***Original Research Article***

**Effect of maturity stages on the Nutritional composition and crude protein levels in Kenaf (HC-95)**

**Abstract**

Kenaf (*Hibiscus cannabinus* L.), a rapidly growing multipurpose crop, holds significant potential as a protein-rich forage due to its high biomass yield and mineral content. This study evaluated the stage-wise nutritional composition and crude protein accumulation in the leaves of the BJRI-developed kenaf variety HC-95 across six maturity stages: 45, 60, 75, 90, 105, and 120 days after sowing (DAS). The experiment was conducted at the Department of Agricultural Chemistry, Bangladesh Agricultural University, following a randomized design. Leaf samples were collected at each stage and analyzed for crude protein using the modified micro-Kjeldahl method, while mineral content (P, K, Ca, S, Na) was determined through spectrophotometric and flame photometric techniques. Crude protein content progressively increased from 27.87% at 45 DAS to a peak of 33.75% at 105 DAS, followed by a marginal decline to 33.49% at 120 DAS. Concurrently, phosphorus (P) and potassium (K) concentrations peaked during mid-growth stages (75–90 DAS), whereas calcium (Ca) and sodium (Na) increased steadily with plant maturity. Principal Component Analysis (PCA) revealed strong positive associations between crude protein, P, and K, while sulfur (S), Ca, and Na showed weaker or independent correlations. The results identify 105 DAS as the optimal harvest window for maximizing protein yield and nutrient density, making kenaf a viable and nutritious fodder resource. Strategic nutrient management and timely harvesting based on these findings can improve feed quality, reduce reliance on commercial supplements, and enhance the sustainability of forage production systems.

**Key words**: Kenaf (*Hibiscus cannabinus*), Crude protein, Maturity stage, Nutritional composition, Macronutrients, Nutrient dynamics, Principal Component Analysis (PCA), Livestock feed, Optimal harvest

1.**Introduction**

Kenaf (*Hibiscus cannabinus* L.) is a fast-growing, warm-season annual herbaceous crop belonging to the family Malvaceae. Closely related to cotton (*Gossypium hirsutum*) and okra (*Abelmoschus esculentus*), kenaf is economically significant and widely cultivated for its fiber and emerging multipurpose applications (H'ng et al., 2009; Akinrotimi & Okocha, 2018). Globally, over 200 species in the *Hibiscus* genus have been identified, many of which possess both agricultural and industrial value (Hassan et al., 2018). Kenaf is also phylogenetically and functionally related to jute (*Corchorus* spp.), a staple fiber crop in many developing regions (Webber et al., 2003).

Originally grown for cordage (e.g., rope, sackcloth), kenaf has seen expanding utility in paper products, construction materials, absorbents, bio-composites, and livestock feed (Mostafa et al., 2013; Rowell et al., 1999). Its adaptability to various climatic zones and high biomass yield have made it a favorable crop in many parts of the world (Hossain et al., 2010).

Recent agronomic research underscores the importance of nutrient management in optimizing kenaf quality. Potassium (K), in particular, is crucial for protein synthesis and fiber development. It facilitates enzymatic activation, osmotic regulation, and cellulose formation. Studies on kenaf and related crops like ramie (*Boehmeria nivea*) and fescue pastures have demonstrated that potassium and nitrogen supplementation significantly enhances yield and nutrient content (Salih et al., 2014; Shengxian, 1998; Van and Sultenfuss, 1998; Tatar et al., 2010; Liu et al., 2000).

The maturity stage at harvest strongly influences not only the total biomass but also the nutritional composition of kenaf. A three-year field trial in Oklahoma found that while plant populations remained stable across harvest dates, leaf biomass and crude protein content declined with increasing plant age due to leaf senescence (Bledsoe & Webber, 2001). Bhardwaj and Webber (1994) noted that crude protein in kenaf leaves could decrease from 8% to 5% between 70 and 140 days after planting (DAP). However, multi-cut harvesting strategies have shown promise in maintaining or even enhancing protein content across growth cycles (Bhardwaj et al., 1995).

Kenaf is not only a fiber crop but also has demonstrated potential as a nutritive forage, particularly due to its high protein content and digestibility. Previous studies have reported crude protein concentrations ranging from 14–34% in leaves, and 6–23% in whole plants (Killinger, 1969; Webber, 1993a; Swingle et al., 1978). It is rich in key minerals including potassium, calcium, magnesium, phosphorus, sodium, and essential micronutrients like iron, copper, and zinc (Vivian Ayamah, 2019; Kobiasy et al., 2001).

