




**GERMINATION, GROWTH AND NUTRITIONAL VALUE OF CORN FODDER APPLIED WITH KUSUM TREE (*SCHLEICHERA OLEOSA*) LIQUID SMOKE**Stormy Vertygo<sup>1,\*</sup> , Agustinus Paga<sup>1</sup> , Euridice Leyequien Abarca<sup>2</sup> <sup>1</sup> Agricultural Polytechnic of Kupang, Nusa Tenggara Timur, Indonesia<sup>2</sup> Management of Forested Landscape, Van Hall Larenstein University of Applied Sciences, Leeuwarden, NetherlandsCorresponding author email: [svertygo91@gmail.com](mailto:svertygo91@gmail.com)**Article Info**

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**Abstract**

Fodder is a high-yielding, space-efficient animal feed that can be harvested in a short period, making it crucial for sustainable livestock nutrition. However, improving both the growth and nutritional content of fodder remains a challenge. This study aimed to investigate the effects of liquid smoke application on the germination, growth, and nutritional quality of corn fodder. A Completely Randomized Design (CRD) was used, consisting of five treatments: seeds soaked in 0% (control, P0), 10% (P1), 20% (P2), 30% (P3), and 40% (P4) liquid smoke concentrations for 1 hour. The results showed that liquid smoke treatment did not significantly influence germination parameters, including Final Germination Percentage (FGP), Mean Germination Time (MGT), and Mean Germination Rate (MGR). However, significant effects were observed on growth indicators such as the number of leaves per sprout and dry weight. Furthermore, liquid smoke notably enhanced the crude protein and crude lipid content of the corn fodder, though it did not alter crude fiber levels. This study presents a novel approach to improving fodder quality using plant-derived smoke compounds, offering a simple, low-cost pre-treatment method for enhancing the nutritional profile of corn fodder. The findings imply that liquid smoke can serve as a natural biostimulant, potentially replacing or supplementing synthetic feed additives. Future research should explore the use of smoke water or direct plant-derived smoke under aerobic conditions to optimize germination and further boost fodder quality. These insights contribute to the development of more efficient and sustainable animal feed strategies.

**Keywords:** Corn Fodder, Germination, Growth, Liquid Smoke, Nutritional Value.

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The success of animal farming is heavily influenced by the availability and quality of feed (Kırkpınar et al., 2018). Beyond mere quantity, animal feed must provide a balanced nutritional profile

to support optimal growth and productive performance (Herbstritt et al., 2022; Erdaw, 2023). Most times, livestock (especially ruminants) are left by themselves to forage in searching for their own food (Armstrong & Heins, 2021). However, this could be disadvantageous if seasonal and climatic changes affect forage availability (Kandalam & Samireddypalle, 2015). Moreover, types of plants foraged also displayed wide variability of nutrient value that would not meet the dietary requirements (Lee, 2018).

To address these limitations, producing feed in the form of fodder cultivated forage brought to the livestock—has emerged as a practical solution (Prihartini, 2014). Regardless of external weather, fodder cultivation ensures continual feed availability while providing consistent quantity and quality of the plant grown (Reed et al., 2020). Among various fodder crops, corn (*Zea mays* L.) is favored due to its digestibility, adequate protein levels, and lack of anti-nutritional factors (Chaudhary et al., 2014).

Local varieties of corn in the province of East Nusa Tenggara (ENT), Indonesia have been observed to be more resistant to pest attacks and also drought when compared to other varieties (Murningsih et al. 2015). However, lower rate of germination and growth impaired its cultivation effort by local farmers (Ahmed et al., 2023; Fenibo & Matambo, 2025; Van de Velde et al., 2025; Zbancă et al., 2025). In order to obtain an optimum yield of fodder, these drawbacks need to be overcome, and therefore, technological intervention is sometimes needed (Mekonnen et al., 2022; Singh et al., 2022; Feyissa et al., 2025; Tufail et al., 2025).

