82 days of rainfall for 10 months.

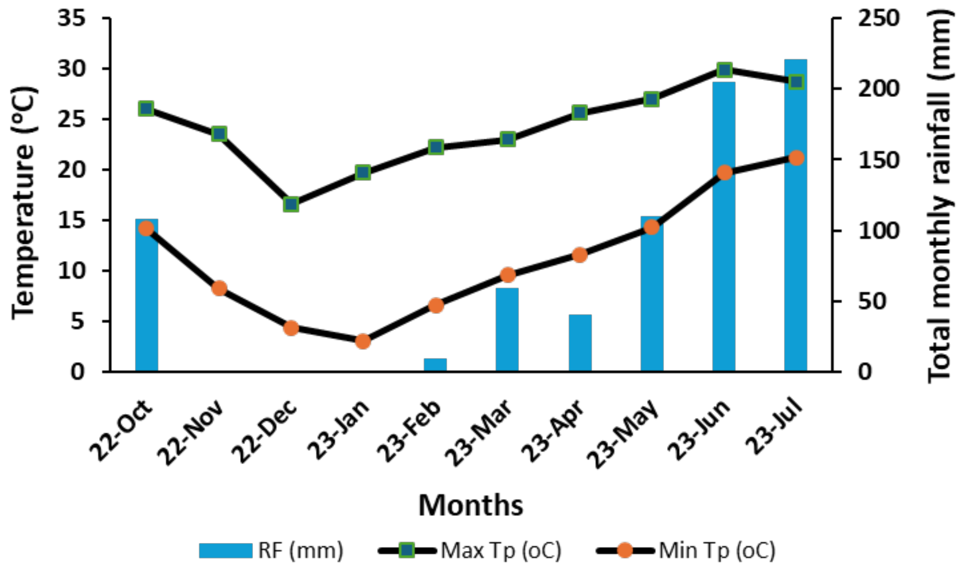


Figure 1: Mean maximum temperatures, mean minimum temperatures, and total monthly rainfall recorded at National Agronomy Research Center, Lalitpur during 2079/80 (2022/23).

2.4 Experimental design and treatments

A field experiment was established in a RCBD design with five replications. The sowing was done in October and the three varieties tested were Stamina 5, Kaituna, and Torlesse. All three varieties were introduced from New Zealand for research purposes. Urea, diammonium phosphate (DAP), and muriate of potash (MOP) were applied at the rate of 20:60:40 kg per hectare respectively. The entire dose of NPK were incorporated as a basal application into the soil during field preparation.

2.5 Sowing and crop management

Before sowing, the experimental plot was ploughed thoroughly in September 2022. After ploughing, Lucerne seeds were sown manually in a continuous line at a row-to-row distance of 40 cm and a targeted depth of 20 mm. The plot size used was 6 (3*2) sq.m. The seed rate of 12 kg per hectare was applied and sowing was done on 1st of October. On the seventh day of sowing, flood irrigation was applied. Since the seeds were sown in winter and the experimental site was dry, irrigation was provided every week. The crop was first harvested for herbage sixty days after sowing (DAS), and the succeeding harvests were taken every 30 days after the previous harvest. The herbage yield (HY)was recorded for half of the plot, and the remaining half was left for seed production. Seeds were hand-picked 3 times during the crop season. Being an indeterminate plant, Lucerne produces seeds that mature at different times, allowing for multiple hand picking. After picking, seeds were threshed manually using a stick. Weeding was done manually as and when required. No records of insects, pests, and diseases were reported.

2.6 Chemical analysis of forage samples

The dry matter (DM) content was calculated using the proximate procedure protocols of the Association of the Analytical Chemists (AOAC) (Horwitz 1975). The nitrogen content (%) was estimated according to AOAC with the modified Kjeldahl method, for each treatment, and then multiplied by the factor of 6.25 to reflect the crude protein content. The fiber components were analyzed as prescribed by Van Soest (1991) for neutral detergent fibers (NDFs), acidic detergent fibers (ADFs), and acid detergent lignin (ADL).