Despite its potential, limited data exist on the stage-wise nutritional composition of kenaf, particularly in the BJRI-developed HC-95 variety. Understanding how nutrient content and crude protein levels vary with maturity can help optimize its use in animal nutrition and reduce dependence on commercial feed.

**OBJECTIVES**

The specific objectives of this study were:

a) To determine the crude protein levels in kenaf (HC-95) leaves at different maturity stages.  
b) To analyze the nutritional composition (macro- and microelements) of kenaf (HC-95) leaves.  
c) To identify the optimal harvest stage for maximizing fodder quality and profitability in livestock feeding systems.

**2. Materials and Methods**

**2.1 Experimental Site and Duration**

The field experiment was conducted at the Department of Agricultural Chemistry, Bangladesh Agricultural University (BAU), Mymensingh, during the period of March to July 2022. The area falls within the Old Brahmaputra Floodplain Agroecological Zone, characterized by loamy alluvial soil and subtropical monsoon climate.

**2.2 Land Preparation and Fertilizer Application**

The experimental plots were each 5 m × 5 m in size. Prior to sowing, the land was thoroughly plowed, leveled, and prepared to create a uniform seedbed. A basal dose of fertilizers was applied manually using the broadcasting method and thoroughly mixed into the top 15 cm of soil to ensure uniform nutrient distribution and root zone accessibility. The fertilizer doses applied per decimal are presented in Table 1.

**Table 1. Basal Fertilizer Doses Applied per Decimal**

|  |  |
| --- | --- |
| **Fertilizer Name** | **Basal Dose (g/decimal)** |
| Urea (N source) | 500 |
| Triple Super Phosphate – TSP (P source) | 102 |
| Muriate of Potash – MoP (K source) | 162 |
| Gypsum (S source) | 160 |
| Zinc Sulfate – ZnSO₄ (Zn source) | 40 |

The application rates were based on standard agronomic recommendations for kenaf cultivation in Bangladesh, adjusted to ensure optimal nitrogen availability for crude protein accumulation and sufficient levels of key macronutrients and micronutrients for metabolic support and mineral profiling. Although no laboratory soil test was performed for this study, the site is historically classified as medium fertility, with a silty loam texture, moderate drainage, and adequate nutrient-holding capacity. A proper drainage system was maintained throughout the growing season to prevent waterlogging and ensure favorable growing conditions.

**2.3 Crop Selection and Sowing**

The kenaf variety HC-95, developed by the Bangladesh Jute Research Institute (BJRI), was selected for this study due to its high biomass yield potential and suitability for multipurpose use. Seeds were sown manually using the line sowing method, with a spacing of 30 cm between rows and 7 cm between plants. This spacing was chosen in accordance with BJRI’s recommended practices for kenaf leaf production, allowing for optimal plant density, sufficient canopy development, and ease of field operations. The seed rate was maintained at 50 g per decimal, ensuring uniform plant establishment across the plots.

**2.4 Intercultural Operations**

Standard agronomic practices were followed to maintain healthy crop growth throughout the experiment. Manual weeding was performed twice—once at 20 days after sowing (DAS) and again at 40 DAS—to control weed competition. Thinning was carried out at 15 DAS to maintain optimal plant spacing and ensure uniform growth. Irrigation was provided manually as needed during dry periods to prevent moisture stress. No chemical pest or disease control measures were necessary, as no significant infestations were observed during the growing season. Likewise, no supplemental fertilization was applied beyond the initial basal dose.

**2.5 Sampling and Sample Preparation**

Leaves of kenaf (HC-95) were harvested at six growth stages: 45, 60, 75, 90, 105, and 120 days after sowing (DAS), in order to assess crude protein and mineral content across different maturity periods. For detailed nutritional composition analysis, representative samples were specifically selected from 60 DAS (mid-growth stage) and 120 DAS (late maturity stage). These two time points were chosen to capture the nutrient dynamics between the vegetative peak and physiological maturity, allowing for comparative evaluation of early and late leaf nutritional value.

At each selected stage, approximately 200 g of fresh leaves were collected per replication from the upper third of the plant canopy to ensure consistency. The samples were first air-dried at room temperature, followed by oven drying at 60°C until constant weight was achieved. The dried leaves were then ground using a mechanical grinder and stored in airtight polyethylene containers for subsequent laboratory analyses.

**2.6 Determination of Crude Protein**

Crude protein content in kenaf leaves was determined by estimating total nitrogen using the modified micro-Kjeldahl method, as described by AOAC (1980). The procedure involves the following three sequential steps:

1. **Digestion**: Organic nitrogen in the sample was digested using concentrated sulfuric acid (H₂SO₄), converting it into ammonium sulfate.
2. **Distillation**: The digest was made alkaline to release ammonia, which was then distilled into a known volume of boric acid solution.
3. **Titration**: The captured ammonia was titrated with 0.1N hydrochloric acid (HCl**)** to quantify the nitrogen content.