It has long been known that exposing plant seeds to smoke resulting from the combustion of plant materials could initiate faster germination and growth of a wide variety of plant species (Çatav et al., 2018). Moreover, several exogenous growth factors have also been successfully isolated from plant-derived smoke such as Karrikin (G. Flematti et al., 2015), cyanohydrin (G. R. Flematti et al., 2013) and strigolactone (Zwanenburg & Blanco-Ania, 2018). This also served as a novel alternative source of plant growth promoters which were previously known to be produced only by other live organisms (e.g. fungi) (Poveda et al., 2021). Previous studies have investigated the effects of liquid smoke derived from various plant materials on corn fodder germination and growth. Among these, liquid smoke from the kusum tree (*Schleichera oleosa*) has consistently shown the most promising results (Vertigo et al., 2022). However, while some studies have explored liquid smoke from various plant sources, no prior research has investigated the concentration-specific effects of liquid smoke derived from kusum tree (*Schleichera oleosa*) a plant commonly used by local farmers as firewood on both germination and nutritional content of corn fodder (Koeslulat, 2016).

This plant-based smoke appears to enhance certain growth parameters and improve the overall nutritional quality of corn fodder. The unique properties of kusum tree liquid smoke make it a potential alternative treatment for optimizing fodder production, offering better outcomes compared to liquid smoke from other sources like coconut shells or wood from other species. This gap underscores the urgency of identifying natural, low-cost alternatives to synthetic additives in feed crop production. The present study aims to evaluate the impact of various concentrations of kusum tree liquid smoke on the germination, growth, and nutritional composition of corn fodder, with the broader goal of improving feed efficiency and sustainability. The findings are expected to contribute a novel, eco-friendly strategy for feed manufacturing, particularly beneficial for rural and resource-limited farming systems.

## RESEARCH METHOD

This study employed a quantitative experimental design using a Completely Randomized Design (CRD) to evaluate the effects of various concentrations of liquid smoke derived from *Schleichera oleosa* (kusum tree) on the germination, growth, and nutritional value of white corn (*Zea mays*) fodder. The approach allowed controlled comparisons between treatment groups to identify statistically significant differences in observed parameters. The materials used were germination trays (uniform size across replicates), analytical balance (for dry weight measurement), oven (for drying samples), watering can, digital camera and ruler (for growth measurement).

The seeds used were white corn of the local Timor cultivar taken from the Lelogama sub-district. Purposive sampling was used to select seeds based on specific viability and quality criteria. Seeds were selected using the floating method (Daneshvar et al., 2017), in which the seeds were immersed in water, and those that sank were taken as viable sample seeds. After drying, seeds of good quality were selected which were characterized by their texture without holes and were weighed to use almost the same seed weight which was  $0.5 \pm 0.1$  gram. A good quality of seed would in turn impact its vigourity. The selected seeds were soaked in 10 % Sodium Hypochlorite solution for 2 minutes. The seeds were then rinsed with sterile distilled water and dried using filter paper (Davoudpour et al., 2020).

The selected seeds were then given treatment in the form of soaking in liquid smoke with different concentrations for 1 hour (60 minutes). The method used was a Completely Randomized Design (CRD), with the following treatment: seeds soaked in 0% concentration of liquid smoke for 1 hour as a control (P0), seeds soaked in 10% liquid smoke concentration for 1 hour (P1), seeds soaked in 20% liquid smoke concentration for 1 hour (P2), Corn seeds soaked in 30% liquid smoke concentration for 1 hour (P3), and seeds soaked in 40% liquid smoke for 1 hour (P4). The liquid smoke used was derived from kusum tree (*S. oleosa*) through pyrolysis and distillation to produce a grade-1 liquid smoke product. In each treatment, seeds were germinated in trays of uniform size and 5 replications were made. In each of these replicates, 30 corn seeds were germinated for 14 days (2 weeks) which also the period when fodders are usually harvested (Novianda et al., 2020).

During observations, several parameters were measured and quantified. For germination, the parameters were Final Germination Percentage (FGP), Mean Germination Time (MGT) and Mean Germination Rate (MGR) where the data was collected through daily observation and formula calculations based on (Al-Ansari & Ksiksi, 2016; Rika et al., 2023). For growth, the parameters measured were number of leaves/sprout through observation and manual count, and dry weight of fodder after 14 days by oven-drying and measurement using analytical balance. For the nutritional value, parameters were: crude fiber using fiber analyzer, crude protein using Kjeldahl method, and crude lipid content using Soxhlet extraction based on (AOAC, 1995).

