

RESEARCH ARTICLE

Smoked Water Enhances the Germination and Seedling Vigour of Forage Legumes

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ABSTRACT

The experiments were conducted at the Department of Processing and Food Engineering Workshop and the Department of Basic Engineering and Applied Sciences at the Agricultural Engineering College and Research Institute in Kumulur. Dried leaves and twigs of neem were burnt under controlled combustion for 5 minutes in the liquid smoke generator unit. The liquid smoke from the combustion of neem leaves and twigs was diluted to different concentrations and used as a pre-sowing treatment for *Stylosanthes* and *Desmanthus* seeds. The treated seeds, along with the controls, were placed for germination tests. The experiments were carried out using four replications of 60 seeds each. Ten days after sowing in *Stylosanthes* and nine days after sowing in *Desmanthus*, germination percentage, root length (cm), shoot length (cm), and vigor index were recorded. The results of the smoke water treatment revealed that *Stylosanthes* seeds soaked in a smoke solution (1:750 dilution) for 2 hours recorded the highest germination, seedling growth, and computed vigor index when compared to the control treatment. On the other hand, *Desmanthus* seeds soaked in hot water at 80 °C for 5 minutes or seeds treated with concentrated H₂SO₄ for 5 minutes followed by soaking in a smoke solution with water at a 1:500 dilution for 2 hours exhibited the highest seed germination and seedling vigor.

Received: 18 Jan 2026

Revised: 10 Mar 2026

Accepted: 13 Apr 2026

Keywords: *Stylosanthes*, *Desmanthus*, Seed dormancy, Smoked water, Germination, Seedling vigour

INTRODUCTION

Forage legumes such as *Stylosanthes* (*Stylosanthes hamata*) and *Desmanthus* (*Desmanthus virgatus* (L) Willd) are important fast-growing crops with multiple uses. They are widely adapted and productive tropical pasture legumes that are commercially used in several agricultural systems across many countries with tropical or subtropical climates (Kavita *et al.*, 2025; Kavita *et al.*, 2015). They can also be easily processed as leaf meal, feed blocks, and feed pellets, and stored for use during lean periods. Additionally, their high

nutritional value and digestibility make them important fodder choices under rainfed and dry land conditions. In India, they are the most important tropical legumes for semi-arid and arid regions, where they are mainly used for livestock production and to restore soil fertility (Cook *et al.*, 2005). Both legumes can be propagated through seeds or vegetative cuttings, with seed germination affected by seed dormancy. The seed coat exerts its germination-restrictive action most of the time by being impermeable to water and/

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or oxygen or by its mechanical resistance to radicle protrusion (Baskin *et al.*, 2020). In legumes, a densely packed layer of palisade cells impregnated with water-repellent compounds (Castillo and Guenni, 2001) causes mechanical resistance of the seed coat. The seed becomes permeable to water only when the coat is disrupted in some way, particularly at the lens (strophiole) region, which is usually the physically weakest part of the seed coat (Salazar *et al.*, 2020).

Smoke has been shown to increase seed germination in more than 1200 species from 80 genera. The main active germination compound in smoke-water derived from burned plant materials and cellulose has been identified as butenolide. Specific water-soluble compounds found in the smoke from burning plant materials (leaves, straws) play an important role in breaking seed dormancy (Iqbal *et al.*, 2016). De Lange and Boucher (1990) revealed that smoke derived from burnt plant material stimulated germination of the South African species *Audouinia capitata*, indicating that the stimulatory molecules were volatile in nature and could be accumulated by bubbling smoke through water to produce “smoke-water”. The identification of the butenolide 3-methyl-2H-furo [2, 3-c] pyran-2-one (KAR1) as the key germination stimulant present in plant-derived smoke is a milestone accomplishment in seed biology (Flematti *et al.*, 2004), which is effective at very low concentrations. This compound has been referred to as Karrikinolide (Commander *et al.*, 2008). The action of smoke in promoting seed germination in many species is attributed to the presence of this compound (Soos *et al.*, 2009). Dixon and Roche (1995) reported that the promotive effect of smoke is independent of seed size, seed shape, seed type, phylogenetic position, geography, and plant life form, whether annual, perennial, herbaceous, fire-sensitive seeder, or fire-tolerant resprouter. Smoke from a wide variety of biotic sources, including wood, straw, mixtures of dry and fresh plant material, and charred wood, can stimulate germination (Brown and Van Staden, 1997). Therefore, the objective of this study was to evaluate the efficacy of smoke-water prepared from dry neem leaves on *Stylosanthes* and *Desmanthus* seed germination and seedling vigor.