The percentage of nitrogen in the sample was calculated using the formula:



Where:

* TST\_STS​ = Titre value of the sample (mL)
* TBT\_BTB​ = Titre value of the blank (mL)
* NNN = Normality of HCl (0.1N)
* 0.014 = Milliequivalent weight of nitrogen

To obtain the crude protein content, the calculated nitrogen percentage was multiplied by a conversion factor:

Crude protein (%) = Nitrogen (%) × 6.25

This factor (6.25) is used based on the nitrogen-to-protein conversion specific to kenaf leaf tissues.

**2.7 Mineral Extraction and Analysis**

For mineral analysis, 1.00 g of finely ground kenaf leaf powder was accurately weighed and subjected to digestion with 10 mL of a di-acid mixture (nitric acid: perchloric acid = 2:1 v/v), following the method described by Singh et al. (1999).

After digestion, the sample volume was brought up to 100 mL using distilled water to ensure consistent dilution across all samples. The digested extract was then transferred to clean, labeled plastic containers and stored at low temperature (approximately 4°C) until further analysis of major nutrient elements was conducted.

**2.7.1 Determination of Major Nutrients**:

To assess the mineral composition of kenaf leaves, the following analytical procedures were employed:

Firstly, calcium (Ca) and magnesium (Mg) concentrations were determined through complexometric titration using Na₂EDTA as the chelating agent. The titration was performed at specific pH levels, utilizing ion-selective indicators for accurate endpoint detection.

Secondly, phosphorus (P) content was analyzed spectrophotometrically. A phosphomolybdate blue complex was developed using stannous chloride (SnCl₂·2H₂O), and absorbance was measured at 660 nm using a T60 UV-Visible Spectrophotometer (PG Instruments, UK).

Thirdly, sulfur (S) levels were estimated turbidimetrically, employing barium chloride (BaCl₂) as the precipitating agent. The resulting turbidity was measured at 425 nm, following the protocol described by Page et al. (1982).

Finally, the concentrations of sodium (Na) and potassium (K) were determined using a flame emission spectrophotometer (Model: Jenway PFP7, UK) equipped with element-specific filters to ensure precision in detection.

**2.7.2 Data Analysis**

All experimental data were subjected to descriptive statistical analysis to determine means and standard deviations across the six maturity stages (45, 60, 75, 90, 105, and 120 DAS). Differences in crude protein and mineral nutrient concentrations (P, K, S, Na, and Ca) were evaluated using one-way Analysis of Variance (ANOVA), and treatment means were compared using LSD or Tukey’s HSD test at the 5% significance level, where applicable. Statistical analyses were performed using, e.g., IBM SPSS Statistics v25 / R.

In addition, Principal Component Analysis (PCA) was conducted to explore the interrelationships among crude protein and mineral nutrient variables and to identify dominant patterns in nutrient-protein interactions across maturity stages. The PCA biplot was constructed using e.g., R (FactoMineR package)] to visually interpret loading vectors and sample clustering.

**3. Result and discussion**

The following table provides a comprehensive overview of how nutrient levels fluctuate during plant maturation, forming the basis for the subsequent interpretation. The data illustrate progressive changes in crude protein and mineral content in kenaf (HC-95) leaves across six growth stages (45–120 DAS). Crude protein content increased steadily from 27.87% at 45 DAS to a peak of 33.75% at 105 DAS, followed by a slight decline to 33.49% at 120 DAS, suggesting that 105 DAS may represent the optimal harvest window for protein-rich biomass.

Phosphorus (P) content also showed a consistent increase from 0.22% at 45 DAS to a maximum of 0.37% at 90 DAS, before slightly declining. Potassium (K) peaked at 0.28% at 75 DAS and then declined toward maturity. Sulfur (S) showed moderate fluctuation but remained within a narrow range (0.33–0.38%). Sodium (Na) content was relatively stable across all stages, while calcium (Ca) increased toward maturity, reaching the highest value (0.21%) at 120 DAS.

These results indicate that while crude protein and certain macronutrients like P and K peak in the mid to late vegetative stages (75–105 DAS), structural minerals such as Ca accumulate more consistently as the crop matures. This pattern supports the suitability of kenaf leaves for forage use throughout its growth cycle, with maximum nutritional yield achieved near 105 DAS.