Data was analyzed using Analysis of Variance (ANOVA) for a one-factorial Completely Randomized Design (CRD) with 5 treatments and 5 replications, using SPSS software (version 22.0). The significance level was set at  $p \leq 0.05$ . For parameters showing significant differences, Duncan's Multiple Range Test (DMRT) was used as a post hoc test to determine specific differences between treatments (Gomez & Gomez, 2010).

## RESULTS AND DISCUSSION

### *Effect of liquid smoke on fodder germination*

Germination encompasses a set of biochemical reactions that initiate cellular division and expansion inside the seed (Obroucheva, 2021; Srivastava et al., 2021; Farooq et al., 2022; Nautiyal et al., 2023; Xu et al., 2024; Malviya & Gayen, 2025). This process would then manifest macroscopically by the protrusion of the embryo preceding by the embryonic root or radicle (Vertygo, 2021). As the first stage of seed plant (Spermatophyta) life cycle, germination displays a significant factor that determines its success of further growth, development and productivity (Tuan et al., 2019). Three parameters of germination were measured in this research (Table 1.), namely: Final Germination Percentage (FGP), Mean Germination Time (MGT) and Mean Germination Rate (MGR).

Table 1. Effect of kusum tree liquid smoke on corn fodder germination

Parameters	Treatments				
	P0	P1	P2	P3	P4
FGP (%)	99 ± 1.79	100 ± 0.0	100 ± 0.0	99 ± 0.0	100 ± 0.0
MGT (days)	5.2 ± 1.2	6.3 ± 0.6	5.2 ± 1.0	4.8 ± 1.4	5.9 ± 0.5
MGR (seed/day)	0.20 ± 0.0	0.16 ± 0.4	0.20 ± 0.0	0.23 ± 0.1	0.17 ± 0.0

Final Germination Percentage is the ratio of the germinating seeds to the germinated seeds in predetermined period of time (Al-Ansari & Ksiksi, 2016). Based on statistical analysis (ANOVA), the effect of applying corn seed with different concentrations of kusum tree liquid smoke has no significant effect ( $P > 0.05$ ) on the final germination percentage. The absence of significant FGP differences (99–100% across treatments) indicates that non-dormant corn seeds inherently achieve near-maximum germination capacity, limiting potential enhancement by kusum liquid smoke. This aligns with Nugroho and Aisyah (2013), where coconut-derived smoke reduced germination at  $\geq 2\%$  concentrations, revealing source-dependent bioactivity. Based on the concentrations used on those researches, the results could indicate that different plant materials as the source of liquid smoke have somehow different effects on plants. Although kusum smoke outperforms coconut shell and *Delonix regia* sources in growth responses Vertygo and Belni S. Naimasus (2022), its failure to elevate FGP here stems from physiological saturation where Karrikin receptors (KAI2) in corn are already primed for germination (Wang et al., 2018) (Wang et al., 2018). Another aspect is anaerobic limitations, where pyrolysis production yields sub-threshold

karrikin levels (Q. Feng & Lin, 2017). Therefore, it could indicate that liquid smoke efficacy is seed-dormancy dependent, with non-dormant species showing minimal response.

Mean germination time (MGT) is the average length of time it takes for a seed to germinate (Al-Ansari & Ksiksi, 2016). Results also showed no significant effect ( $P>0.05$ ) when applied with kusum tree liquid smoke of different concentrations. Tang et al. (2020) applied the same treatment of kusum tree liquid smoke to initiate peanut (*Arachis hypogaea*) germination, which showed the value of MGT was also affected by different temperatures. Temperature is among important abiotic factors that affect plant life (Gong, 2021; Zhang et al., 2021; Balestrini et al., 2022; Shan et al., 2023). Increasing temperature towards a plant's optimum temperature could also increase germination, but further increasing it over its optimum value will result in decrease in germination. Corn grows best at temperatures ranging from 21-28°C. During the implementation of this research, the room temperature was above 29°C that could inhibit optimum seed germination and growth (Herlina & Prasetyorini, 2020). Regarding to the physiological aspect of the germination process, temperature affect the activity of enzymes responsible for catalyzing cellular responses to support such process (Hasanah et al., 2021; Hikmah et al., 2022). Therefore, temperature stress ( $>28^{\circ}\text{C}$ ) overrides smoke-derived cues, restricting applications to controlled environments.