MATERIAL AND METHODS

The experiments were conducted at the Department of Processing and Food Engineering Workshop and the Department of Basic Engineering and Applied Sciences

at the Agricultural Engineering College and Research Institute, Kumulur, Tiruchirappalli, Tamil Nadu.

Seed Source

Truthfully labeled seeds of *Stylosanthes* and *Desmanthus* (Var. CO2) were received from the Department of Forage Crops, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, and stored at ambient temperature (28–30 °C).

Smoke-Water Preparation

Neem leaves (*Azadirachta indica* A Juss) were collected from an avenue plantation at the Agricultural Engineering College and Research Institute, Kumulur, Tiruchirappalli, Tamil Nadu, and stored in a 25-litre plastic barrel. The liquid smoke generator unit consists of a bee smoker with a bellows, a smoke collection hose, a side-arm flask, an aspirator, a power unit, and a control valve. The smoke from 50 grams of dried leaves is produced by a controlled combustion process using the bee smoker with a bellows unit for 15 minutes. The smoke is collected through the hose and dissolved in distilled water, which is kept in the side arm flask (500 ml capacity). The rate of smoke flow is controlled by an aspirator through a control valve. A 12 Volts DC motor powers the aspirator. The concentration of liquid smoke is controlled by regulating the flow of smoke into the side-arm flask (Plate 1). This smoke extract was filtered through Whatman No. 1 filter paper and used as the stock solution.

Germination Experiments

a. *Stylosanthes*

Stylosanthes seeds were soaked in 7 different concentrations of smoke-water, namely: T₁ - Dry seed (control), T₂ - Water soaking for 2 hours, T₃ - 100% smoke solution soaking for 2 hours, T₄ to T₇ - Soaking smoke solution + water 1:1000, 1:750, 1:500, and 1:250 dilutions for 2 hours.

b. *Desmanthus*

i. Experiment 1

Desmanthus (Var. CO2) seeds were soaked in 7 different concentrations of neem leaves derived smoke-water, namely: T₁ - Dry seed (control), T₂ - Seeds treated in hot water at 80 °C for 5 minutes followed by soaked in cold water for 12 hours, T₃ - Seeds treated in hot water at 80 °C for 5 minutes followed by 100% smoke solution soaking for 12 hours, T₄ to T₇ - Seeds

treated in hot water at 80 °C for 5 minutes followed by soaking smoke solution + water 1:1000, 1:750, 1:500, and 1:250 dilutions for 12 hours.

ii. Experiment 2

Desmanthus (Var. C02) seeds were soaked in 8 different concentrations of neem leaves derived smoke-water, namely: Dry seed (control), T₁, Seeds treated with conc. H₂SO₄ for 5 minutes (T₂), T₂ + Seeds soaked with water 2 hrs. (T₃), T₂ + 100% smoke solution soaking for 2 hrs. (T₄), T₂ + Soaking smoke solution + water 1:1000, 1:750, 1:500, and 1:250 dilutions soaking for 2 hrs. (T₅ to T₈)

The treated seeds of stylosanthes and desmanthus were placed for germination using the roll towel method, with 60 seeds per replication. The experiment was laid out in a completely randomized block design. Ten days after sowing in Stylosanthes and nine days after sowing in Desmanthus, percent germination was computed (ISTA, 1999). Ten seedlings from each replication were randomly taken, and root and shoot lengths (cm) were measured. The vigor index was derived from the following formula (Abdul Baki and Anderson, 1973):

$$\text{Vigor Index} = \text{Germination (\%)} \times \text{Total seedling length (cm)}$$