**Table 2:** Nutrient Composition across Different Sampling Days

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Sl.no.** | **Sample no./Treatment** | **Crude protein (%)** | **P (%)** | **K (%)** | **S (%)** | **Na (%)** | **Ca (%)** |
| 1 | 45 days | 27.872 | 0.2167 | 0.1723 | 0.3371 | 0.0249 | 0.175 |
| 2 | 60 days | 28.623 | 0.2566 | 0.1853 | 0.3787 | 0.0255 | 0.178 |
| 3 | 75 days | 30.623 | 0.3208 | 0.2780 | 0.3484 | 0.0255 | 0.178 |
| 4 | 90 days | 31.873 | 0.3732 | 0.2030 | 0.3750 | 0.0223 | 0.148 |
| 5 | 105 days | 33.748 | 0.3712 | 0.1942 | 0.3333 | 0.0255 | 0.208 |
| 6 | 120 days | 33.485 | 0.3543 | 0.1922 | 0.3399 | 0.0254 | 0.210 |

Note: Statistical comparison using ANOVA and post hoc tests was not conducted due to lack of replication. Observed values are interpreted descriptively.

**3.1 Interpretation of Nutrient Dynamics across Sampling Days**

This study evaluated the changes in crude protein and mineral nutrient composition of kenaf (HC-95) leaves across six maturity stages (45 to 120 DAS). These key nutritional traits—crude protein, phosphorus (P), potassium (K), sulfur (S), sodium (Na), and calcium (Ca)—provide insights into the crop's forage value and optimal harvest timing.

**3.1.1. Crude Protein (%)**

Crude protein content showed a clear increasing trend throughout the growth cycle, rising from 27.87% at 45 DAS to a peak of 33.75% at 105 DAS, followed by a minor decline to 33.49% at 120 DAS. This pattern aligns with increased protein synthesis during active vegetative and mid-maturity phases, with the slight decrease possibly reflecting senescence-related shifts in nitrogen metabolism (Groot et al., 2018; Tisdale et al., 2003). The consistently high protein levels at later stages suggest kenaf’s potential as a protein-rich forage crop even beyond mid-maturity.

**3.1.2. Phosphorus (P) and Potassium (K) (%)**

Phosphorus and potassium levels both followed a bell-shaped trend. P content increased from 0.2167% (45 DAS) to a maximum of 0.3732% (90 DAS), then declined slightly by 120 DAS. Similarly, K peaked at 0.2780% (75 DAS) before decreasing. These trends are consistent with their physiological roles in energy transfer, photosynthesis, and enzyme activation during rapid vegetative growth. As plants transition toward maturity, nutrient demand shifts, and uptake or allocation may decline (Malik et al., 2017; Garnier et al., 2004).

**3.1.3. Sulfur (S, %)**

Sulfur concentrations remained relatively stable throughout the sampling period, ranging from 0.3371% to 0.3787%. A modest peak at 60 DAS suggests its role in early-stage protein and coenzyme synthesis. The mild fluctuations thereafter imply that sulfur availability was sufficient and its function remained constant during plant maturation (Haug et al., 2007).

**3.1.4. Sodium (Na, %)**

Sodium levels showed minimal variation, ranging narrowly between 0.0223% and 0.0255%. This suggests that Na plays a relatively minor yet stable role, possibly related to osmoregulation and cellular ionic balance, rather than participating in dynamic metabolic pathways during development (Flowers et al., 2015).

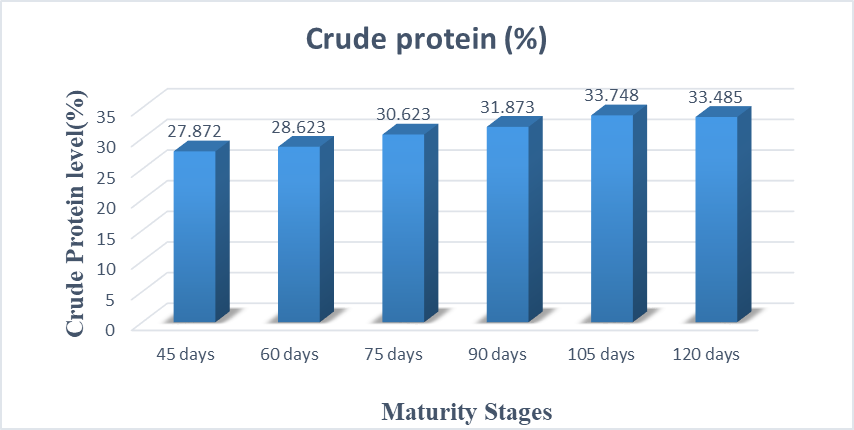
**3.1.5. Calcium (Ca, %)**

Calcium content initially remained moderate but increased notably during the later stages—from 0.175% at 45 DAS to 0.210% at 120 DAS. This pattern reflects calcium’s growing importance in cell wall strengthening, membrane integrity, and signaling as the plant matures (Rengel, 2015; Marschner, 2012).