2.7 Measurements and data recording

Five plants were selected randomly from half of each plot and were tagged to record the yield attributes at each harvest. At 60 DAS, all plants including the 5 tagged plants were cut at a height of 15 cm above ground to record HY from half of the plot in each plot. After the crop was harvested, it was allowed to regenerate for the subsequent cuttings, 5 plants from each sowing were tagged again. HY was measured by weighing the fresh biomass, which was then dried in a hot air oven at 65°C for 72 hours until a constant weight was obtained to determine the DM content for each harvest. A total of eight cuts were taken from each variety, and all their yields were summed up to get the final HY. The dry matter percentage was then calculated using the formula given by Devkota et al. (2015).

$$DM\% = \frac{\text{Dry weight}}{\text{Fresh weight}} * 100$$

Dry matter yield (DMY) was then estimated using DMY (kg ha-1) = HY (kg ha^{-1}) * DM (%) content. Total HY and total DMY were calculated by adding the values across all eight harvests. Plant height was measured from ground level to the tip of the fully opened leaves in centimeters from the 5 randomly tagged plants in each experimental plot. The total number of leaves was recorded at each harvest before taking the HY. Leaf area index (LAI) was calculated using the formula as suggested by Tanko and Hassan (2016). Similarly, stems per plant were recorded by counting all the stems of each of the 5 tagged plants separately before each harvest and then the value was averaged.

2.8 Statistical analysis

The effect of varieties, cutting management, and their interactions on herbage yield, seed yield, and related yield attributes of lucerne were analysed using Analysis of Variance (ANOVA) under the General Linear Model (GLM) procedure in GenStat 19th Edition (VSN International 2019). Significance levels were determined at $p < 0.05$, $p < 0.01$, and $p < 0.001$, corresponding to 5%, 1%, and 0.1% levels, respectively, where significant differences were observed, mean comparisons among factor levels were conducted using Fisher's unprotected least significant difference (LSD) test.

3 Results

3.1 Nutrient Analysis

Table 1 shows the nutrient composition of three varieties of Lucerne namely, Stamina 5, Kaituna and Torlesse. All three varieties have high crude protein (CP) content ranging from 23.9-24.7% indicating potential forage for improving livestock nutrition. All three varieties show similar values in terms of neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) making it digestible and efficient forage for ruminant feeding.

Table 1: Nutrient content of different Lucerne varieties as provided by National Animal Nutrition Research Center Laboratory, Nepal Agricultural Research Council, Khumaltar, Lalitpur.

Varieties	**CP %**	**NDF %**	**ADF %**	**ADL %**
Stamina 5	23.9	42.9	24.2	7.2
Kainuna	24.7	41.4	24.5	6.7
Torlesse	24.7	42.0	23.7	6.2

3.2 Main effect means of varieties and cutting management on yield attributes of Lucerne

There was a significant variation among the varieties for plant height and LAI ($p > 0.001$) for all the varieties (Table 2). Among all tested varieties, Stamina 5 had the tallest plants with highest LAI followed by Torlesse and the smallest plants were recorded from Kaituna. In contrast, leaf number and stem number did not vary among the varieties (Table 2).
There was a significant difference between the cutting management for all the yield attributes (plant height, LAI, leaf number and stem number) ($p < 0.001$) for all the varieties (Table 2). Plant height and LAI showed an increasing trend with successive cuts. Leaf number and stem number peaked around the 4th cut but declined from then onwards significantly. This decline may be an indication of loss of overall plant vigor or yield potential with extended cutting, which shows that the leaf number and stem numbers were at a declining phase leading to reduced yield after certain harvest.

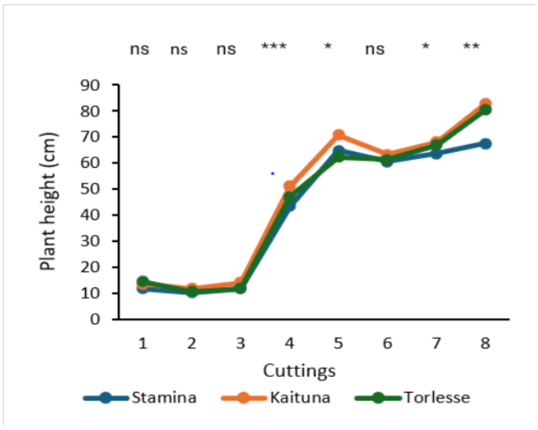
Table 2: Main effect means of varieties and cutting management on yield attributes of Lucerne.