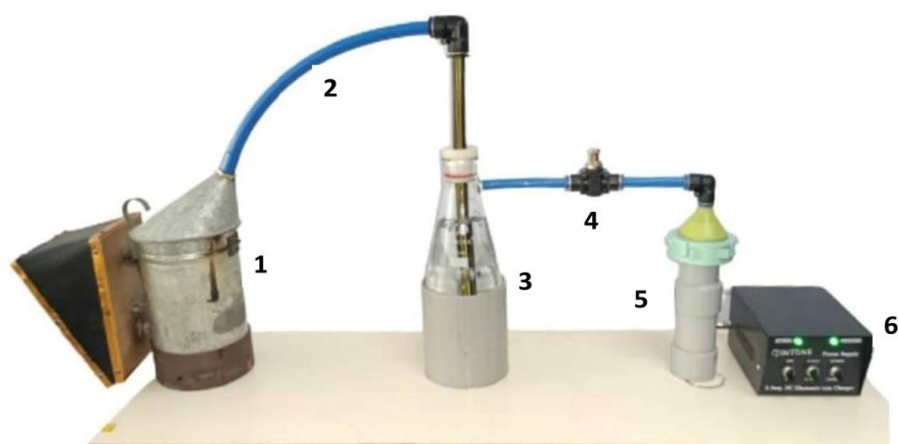
Statistical Analysis

The data were examined using the F-test of significance, as described by Panse and Sukhatme

(1999). To analysis, percentage data were transformed to angular (arc-sine) values when necessary. The critical differences (CD) were calculated at the 5% probability level.

RESULTS AND DISCUSSION

The results of neem leaf-derived smoke water soaking on the germination and seedling vigor of Stylosanthes revealed that smoke water successfully enhanced germination. The seed germination percentage was highest (60%) in seeds soaked in a smoke solution with a 1:750 water dilution for 2 hours, followed by seeds soaked in a smoke solution with a 1:500 water dilution, which recorded 45% germination. The lowest germination rate (17%) was in seeds soaked in a 100% smoke solution for 2 hours. Compared to the control, the germination enhancement was 21% in seeds soaked in a smoke solution with a 1:750 water dilution for 2 hours. However, higher concentrations of smoke water treatments did not show a significant increase in germination (Table 1; Fig.1). The results for the effect of neem leaf-derived smoke water soaking on the germination and seedling vigor of desmanthus seeds, soaked in hot water followed by smoke water soaking, showed that the maximum seed germination percentage (55.75%) was in seeds soaked in hot water at 80 °C for 5 minutes, followed by soaking in a smoke solution with a 1:500 water dilution for 12 hours. Seeds soaked in hot water at 80 °C for 5 minutes followed by soaking in a smoke solution with a 1:250 water dilution for 12 hours recorded 49.75%



1. Bee smoker with bellows 2. Smoke collection hose
3. Side-arm flask 4. Control valve 5. Aspirator (DC motor-12volts)
6. Power unit

Plate -1 Liquid Smoke Generator



Table 1. Effect of Neem leaves - derived smoke water on germination and seedling vigor of Stylosanthes

Treatments	Germination (%)	Root length (cm)	Shoot length (cm)	Vigour index
T ₁ - Dry seed (control)	39 (38.64)	1.50	4.27	225
T ₂ - Water soaking for 2 hours	25 (30.00)	1.49	4.40	147
T ₃ - 100% smoke solution soaking for 2 hours	17 (24.35)	1.91	4.44	108
T ₄ - Soaking smoke solution + water (1:1000 dilutions) for 2 hours	39 (38.64)	0.86	3.54	127
T ₅ - Soaking smoke solution + water (1:750 dilutions) for 2 hours	60 (42.13)	1.86	4.07	355
T ₆ - Soaking smoke solution + water (1:500 dilutions) for 2 hours	45 (39.23)	2.31	3.98	283
T ₇ - Soaking smoke solution + water (1:250 dilutions) for 2 hours.	40 (38.46)	1.51	3.86	220
Mean	21.85 (38.65)	1.63	4.08	210
S.Ed	0.87	0.14	0.29	13.38
CD (P = 0.05)	1.80	0.29	0.60	27.83

(Figures in parentheses indicate arcsine values)