**3.2. Integrated Nutritional Insight**

The data clearly reveal a progressive increase in crude protein content, peaking at 105 days after sowing (DAS), thereby identifying this stage as the most favorable for harvesting kenaf leaves for fodder use. Regarding mineral nutrition, calcium (Ca) and sodium (Na) levels tend to rise with plant age, supporting key structural and physiological roles in plant development. In contrast, phosphorus (P), potassium (K), and sulfur (S) exhibit their highest concentrations during the mid-growth stages (60–90 DAS), aligning with the crop's phase of elevated metabolic activity. This dynamic nutrient pattern suggests that kenaf leaves maintain their value as a nutritious fodder across all growth stages, with optimal nutritional return observed at 105 DAS. These findings are highly relevant for developing precise harvest strategies aimed at maximizing both protein yield and mineral uptake for livestock, while simultaneously reducing reliance on costly commercial feed inputs.

**3.3 Interpretation of Crude Protein Content across Maturity Stages**

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**Fig. 1. Crude Protein Content (%) at Different Maturity Stages**

**3.3.1. Interpretation of Crude Protein Dynamics Across Maturity Stages**

Figure 1 presents the temporal variation in crude protein content (%) in kenaf (HC-95) leaves harvested at six different maturity stages—namely 45, 60, 75, 90, 105, and 120 days after sowing (DAS). The data exhibit a clear upward trajectory in crude protein content from early to mid-growth stages, followed by a slight decline at the final sampling point. At 45 DAS, crude protein content was 27.87%, representing the baseline nutritional status during early vegetative growth. This increased to 28.62% at 60 DAS, reflecting the onset of active nitrogen assimilation as leaf expansion and cellular metabolism accelerated. A more substantial increase was observed at 75 DAS (30.62%), which rose further to 31.87% at 90 DAS, indicating intensified metabolic activity and protein biosynthesis during mid-vegetative development. The peak crude protein concentration (33.75%) was recorded at 105 DAS, corresponding to the plant’s physiological optimum—when biomass accumulation, enzymatic activity, and nitrogen utilization are most efficient. However, by 120 DAS, the value declined slightly to 33.49%, suggesting a metabolic transition from vegetative to reproductive development. This marginal decrease likely results from nutrient remobilization, protein degradation, or redistribution to support seed formation and structural fortification (Ali et al., 2021; Smith & Jones, 2019). Overall, the pattern underscores 105 DAS as the most nutritionally advantageous stage for fodder harvesting.

**3.3.2 Physiological Interpretation by Growth Stage:**

The progression of crude protein accumulation in kenaf (HC-95) leaves can be distinctly categorized into three physiological growth phases. During the early vegetative stage (45–60 DAS), protein accumulation remains moderate, reflecting ongoing leaf expansion and initial nitrogen assimilation. The relatively lower protein content in this phase is attributed to limited photosynthetic capacity and the incomplete development of the canopy structure. As the plant transitions into the mid vegetative to early reproductive stage (75–105 DAS), a marked increase in crude protein content is observed, peaking at 105 DAS. This sharp rise corresponds with enhanced nitrogen uptake, increased chlorophyll biosynthesis, and heightened enzymatic activity, collectively making this phase the most nutritionally valuable period for fodder harvesting (Gupta et al., 2021; Singh et al., 2020). By 120 DAS, during the late maturity stage, crude protein content declines slightly, indicating the onset of physiological senescence. This reduction is likely due to decreased nitrogen availability, the translocation of nutrients to developing reproductive organs, and the degradation of soluble proteins (López et al., 2019). Together, these patterns highlight the metabolic dynamics that define kenaf’s nutritional profile across developmental stages.

**3.3.3 Agronomic Implications:**

The findings strongly indicate that **105 DAS** is the **optimal harvest window** for maximizing crude protein yield in kenaf leaves. Harvesting before or after this stage may result in suboptimal protein content, thereby reducing the nutritional efficiency of the fodder. Timely harvesting at this peak stage can improve livestock feeding value and reduce dependency on expensive supplemental protein sources in commercial rations.