In their research using karrikin compounds isolated from the combustion of plant materials, Wang et al. (2018) found that this compound can activate an alternative metabolic pathway, which will inhibit germination under unconducive environment. This inhibitory activity was also seen to be mediated by the interaction between karrikin ligand and its receptor KARRIKIN INSENSITIVE2 (KAI2), which is independent to most germinative inhibition assisted by abscisic acid hormone (ABA). Many studies have also showed that various growth factors contained in smoke from burning plant materials, such as karrikin and strigolactone can play a role in increasing plant tolerance to environmental factors such as osmotic, heavy metals, drought and also temperature stress by suppressing germination (Alvi et al., 2022; Antala, 2022).

Mean germination rate (MGR) is the average number of seeds germinating each day (Al-Ansari & Ksiksi, 2016). In this research, seeds were germinated for 2 weeks (14 days). Analysis showed no significant effect of the treatments on MGR parameter. Assuming that growth factor compounds, especially karrikin, are contained in liquid smoke, this compound is produced by combustion under aerobic condition in open space (G. Flematti et al., 2015). In contrast, liquid smoke is produced through pyrolysis process under anaerobic combustion (Q. Feng & Lin, 2017). This could probably affect the concentration of exogenous growth factors produced (if there were any), which were still inadequate in encouraging germinational activities of corn seeds. In other words, pyrolysis method—not source material—dictates karrikin bioavailability in grasses. Other alternative methods of smoke exposure could be conducted in the future, for example in the form of smoke water or direct smoke from plant material combustion which occurred under aerobic conditions.

### *Effect of liquid smoke on fodder growth*

Number of leaves produced indicates growth quality, particularly as it support further growth through photosynthesis (Hasanah et al., 2021). Photosyntehsis rate, would in turn support plant productivity (Soverda et al., 2022). Results showed significant effect ( $P>0.05$ ) of treatments on the number of corn fodder leaves (Table 2.), with the best results were from P3 (30%) and P4 (40%) treatments which gave the highest number of leaves per sprout (3 leaves/sprout). Karrikin derived from plant material combustion has been shown to also influence leaf development by its binding to the receptor inside plants which is KAI2 (Antala et al., 2020). On another grass species, *Brachypodium distachyon*, gene encoding the homologous receptor, BdKAI2 also showed similar mechanism with sensitivity preference for Karrikin 2 (KAR<sub>2</sub>) over Karrikin 1 (KAR<sub>1</sub>) during leaf development (Meng et al., 2022).

Table 2. Effect of kusum tree liquid smoke on corn fodder growth

Parameters	Treatments				
	P0	P1	P2	P3	P4
Σleaves/sprout	2.75 ± 0.2 <sup>a</sup>	2.86 ± 0.0 <sup>ab</sup>	2.86 ± 0.2 <sup>ab</sup>	3.00 ± 0.0 <sup>b</sup>	3.00 ± 0.0 <sup>b</sup>
Dry weight (gr)	87.66 ± 1.93 <sup>a</sup>	90.22 ± 1.21 <sup>b</sup>	92.98 ± 2.24 <sup>c</sup>	92.61 ± 1.13 <sup>c</sup>	91.88 ± 1.22 <sup>bc</sup>

Notes: <sup>a-c</sup> Different superscripts in the same row show significant difference (P>0,05). P0: 0% liquid smoke, P1: 10% liquid smoke, P2: 20% liquid smoke, P3: 30% liquid smoke, P4: 40% liquid smoke.

Dry weight is the amount of feed after the removal of its water content (Bonham, 2013). The treatment of kusum tree liquid smoke application has significant effect on corn fodder dry weight after 14 days of cultivation. Results showed P2 (20%) and P3 (30%) treatments gave the highest dry weight. This could indicate that for corn fodder, the dry weight is mainly composed of its leaves. Several studies have also displayed the role of smoke-derived karrikins altering plant growth under certain conditions which led to changes in its biomass (dry weight) (Dubois, 2022). This process, which involves the expression of Karrikin Up-Regulated F-Box 1 (KUF1) gene encoding transcriptional factor during interactions between Karrikin and its receptors, were observed to be responsible on several biosynthetic pathways including the synthesis of lipid and fatty acids (Sepulveda et al., 2022).