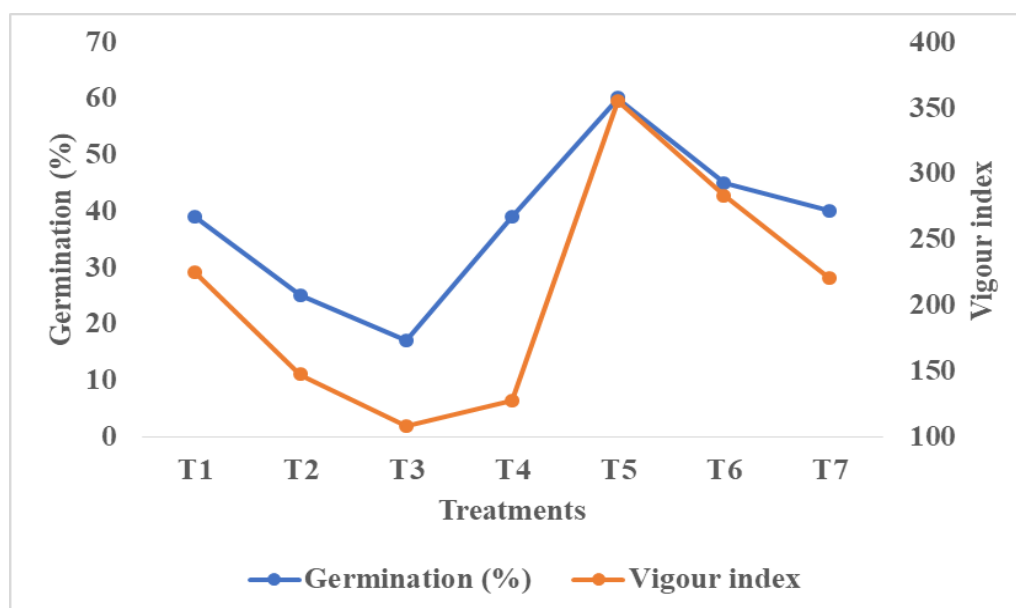


Fig. 1. Effect of Neem leaves - derived smoke water on germination and seedling vigour of Stylosanthes

Treatments

T₁ - Dry seed (control)

T₂ - Water soaking for 2 hours

T₃ - 100% smoke solution soaking for 2 hours

T₄ - Soaking smoke solution + water (1:1000 dilutions) for 2 hours

T₅ - Soaking smoke solution + water (1:750 dilutions) for 2 hours

T₆ - Soaking smoke solution + water (1:500 dilutions) for 2 hours

T₇- Soaking smoke solution + water (1:250 dilutions) for 2 hours.



germination, while the control treatment (dry seed) did not show a significant increase in germination had a minimum germination of 8.75%. However, (Table 2; Fig. 2).
higher concentrations of smoke-water treatments

Table 2. Effect of Neem leaves - derived smoke water on germination and seedling vigour of Desmanthus

Treatments	Germination (%)	Root length (cm)	Shoot length (cm)	DMP (g/10 seedling)	Vigour index
T ₁ - Dry seed (control)	8.75 (17.21)	2.70	4.83	0.015	68
T ₂ - Seeds treated in hot water at 80° C for 5 minutes followed by soaked in cold water for 12 hours	37.92 (38.01)	3.73	5.24	0.015	353
T ₃ - Seeds treated in hot water at 80° C for 5 minutes followed by 100% smoke solution soaking for 12 hours	28.75 (32.42)	3.33	4.67	0.016	231
T ₄ - Seeds treated in hot water at 80° C for 5 minutes followed by soaking smoke solution + water (1:1000 dilutions) for 12 hours	46.67 (43.10)	3.01	4.81	0.016	380
T ₅ - Seeds treated in hot water at 80° C for 5 minutes followed by soaking smoke solution + water (1:750 dilutions) for 12 hours	47.91 (43.80)	3.58	5.25	0.014	387
T ₆ - Seeds treated in hot water at 80° C for 5 minutes followed by soaking smoke solution + water (1:500 dilutions) for 12 hours	55.75 (48.30)	3.91	5.26	0.018	508
T ₇ - Seeds treated in hot water at 80° C for 5 minutes followed by soaking smoke solution + water (1:250 dilutions) for 12 hours.	49.75 (44.86)	3.52	4.94	0.015	421
Mean	39.36 (38.86)	3.40	4.99	0.015	335
S.Ed	3.61	0.52	0.29	0.0018	47.39
CD (P = 0.05)	7.50	NS	NS	NS	98.56

(Figures in parentheses indicate arcsine values)