Main effect means	**Plant height (cm)**	**Leaf number**	**LAI**	**Stem number**
Overall mean	44.47	30.00	2.59	8.32
Variety				
Stamina 5	47.05	28.7	2.78	8.38
Katuna	41.85	30.7	2.42	8.32
Torlesse	44.52	30.6	2.58	8.28
Significance of linear trend (p value)	<0.001	ns	<0.001	ns
LSD (0.05)	1.206	1.9	0.15	0.259
Cuttings				
1	13.38	10.87	2.31	6.67
2	11.13	20.26	2.23	7.38
3	12.79	16.43	2.17	5.83
4	41.33	35.50	3.03	9.50
5	66.05	45.08	3.00	8.89
6	61.31	33.98	2.73	10.44
7	66.36	44.69	2.92	13.12
8	76.95	34.89	2.41	10.44
Significance of linear trend (p value)	<0.001	<0.001	<0.001	<0.001
LSD (0.05)	1.97	3.1	0.25	0.42
Significance of interactions of linear contrasts (p value)				
	<0.001	0.009	0.01	<0.001
Var × Cutting LSD (0.05)	3.41	5.37	0.15	0.73
CV%	6.1	14.3	13.1	7.0

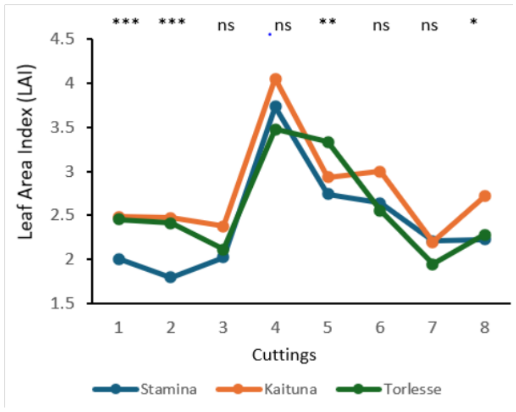
Note: LSD = Least Significant Difference; CV = Coefficient of Variation. Lettering has been assigned using the unrestricted LSD procedure; means with no letters in common (in the same column) are significantly different at the 5% level $p < 0.05$.

3.3 Interactions

A significant interaction between varieties and cutting management was observed as plant height and LAI increased with every cutting ($p < 0.001$) (Table 2). The interactions are shown in Figure 2 to Figure 5 suggesting that the response of each variety to repeated cutting was not uniform. A highly significant interaction ($p < 0.001$) between the plant height and varieties was observed during the fourth cutting and a significant difference was observed during the fifth cut ($p < 0.05$) and 8th cut ($p < 0.01$) between varieties and cutting management (Figure 2a). There was a highly significant interaction between LAI and varieties ($p < 0.001$) during the first and second cuts. A significant interaction ($p < 0.05$) was also observed during the 5th and 8th cut ($p < 0.01$) (Figure 2b). Similarly, there was a strong interaction ($p < 0.001$) between the stem number among the varieties during the third, fifth and sixth cuts. Likewise, significant difference ($p < 0.05$) was recorded in the fourth cut, and seventh cutting (Figure 3a). Further, a highly significant difference ($p < 0.001$) between leaf number and varieties was found during the second and eighth cut. A significant variation ($p < 0.01$) was also recorded during the fifth cut (Figure 3b).