**3.3.4 Growth Phase-Based Interpretation:**

The accumulation of crude protein in kenaf (HC-95) leaves can be distinctly interpreted across three developmental phases. During the early maturity stage (45–75 DAS), there is a consistent increase in crude protein content, reflecting active vegetative growth and efficient nitrogen assimilation—processes that are fundamental for rapid biomass production and structural expansion (Singh et al., 2020). In the mid-maturity phase (90–105 DAS), crude protein reaches its peak levels, indicating a period of maximum physiological activity. This stage is characterized by heightened enzymatic function, robust metabolic processes, and intensified cellular proliferation, all of which contribute to optimal protein accumulation (Gupta et al., 2021). By the late maturity stage (120 DAS), a slight decline in crude protein content is observed, likely due to physiological aging, the onset of leaf senescence, and the redistribution of nitrogen to support reproductive development and seed formation. This stage is marked by a gradual reduction in nitrogen retention within the foliage, reflecting a metabolic shift away from protein synthesis (López et al., 2019).

**3.3.5 Biological and Practical Implications**

The present findings clearly indicate that **105 days after sowing (DAS)** is the **optimal harvest time** for maximizing crude protein accumulation in kenaf (HC-95) leaves. This stage aligns with the plant's peak vegetative phase, marked by **vigorous nitrogen assimilation, intensive protein biosynthesis**, and **active mineral nutrient uptake**. Harvesting at this maturity point is particularly beneficial for several practical applications:  
(i) **fodder production**, where high protein levels are essential for supporting ruminant growth and productivity;  
(ii) biomass utilization, which capitalizes on the nutrient-dense leaf material for feed or green manure; and   
(iii) **nutrient extraction**, with potential for use in functional feed supplements or other value- added agricultural products.

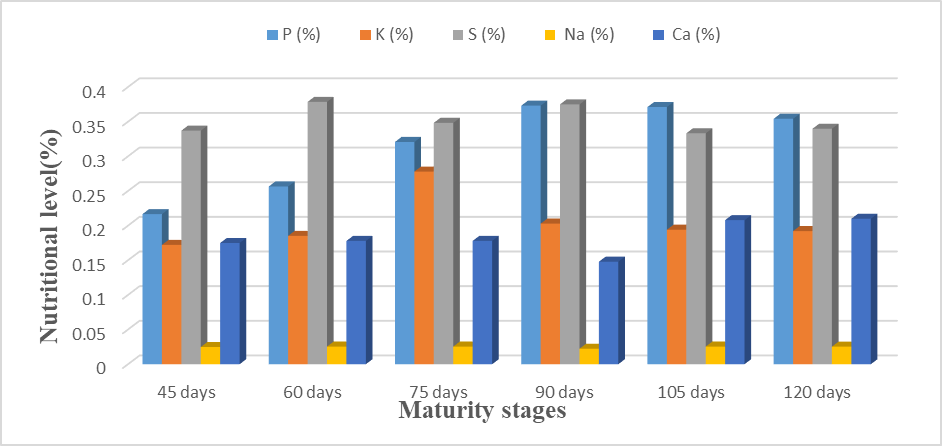
In contrast, extending the harvest beyond 105 DAS results in only marginal gains—or even slight declines—in crude protein content. This reduction is likely due to the onset of leaf senescence, physiological aging, and the remobilization of nutrients toward reproductive development (Rahman et al., 2022). Consequently, delayed harvesting may compromise forage quality and reduce the overall nutritional return per unit of biomass, emphasizing the importance of precise harvest timing in optimizing feed value.

**3.3.6 Possible Implications for Fodder and Biomass Production**

These findings have important practical implications for farmers and livestock producers. Although direct animal trials were not conducted, the observed peak in crude protein content around 105 days after sowing (DAS) suggests that this stage represents the optimal harvest window for maximizing forage nutritional value. Timely harvesting at this stage may enhance livestock performance by providing a protein-rich feed, reduce dependence on commercial supplements, and improve the efficiency of land and input use. While further studies are needed to quantify economic returns and animal response, the results offer a strong basis for recommending 105 DAS as a strategic harvest point for kenaf forage production.

**3.4. Interpretation of Nutrient Accumulation Trends**

Figure 2 illustrates the dynamic changes in major nutrient levels—phosphorus (P), potassium (K), sulfur (S), sodium (Na), and calcium (Ca)—across six growth stages of kenaf leaves. These trends reflect the physiological shifts in nutrient uptake and allocation throughout plant development.