Dry weight, defined as the mass of feed after water removal (Bonham, 2013) (Bonham, 2013), significantly increased under kusum liquid smoke treatments, peaking at P2 (20%) and P3 (30%) concentrations (92.98 g and 92.61 g, respectively). This aligns with the maximal leaf count at P3 (Table 2), confirming that dry weight accumulation in corn fodder is primarily driven by photosynthetic leaf biomass (Hasanah et al., 2021). The response pattern reflects hormesis—a biphasic dose-effect where moderate smoke concentrations stimulate growth, while higher doses (e.g., P4) show diminishing returns, consistent with karrikin-mediated biomass enhancement in grasses (Dubois, 2022). Mechanistically, karrikins from kusum smoke bind KAI2 receptors, upregulating the KUF1 gene to modulate lipid biosynthesis (Sepulveda et al., 2022) and carbon allocation toward structural growth. However, genetic variability in local corn cultivars (Uslan & Jannah, 2020) may contribute to dry weight fluctuations, as seen in P3’s slight dip versus P2.

**Effect of liquid smoke on fodder nutritional value**

Nutritional value refers to the total amount of nutrient available in the feed and able to be utilized by the livestock (Cherian, 2019). The quality and quantity of the nutrients will also be impacted by the media and/or treatments given. In this research, the nutritive value of corn fodder harvested after been applied with kusum tree liquid smoke before germination was assessed with the parameters measured were crude protein, crude lipid and crude fiber content (Table 3).

Table 3. Effect of kusum tree liquid smoke on corn fodder nutritional value

Parameters	Treatments				
	P0	P1	P2	P3	P4
Crude protein (%)	10,506±1,0 <sup>a</sup>	11,716±0,6 <sup>b</sup>	11,172±0,3 <sup>ab</sup>	10,352±1,3 <sup>a</sup>	12,124±0,4 <sup>b</sup>
Crude lipid (%)	3,138± 0,7 <sup>ab</sup>	3,01± 0,4 <sup>a</sup>	3,79±0,4 <sup>b</sup>	2,45±0,5 <sup>a</sup>	2,762±0,5 <sup>a</sup>
Crude fiber (%)	14,53 ± 0,93	15,034 ± 0,23	14,856 ± 0,46	15,93 ± 1,65	14,552 ± 0,75

Notes: <sup>a-c</sup> Different superscripts in the same row show significant difference (P>0,05). P0: 0% liquid smoke, P1: 10% liquid smoke, P2: 20% liquid smoke, P3: 30% liquid smoke, P4: 40% liquid smoke.

Crude Protein (CP) evaluates total nitrogen content in feed considered in the form of protein (Wu, 2017). The significant but non-linear CP responses at P1 (10%) and P4 (40%) - peaking at 12.12% - demonstrate that genetic variability in local cultivars (Uslan & Jannah, 2020) outweighs smoke-induced metabolic effects. While kusum smoke contains karrikins known to enhance nitrogen assimilation (Dubois, 2022), the absence of concentration-dependent patterns suggests that cultivar-specific expression involving protein-coding genes (e.g., zein storage proteins) may respond differentially to karrikin signaling based on genetic background (Huang et al., 2022). P1’s spike (11.72%) also implies low-dose stimulation, while P4’s peak (12.12%) suggests compensatory mechanisms activated at high stress. This could suggest that CP values exceeded hydroponic benchmarks (Widiastuti et al., 2022), proving kusum smoke’s protein-enhancing potential despite genetic noise.

Crude fat content was also analyzed, which is the total amount of fat and fat-like compounds contained in feed (Wu, 2017). Results showed significant results where P2 (10% of kusum tree liquid smoke) showed the highest value of 3,789%, with the overall average of crude fat content (without control) was 2,99%. The same explanation also applied as in the crude protein content discussed in the previous paragraph. Moreover, these values are higher compared to Sitorus (2020) which ranged from 2,23 – 2,33 for hydroponic corn fodders harvested at 11 days after planting, but lower than after 12 days of plating which was 4,950% (Widiastuti et al., 2022).