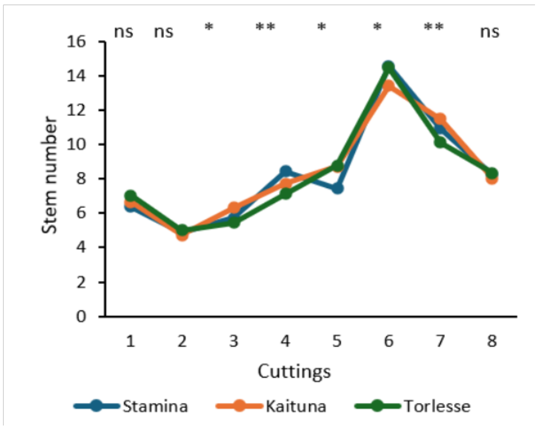


(a)

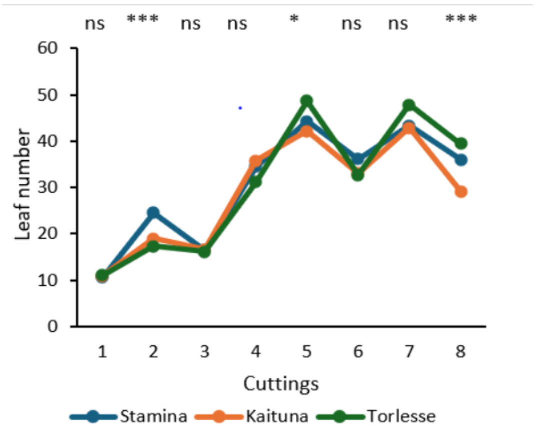


(b)

Figure 2: Interaction between (a) plant height and cutting management (b) leaf area index and cutting management.



(a)



(b)

Figure 3: Interaction between (a) stem number and cutting management (b) leaf number and cutting management.

3.4 Main effect means of varieties and cutting management on HY, DMY and seed yield attributes of Lucerne

There was a significant difference in total DMY among the Lucerne varieties (Table 4) although no significant differences was observed in HY. Among all varieties, Stamina 5 produced the highest DMY which was 20% higher Torlesse and 4% than Kaituna ($p < 0.027$). Similarly, Stamina 5 also recorded the highest seed yield exceeding Torlesse by 20% and Kaituna by 14% ($p < 0.005$).

Table 3: Effect of varieties on HY (t/ha), DMY (t/ha), and seed yield (kg/ha).

Variety	**HY (t/ha)**	**DMY (t/ha)**	**Seed Yield (kg/ha)**
Overall mean	**38.96**	**10.64**	**31.4**
Stamina 5	39.98	11.41	34.8
Katuna	36.25	9.51	28.9
Torlesse	40.67	11.01	30.5
Significance of linear trend (p value)	ns	<0.027	<0.005
LSD (0.05)	4.651	1.384	3.26
CV%	8.7	9.4	7.5

Note: HY = Herbage Yield; DMY = Dry Matter Yield; t/ha = tons ha-1; LSD = Least Significant Difference; CV = Coefficient of Variation. Lettering has been assigned using the unrestricted LSD procedure; means with no letters in common (in the same column) are significantly different at the 5% level ($p < 0.05$).

4 Discussion

4.1 Nutrient composition

This is reflected in the nutrient analysis of the tested three Lucerne varieties Stamina 5, Kaituna, and Torlesse. All three varieties have a high crude protein content ranging from 23.9 to 24.7% and could be a potential forage to enhances the livestock productivity in Nepal (Table 1). Higher protein in Lucerne makes the crop valuable during the scarce winter period (Singh et al., 2019) due to its contribution through high protein supplement to increase productivity per unit area. Further similar content of fiber fractions like neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) between the varieties indicate that all three varieties serve as a digestible and effective source of roughages within ruminant diet which is similar to the findings of (Galyean et al., 2014).

4.2 Effect of varieties on yield attributes

There was a significant difference among the tested varieties for plant height and LAI, with Stamina 5 producing the tallest plants and highest LAI. This suggests that Stamina 5 had superior vegetative growth and canopy development resulting in higher DMY. Similar results have been reported by Mondal et al. (2014) in soybean. In contrast, Kaituna had the shortest plants and lowest LAI, indicating lower biomass accumulation which is at par with the findings of Falster et al. (2011) who reported that shorter plants with smaller LAI produce lower biomass.