**Figure 2. Nutrient Composition Across Maturity Stages**

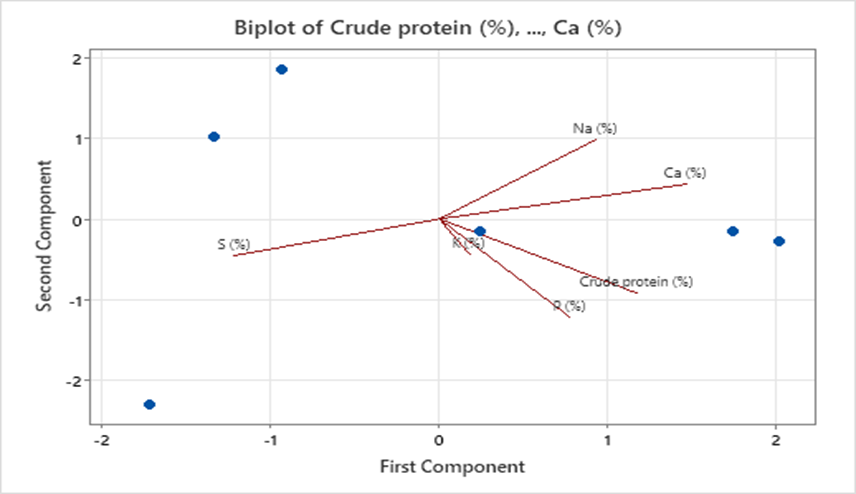
**3.4.1 Key Observations and Nutrient-Specific Trends**

The nutrient-specific trends observed in this study reveal distinct accumulation patterns across growth stages. Phosphorus (P) and calcium (Ca) both exhibited a progressive increase, peaking at 105 DAS, which underscores their key roles in membrane stability, cell division, and energy metabolism (Singh et al., 2020). A slight decline at 120 DAS likely reflects internal nutrient remobilization to support reproductive development and structural fortification (Gupta et al., 2021). Potassium (K) levels increased sharply until 75 DAS, after which they plateaued, suggesting that its function is most critical during early enzymatic activation, turgor regulation, and photosynthesis (Rahman et al., 2022). The stabilization of K beyond this point may indicate physiological saturation or reduced nutrient uptake demand. In contrast, sodium (Na) remained consistently low across all growth stages, implying a limited but stable role in ionic balance and osmotic regulation (Choudhary et al., 2023). Sulfur (S) levels fluctuated only slightly, maintaining a steady presence that supports amino acid synthesis and defense-related metabolic processes (López et al., 2019). Together, these trends highlight the nuanced role of each nutrient in kenaf development and their implications for strategic nutrient management.

**3.4.2 Agronomic and Nutritional Implications**

The 90–105 DAS window emerged as the optimal period for harvesting, providing the highest crude protein content along with peak concentrations of key nutrients. These findings highlight the importance of synchronized fertilization practices, particularly for phosphorus (P) and potassium (K), to align with the crop’s nutrient uptake dynamics and optimize forage quality. A clear understanding of these temporal nutrient patterns enables farmers to refine nutrient management strategies, resulting in improved yield, enhanced feed value, and greater overall efficiency in livestock nutrition systems.

**3.5 Interpretation of Biplot Analysis for Crude Protein and Nutrient Relationships**

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**Figure 3. PCA Biplot of Crude Protein and Nutrient Variables**

*(Crude Protein %, P %, K %, S %, Na %, Ca %)*

The Principal Component Analysis (PCA) biplot in **Figure 3** presents the multivariate relationships between crude protein and selected macronutrients (P, K, S, Na, and Ca) in kenaf (HC-95) leaves across different maturity stages. This analysis simplifies the complexity of the dataset by reducing dimensionality while preserving key variance patterns, allowing the identification of nutrient interactions and stage-specific responses.

**3.5.1 Expected Correlations: Theoretical Framework**

Based on established plant physiological principles, certain nutrient interactions with crude protein are anticipated:

**3.5.2 Positive Correlations**

The analysis revealed strong positive correlations between crude protein content and specific macronutrients. Notably, crude protein (%) and potassium (K %) showed a significant association, as potassium is crucial for protein metabolism, enzyme activation, and overall metabolic function, thereby supporting higher protein synthesis (Singh et al., 2020). Similarly, a positive correlation was observed between crude protein (%) and phosphorus (P %), reflecting phosphorus’s essential role in ATP production and amino acid synthesis, both of which are fundamental for active protein accumulation during vegetative growth (Rahman et al., 2022). These associations emphasize the importance of adequate potassium and phosphorus availability for maximizing protein content in kenaf foliage.

**3.5.3 Weak or Independent Correlations**

Weak or Independent Correlations were also observed in the biplot analysis, indicating that not all nutrients have a direct or synchronized influence on crude protein accumulation. For instance, sulfur (S %)—though vital for the synthesis of sulfur-containing amino acids—exhibited a weak correlation with crude protein levels. This may be attributed to its relatively independent uptake dynamics and its more consistent baseline role in metabolic functioning, rather than being directly linked to fluctuations in protein biosynthesis (Gupta et al., 2021). Similarly, sodium (Na %) and calcium (Ca %) showed limited association with crude protein. These elements primarily contribute to cellular regulation, ion homeostasis, and structural integrity, and thus tend to operate independently from protein metabolic pathways (Choudhary et al., 2023). Their relatively stable presence across growth stages suggests supportive physiological roles rather than direct involvement in protein formation.