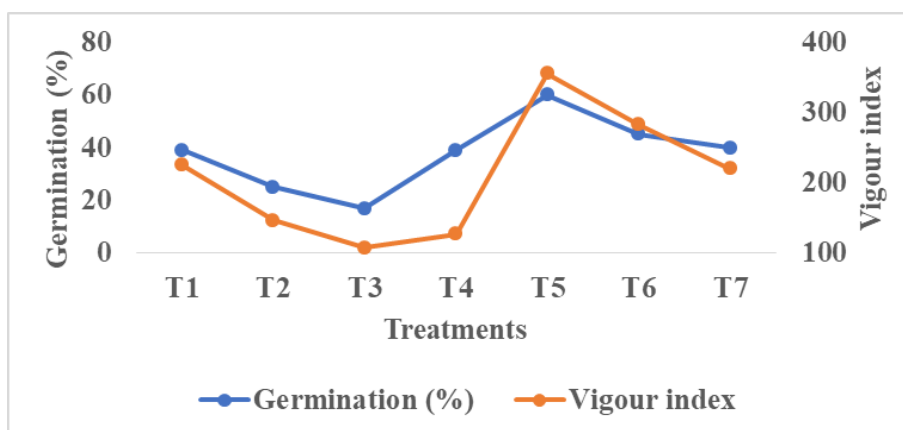


Fig. 2. Effect of Neem leaves - derived smoke water on germination and seedling vigour of Desmanthus



Treatments

T₁ - Dry seed (control)

T₂ - Seeds treated in hot water at 80 °C for 5 minutes followed by soaked in cold water for 12 hours

T₃ - Seeds treated in hot water at 80 °C for 5 minutes followed by 100% smoke solution soaking for 12 hours

T₄ - Seeds treated in hot water at 80 °C for 5 minutes followed by soaking smoke solution + water (1:1000 dilutions) for 12 hours

T₅ - Seeds treated in hot water at 80 °C for 5 minutes followed by soaking smoke solution + water (1:750 dilutions) for 12 hours

T₆ - Seeds treated in hot water at 80 °C for 5 minutes followed by soaking smoke solution + water (1:500 dilutions) for 12 hours

T₇ - Seeds treated in hot water at 80 °C for 5 minutes followed by soaking smoke solution + water (1:250 dilutions) for 12 hours.

The results for Desmanthus seeds soaked in different concentrations of H₂SO₄, followed by smoke water soaking, showed that neem leaf-derived smoke water soaking successfully enhanced germination and seedling vigor. Among the treatments, seeds treated with concentrated H₂SO₄ for 5 minutes, followed by soaking in a smoke solution at a 1:500 water dilution for 2 hours, recorded the highest seed germination (56.25%). Seeds treated with concentrated H₂SO₄ for 5 minutes, followed by soaking in a smoke solution at a 1:250 water dilution for 2 hours, recorded 48.34% germination, while the control treatment had a minimum germination rate of 9.17%. The seedling vigor values followed the same trend as the germination percentage (Table 3; Fig.3).

Neem leaf-derived smoke-water soaking of Stylosanthes seeds significantly increased seedling growth. Among the treatments, the longest root length (2.31 cm) was recorded in seeds soaked in a 1:500 water dilution of smoke for 2 hours, followed by seeds soaked in a 100% smoke solution for 2 hours (1.91 cm). The shortest root length was observed in seeds soaked in a smoke solution with a 1:1000 water dilution (0.86 cm). Regarding shoot length, seeds soaked in a 100% smoke solution for 2 hours had the longest shoot length (4.44 cm), followed by seeds soaked in water for 2 hours (4.40 cm). The shortest shoot length was recorded in the control treatment (4.27 cm). Seedling vigor index values reflected the same trend as the germination percentage. Seeds

Table 3. Effect of Neem leaves derived smoke water on germination and seedling vigour of desmanthus

Treatments	Germination (%)	Root length (cm)	Shoot length (cm)	Vigour index
T ₁ - Dry seed (control)	9.17 (17.63)	3.26	4.25	69
T ₂ - Seeds treated with conc. H ₂ SO ₄ for 5 minutes	36.25 (37.02)	3.11	4.78	286
T ₃ - T ₂ + Seeds soaked with water 2 hrs.	34.58 (36.02)	3.37	4.54	267
T ₄ - T ₂ + 100% smoke solution soaking for 2 hrs.	31.67 (34.25)	2.78	5.86	243
T ₅ - T ₂ + Soaking smoke solution + water (1:1000 dilutions) soaking for 2 hrs.	35.76 (36.73)	2.68	4.42	254
T ₆ - T ₂ + Soaking smoke solution + water (1:750 dilutions) soaking for 2 hrs.	41.25 (39.96)	2.73	5.21	328
T ₇ - T ₂ + Soaking smoke solution + water (1:500 dilutions) soaking for 2 hrs.	56.25 (48.59)	1.97	4.86	385
T ₈ - T ₂ + Soaking smoke solution + water (1:250 dilutions) soaking for 2 hrs.	48.34 (44.05)	2.86	4.43	352
Mean	36.66	2.84	4.79	273
S.Ed	2.51	0.24	0.45	12.19
CD (P = 0.05)	5.18	0.50	0.92	25.15