4.3 Effect of cutting management on yield attributes

Cutting management had a highly significant impact on all measurable yield attributes, including plant height, LAI, leaf number and stem number. Plant height and LAI increased progressively with each successive cut, reporting strong regrowth potential and canopy development. However, stem numbers declined after the 7th harvest causing lower number of leaves which might be due to physiological stress and reducing growth capacity which indicates that excessive harvesting without adequate recovery periods might have led to reduced overall productivity. This finding aligns with the study of Portugal et al. (2021) and Vinther (2006) in Sorghum, and Perennial Rye grass and clover sward respectively where frequent grazing management was found to reduced leaf mass ultimately leading to reduced herbage yield. This finding highlighted the critical importance of well-planned cutting intervals to balance regrowth and maintain yield potential for optimizing both herbage and seed yield.

4.4 DMY and seed production

For DMY and seed production, varietal differences were statistically significant, where Stamina 5 outperformed both the varieties. This might be because Stamina 5 had better adaptability, taller plants and higher leaf area that led to higher photosynthetic activity that improved the accumulation of dry matter by the plants and ultimately higher DMY and seed yield as reported by Richards et al. (2000) and Siloriya et al. (2014). Similar results have been reported by Pariyar (2007) indicating taller plants had increased seed yield in oat.
Among the varieties, Stamina 5 produced 19.7% higher DMY than the Kaituna and 17.3% higher DMY than the Torlesse. Although sown on the same date, regrowth has been varied among the three varieties which might be because higher growth speed in Stamina 5 was better able to mobilize ground resources as supported by taller plants and higher LAI. This is in line with the findings of Meiss et al. (2008) who mentioned similar results in nine plant species. Likewise, Stamina 5 produced the highest seed yield, which was significantly greater by 20.4% than Kaituna and 14.1% than Torlesse. This variation in seed yield among the varieties could be attributed to difference in genetic makeup and adaptability leading to improved biomass accumulation. Similar findings were reported by Rivas Jacobo et al. (2020) in Lucerne and Jahn et al. (2011) in rice. The lowest seed yield in Kaituna might be the result of lower biomass due to cutting stress (Herbert, 2018). The significant interactions between variety-by-cutting management ($P < 0.001$) for all the characters highlighted that the performance of each variety varied across harvests. Plant height, LAI, number of stems, and number of leaves were affected in different ways by variety and frequency of cutting, with Stamina 5 showing the superior performance.
In 2023, Nepal achieved a significant milestone by producing Lucerne seeds at the National Pasture and Forage Research Program, 2024). Although the seed yield is considerably low as compared to the global average, this makes a promising beginning for Lucerne cultivation in Nepal. The global average is 250 - 400kg/ha (Genter, et al.,1997) as compared to the 30 - 35 kg/ha in Nepal. This low seed production observed in Nepal might be primarily attributed to low seed sets, which was further complicated by heavy rainfall during seed maturation period, which often caused to premature seed germination within the plant. Similar findings regarding seed quality effected by adverse climatic conditions have also been reported by (Maity et al., 2023). Therefore, to enhance lucerne seed production in the future certain management practices must be adopted to address the issues.

5 Conclusion

These preliminary results showed that Stamina 5 stands as a most suitable cultivar for both seed and herbage yield in the mid-hills of Nepal, making it a right choice for livestock farmers. The results also showed that proper cutting management is essential to help in maximizing the yield, besides maintaining herbage quality through harvests. Moreover, the results have a significant implication on Lucerne production in Nepal, especially in climate-susceptible areas where productive legume forages are most critically needed. However, multi-year and multi-location statistics are needed to validate and confirm the results further.

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Author's contribution

The overall preparation of the manuscript, including research design, data collection, analysis, interpretation, conceptualization, and original writing was done by the first author. Monitoring, review and editing was done by the second author, and review and editing was done by the third author. All authors have read and agreed to the publication of this manuscript.

Conflict of interest

The authors declare that there are no conflicts of interest related to the preparation or publication of this research work.

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