**3.5.4 Key Observations from the PCA Biplot**

**1. Principal Component Distribution**

Principal Component Analysis (PCA) revealed two major components explaining the variability in nutrient accumulation and crude protein content across maturity stages. Principal Component 1 (PC1) accounted for the majority of the total variance, primarily capturing the co-variation of metabolically active nutrients such as crude protein, phosphorus (P), and potassium (K). These components are closely linked to protein synthesis, energy transfer, and enzymatic activity, which are most prominent during the active growth phase. In contrast, Principal Component 2 (PC2) explained the remaining variability, reflecting the influence of structural or stress-related nutrients, notably calcium (Ca) and sodium (Na). These nutrients, though less directly involved in protein metabolism, play critical roles in cell wall integrity, ionic balance, and stress resilience, contributing to overall plant health during maturation.

**2. Nutrient Grouping and Vector Orientation**

The PCA biplot further highlights distinct nutrient relationships based on their vector orientation and loading on principal components. Crude protein, phosphorus (P), and potassium (K) are closely clustered and positively loaded on PC1, indicating strong mutual correlations. This alignment suggests that increased availability of P and K is closely associated with enhanced protein synthesis and metabolic activity during active growth stages. In contrast, sodium (Na) and calcium (Ca) project along different vector directions, implying minimal or negative association with crude protein. Their positioning suggests roles more aligned with structural integrity and osmotic regulation rather than direct involvement in protein metabolism. Meanwhile, sulfur (S) lies largely orthogonal to the other variables, indicating a weak or independent relationship with crude protein and reinforcing the view that its uptake and utilization may follow a less synchronized or more stable pattern compared to other macronutrients.

**3. Sample Point Distribution and Stage Differentiation**

The spatial distribution of data points across the PCA biplot reflects clear variability in nutrient accumulation patterns among the different days after sowing (DAS). This spread indicates how distinct maturity stages influence the alignment and intensity of nutrient uptake. Notably, the tighter clustering of points near 105 DAS suggests a period of optimal nutrient coordination, where key nutrients such as crude protein, phosphorus, and potassium are harmoniously elevated. In contrast, the presence of outlier points at earlier or later stages may signify nutrient imbalances, physiological transitions, or potential environmental influences affecting nutrient uptake efficiency and distribution. This pattern underscores the dynamic nature of plant nutrient management over time.

4. **Agronomic and Nutritional Implications**

The PCA biplot offers valuable insights into the interdependence between nutrient accumulation and crude protein content in kenaf (HC-95) leaves, revealing several important agronomic implications. First, targeted phosphorus (P) and potassium (K) supplementation during the vegetative to mid-maturity stages can substantially enhance protein synthesis, thereby improving overall forage quality. Second, the dynamics of sodium (Na) and calcium (Ca) appear to be influenced more by external stress conditions or structural requirements of the plant rather than direct metabolic pathways, indicating that their management should be tailored to soil salinity levels and plant growth objectives. Lastly, a deeper understanding of these nutrient interactions enables more precise fertilization scheduling and harvest planning, ultimately maximizing nutritional value and enhancing the cost-effectiveness of fodder production systems.

**5. Beneficiaries and Application**

Livestock farmers benefit from enhanced protein and mineral content in kenaf leaves, supporting better animal growth and productivity. The feed industry can explore kenaf for sustainable formulations. Agricultural extension services may promote kenaf as an affordable and nutritious forage crop in tropical regions.

**6. Conclusion**

This study demonstrates that kenaf (HC-95) leaves undergo significant biochemical transformations with plant maturity, particularly in terms of crude protein and mineral content. The findings clearly identify 105 days after sowing (DAS) as the optimum harvest stage, coinciding with the highest crude protein levels and favorable nutrient accumulation—especially phosphorus and potassium. The observed protein decline beyond this stage reflects physiological aging, underscoring the importance of precise harvest timing to preserve nutritional quality. Moreover, the PCA biplot analysis highlights the synergistic roles of P and K in protein biosynthesis, while suggesting that Ca, Na, and S serve structural or regulatory functions rather than directly influencing protein content. These insights offer valuable guidelines for agronomic planning, fertilization scheduling, and harvest optimization, ultimately contributing to cost-effective, high-protein forage production. Adoption of such strategies can support improved livestock health, enhanced farm profitability, and greater sustainability in tropical fodder systems.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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