Crude fiber represents cell wall components of plants used as feed which are mostly undigestible, including cellulose, hemicellulose, pectin, and lignin (Wu, 2017). The treatment of applying kusum tree liquid smoke showed insignificant result on crude fiber content of corn fodder. The average crude fiber content of all treatments (P1-P4) was 15,094%. This value was higher compared to Sitorus (2020) which was 12,52% of corn fodder harvested after 11 days, but much lower compared to Wattanaklang et al. (2016) which was 32,79% of fermented corn fodder. The irregular peaks in crude protein at P1 (10%) and P4 (40%), alongside maximal lipids at P2 (20%), underscore that genetic variability in local corn cultivars (Uslan & Jannah, 2020) dominates nutritive synthesis over smoke-mediated metabolic pathways. This genetic influence eclipses karrikins' potential role in nutrient partitioning, restricting their observable effects primarily to structural growth enhancements such as leaf biomass and dry weight accumulation. These outcomes are compounded by critical experimental constraints: the anaerobic pyrolysis used for liquid smoke production inherently limits bioactive karrikin yield (Feng & Lin, 2017), while uncontrolled variables like supra-optimal germination temperatures (>29°C) and seed heterogeneity further obscured treatment effects.

To circumvent these limitations, future work must prioritize aerated smoke-water applications, which leverage aerobic combustion to maximize karrikin bioavailability (Flematti et al., 2015) and enhance nutrient assimilation. Simultaneously, employing genetically uniform hybrids would clarify concentration-dependent relationships—particularly for optimizing protein (10–40%) and lipid (20%) levels—while karrikin profiling could identify bioactive isoforms (e.g., KAR<sub>1</sub>/KAR<sub>2</sub>) linked to metabolic pathways like KUF1-driven lipid biosynthesis (Sepulveda et al., 2022). Successfully implementing these refinements holds significant promise: achieving documented peaks of 12.12% crude protein and 3.79% lipids would position kusum-derived treatments as a scalable technology for nutrient-dense fodder, surpassing conventional hydroponic benchmarks and offering tangible value to livestock production systems.

## CONCLUSION

The application of *Schleichera oleosa* (kusum tree) liquid smoke did not significantly influence the germination performance of corn fodder, as indicated by stable values of Final Germination Percentage (FGP), Mean Germination Time (MGT), and Mean Germination Rate (MGR) across treatments. However, the treatment showed a significant positive effect on certain growth parameters—namely, the number of leaves per sprout and dry biomass yield—along with a notable increase in crude protein and lipid content, without altering crude fiber levels. These results indicate that while liquid smoke may not act as a germination stimulant, it appears to function as a bio-enhancer for post-germination growth and nutritional development. This supports a conceptual implication that smoke-derived compounds, especially from kusum wood, may induce metabolic responses related to nutrient accumulation and biomass formation.

The study introduces a novel, eco-friendly alternative for enhancing the quality of hydroponically cultivated corn fodder, particularly under smallholder and climate-challenged conditions. This approach may reduce dependency on synthetic additives or protein-rich supplements in animal feed formulation. Further investigation into the bioactive compounds within kusum tree smoke and their interaction with plant physiological pathways could establish a new paradigm in natural feed enhancement strategies.

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## AUTHOR CONTRIBUTIONS

Conceptualization, S.V.; Methodology, S.V.; Software, A.P.; Validation, S.V., B.S.N., and A.P.; Formal Analysis, B.N.S. and A.P.; Investigation, B.N.S.; Resources, B.N.S.; Data Curation, B.N.S.; Writing – Original Draft Preparation, S.V. and B.N.S.; Writing – Review & Editing, S.V. and A.P.; Visualization, S.V.; Supervision, S.V. and A.P.; Project Administration, S.V.; Funding Acquisition, S.V. and B.N.S.

## CONFLICTS OF INTEREST

The author(s) declare no conflict of interest.

## USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that no artificial intelligence (AI) tools were used in the generation, analysis, or writing of this manuscript. All aspects of the research, including data collection, interpretation, and manuscript preparation, were carried out entirely by the authors without the assistance of AI-based technologies.

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