(Figures in parenthesis indicate arcsine values)

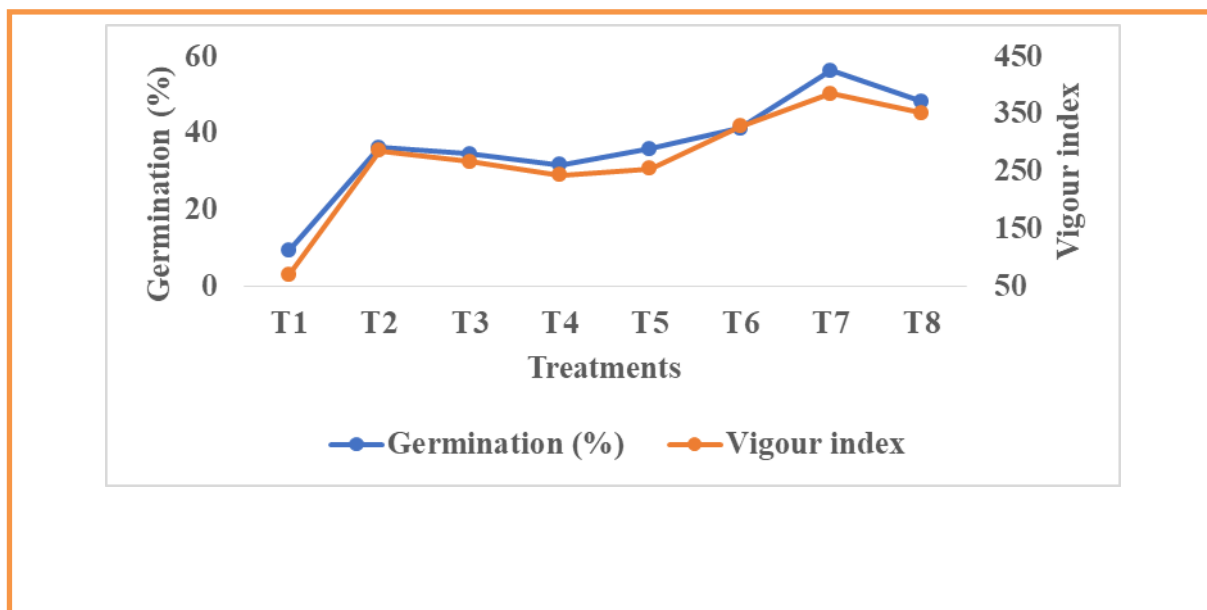


Fig. 3. Effect of Neem leaves - derived smoke water on germination and seedling vigour of Desmanthus

Treatments

- T₁ - Dry seed (control)
- T₂ - Seeds treated with conc. H₂SO₄ for 5 minutes
- T₃ - T₂ + Seeds soaked with water 2 hrs.
- T₄ - T₂ + 100% smoke solution soaking for 2 hrs.
- T₅ - T₂ + Soaking smoke solution + water (1:1000 dilutions) soaking for 2 hrs.
- T₆ - T₂ + Soaking smoke solution + water (1:750 dilutions) soaking for 2 hrs.
- T₇ - T₂ + Soaking smoke solution + water (1:500 dilutions) soaking for 2 hrs.
- T₈ - T₂ + Soaking smoke solution + water (1:250 dilutions) soaking for 2 hrs.

soaked in a smoke solution at a 1:750 water dilution for 2 hours had the highest vigor index (355), followed by those soaked in a smoke solution with a 1:500 water dilution for 2 hours (283). The lowest vigor index was observed when the seeds were soaked in a smoke solution at a 1:1000 water dilution for 2 hours. In *Desmanthus*, seedling growth, dry matter production, and vigor index values followed the same trend as the germination percentage when the seeds were soaked in hot water or different concentrations of H₂SO₄, followed by soaking in smoke water with different concentrations of neem leaf-derived smoke water.

The current study clearly shows that plant-derived smoke water treatments, particularly smoke water obtained from neem leaves, effectively improved seed germination and seedling vigor of *Stylosanthes* and *Desmanthus*. Consistent with these results, previous studies have shown that more than 4,000 compounds have been identified in smoke. Furthermore, different

compounds or combinations of two or more compounds may be responsible for smoke-stimulated germination (Flematti *et al.*, 2009). Smoke interacts chemically with inhibitors in the seed coat, endosperm, or embryo to enhance seed germination, and this stimulation of germination is due to smoke-specific signal molecules, possibly promotive hormones (Baldwin *et al.*, 1994). Nitric oxide (NO) and related nitrogen oxides have been reported to stimulate seed germination (Brown and Van Staden, 1997; Govindaraj *et al.*, 2016). The biologically active compound 3-methyl-2H-furo[2,3-c]pyran-2-one, known as butenolide, was isolated from burnt cellulose (Flematti *et al.*, 2004) and from plant-derived smoke (Van Staden *et al.*, 2004). Although this compound is now recognized as a germination cue for many naturally occurring species, whether or not exposed to smoke, it also shows strong potential for agricultural and horticultural crops. The effects of various concentrations



of aqueous smoke solutions resemble hormonal responses and closely match those of gibberellic acids rather than potassium nitrate (Adkins and Peters, 2001). Studies on *Nicotiana attenuata* seeds (Schwachtje and Baldwin, 2004) demonstrated that smoke alters endogenous GA and ABA patterns during germination. Smoke can substitute for red light (640 nm) in the germination of *Lactuca sativa*, promoting germination in darkness or in far-red (730 nm) light (Van Staden *et al.*, 1995).

The current results closely align with the findings of Light *et al.* (2009). They reported that smoke from burning plant material stimulates seed germination in numerous species worldwide. Smoke-water may act on the seed coat in a manner similar to scarification, allowing the dormant embryo to more efficiently absorb water and oxygen (Egerton-Warburton, 1998). An important component of biomass smoke is ethylene, a chemical cue known to stimulate seed germination. Ethylene promotes radial cell expansion, increases seed respiration, and aids in endosperm rupture (Kucera *et al.*, 2005). The present study confirms previous observations that smoke water or smoke-derived compounds can improve germination and promote the growth of agricultural and horticultural crops such as red rice (Doherty and Cohn, 2000), indigenous maize (Modi, 2004), indigenous rice (Kulkarni *et al.*, 2006), commercial bean (Van Staden *et al.*, 2006), okra and tomato (Kulkarni *et al.*, 2007), onion (Kulkarni *et al.*, 2010), and papaya (Chumpookam *et al.*, 2012). Pillai *et al.*, (2024) found that Karrikinolide (KAR1) can stimulate root growth, a trend also observed in tomato (Taylor and Van Staden, 1998) and rice (Sparg *et al.*, 2005).

CONCLUSION

This study concludes that smoke-water treatments affect the permeability of the seed coat, thereby impacting germination. Among the treatments tested, *Stylosanthes* seeds soaked in a neem leaf smoke-water solution at a 1:750 dilution for 2 hours showed the highest germination percentage (60%), seedling growth, and computed vigor index, compared to the control treatment (39% germination). In contrast, *Desmanthus* seeds soaked in hot water at 80°C for 5 minutes, or seeds treated with concentrated H₂SO₄ for 5 minutes followed by soaking in a smoke solution with water at a 1:500 dilution for 2 hours, exhibited the highest seed germination and seedling vigor.

ACKNOWLEDGMENTS

Conflict of Interests

Funding and Acknowledgment:

The authors would like to express their gratitude to Tamil Nadu Agricultural University (TNAU) for providing the necessary financial support and facilities during the research process. Their assistance was instrumental in the successful completion of this study.

Ethics Statement:

This research involved studies on plants/inanimate objects; therefore, no ethical approval was required.

Originality and Plagiarism:

We confirm that this manuscript is an original work. All sources used have been properly cited, and no part of this work has been previously published.

Consent for Publication:

All authors have reviewed the final version of the manuscript and have given their explicit consent for its publication in this journal.

Competing Interests:

The authors declare that there are no potential conflicts of interest, financial or otherwise, that could affect the research or its interpretation.

Data Availability:

All data supporting the conclusions of this manuscript are included within the article. No additional datasets were generated or required for this study.

Author Contributions:

This work was carried out in collaboration among all authors. Masilamani & Rajkumar did conceptualization of the study, methodology development, and primary writing of the article. Sivakumar & Jeya Chandra conducted the experiments, drafted the initial manuscript, and prepared the writing in the required draft format.

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