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Conservation and sustainable development of *Stephania brachyandra* Diels: Cultivation, management and preliminary processing techniques

Thao Van Duong[1], Quang Van Nguyen[2]*

[1]Department of Research and International Affairs, Thai Nguyen University of Agriculture and Forestry, Thai Nguyen City, Vietnam.
[2]Center for Advanced Technology Development, Thai Nguyen University, Thai Nguyen City, Vietnam.

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ABSTRACT

Stephania brachyandra Diels is a valuable medicinal plant containing rotundin, an alkaloid known for its sedative and analgesic properties. However, overexploitation has posed significant threats to its natural populations. This study aims to develop sustainable techniques for cultivation, care, and post-harvest preservation. Four experiments were conducted to evaluate the effects of planting density, fertilization, pest management, and storage methods on tuber growth and yield. Results showed that low planting density (28,000–42,000 plants/ha) significantly improved tuber development in the long term, while medium density was advantageous during early growth stages. The application of nitrogen (N), phosphorus (P), and potassium (K) fertilizers, especially when combined with organic amendments, enhanced both tuber size and soil fertility. Caterpillars were the only pests observed, and their impact on overall yield was minimal. Moreover, storing tubers in sand or mesh bags at controlled temperature and humidity reduced post-harvest weight loss and extended storage life. This study offers a scientific basis for the conservation and sustainable development of *S. brachyandra* Diels, contributing to the long-term availability of this important medicinal resource.

1. INTRODUCTION

Vietnam's natural conditions have nurtured an exceptionally diverse ecosystem, which includes numerous valuable medicinal plants with significant potential for pharmaceutical applications. One such plant is *Stephania brachyandra* Diels. The tuber of this species contains over 30 identified alkaloids, including cepharanthine, glaucine, menispermine, rotundin, and stepharine [1,2]. Due to these bioactive compounds, *S. brachyandra* Diels has been widely used in traditional Eastern medicine to treat various conditions related to the nervous, digestive, and respiratory systems [3]. Among these alkaloids, rotundin stands out for its pronounced effects on the nervous system. It interacts with both opioid and dopamine receptors, offering therapeutic benefits, such as pain relief, anxiety reduction, and stress mitigation [4,5]. Notably, experimental studies have shown that rotundin derived from *S. brachyandra* Diels causes minimal side effects, making it a safer alternative to many synthetic drugs [6]. These findings underscore the significant medicinal value of this plant in enhancing public health and well-being. *S. brachyandra* Diels belongs to the family Menispermaceae. It is a perennial climbing plant with cymose inflorescences, producing drupe-like fruits each containing one seed. The species is primarily propagated through tuber division, which is preferred over seed propagation due to low germination rates and greater uniformity [7,8].

Although there are currently no official statistics on its global production, estimates suggest the total annual output is below 300 tons, with Vietnam contributing under 100 tons – sourced through a mix of wild harvesting and small-scale cultivation [9,10]. This limited supply, coupled with rising demand, highlights the urgent need for conservation strategies and sustainable farming models.

One of the main challenges in the conservation and utilization of *S. brachyandra* Diels is its overexploitation. In recent years, the natural population of this species has declined rapidly [11,12]. The increasing demand for medicinal raw materials exacerbates this issue, not only threatening the sustainable supply chain for the pharmaceutical industry but also posing risks to the long-term development of Vietnam's medicinal plant sector [13,14]. To address this, several researchers have initiated propagation studies. In 2018, Nguyen Hong Ngoc tested two propagation methods – tuber cuttings and seed sowing – on a 1,000 m^2 pilot plantation in Phu Tho Province [15]. In 2020, Nguyen Thi Sen and colleagues published research on *in vitro* propagation of *S. brachyandra* Diels [16]. However, overall research on the conservation of this species remains limited. Furthermore, aspects related to sustainable development, although increasingly prioritized in recent years, are rarely addressed in these studies.

Hence, research focusing on technical cultivation processes and sustainable development strategies for *S. brachyandra* Diels is both

**Corresponding Author:*
Quang Van Nguyen,
Center for Advanced Technology Development,
Thai Nguyen University, Tan Thinh Ward, Thai Nguyen City, Vietnam.
E-mail: nguyenvanquangkn@tuaf.edu.vn

urgent and timely. Establishing a sustainable conservation model for *S. brachyandra* Diels is essential to protect and maintain this valuable medicinal resource. Proposed solutions should include the development of optimal cultivation, care, and harvesting techniques that preserve the plant's natural habitat while ensuring a reliable supply of raw materials for pharmaceutical use. Such practices would also alleviate the pressure on wild populations. A well-designed conservation and restoration strategy would not only prevent the depletion of this species but also foster long-term development opportunities for rural communities. Moreover, creating a scientifically validated cultivation model will guarantee the quality of medicinal materials and pharmaceutical products, bringing economic benefits to local farmers and supporting the growth of Vietnam's medicinal plant industry.

In conclusion, with a sustainable conservation approach and a science-based cultivation process, the development of *S. brachyandra* Diels can contribute to environmental protection while enhancing economic value and improving community health. This research direction lays the foundation for the present study on the conservation and sustainable development of *S. brachyandra* Diels: Cultivation, care, and preliminary processing techniques.

2. RESEARCH METHODOLOGY

2.1. Development of an *Ex Situ* Conservation Model

The study was conducted in La Hien Commune, Vo Nhai District, Thai Nguyen Province, over an area of 5,000 m^2. The research process involved terrain analysis, soil quality assessment, and botanical evaluations to select the most suitable planting materials. The crop was established at a planting density of 50,000 plants/ha, aiming to optimize land utilization and ensure favorable conditions for plant growth.

2.2. Experimental Design

- Experiment 1: Investigation of the effect of planting density on the growth of *S. brachyandra* Diels

This experiment evaluated the impact of different planting densities on the growth of *S. brachyandra* Diels. Four planting densities were tested, as outlined below:

- Formula 1: 0.6 × 0.6 m (N = 28,000 plants/ha)
- Formula 2: 0.6 × 0.4 m (N = 42,000 plants/ha)
- Formula 3: 0.6 × 0.32 m (N = 50,000 plants/ha)
- Formula 4: 0.4 × 0.4 m (N = 62,000 plants/ha).

Each density treatment was replicated 3 times, with each experimental plot covering an area of 50 m^2. The total experimental area was 600 m^2 (three replications × four planting densities). The layout of the experimental plots is presented in Table 1.

To assess the effects of planting density, key growth indicators, such as average stem diameter and height were recorded at 12, 24, and 30 months after planting.

- Experiment 2: investigation of the effect of fertilizer formulations on the growth of *S. brachyandra* Diels.

This experiment examined the impact of different fertilization treatments on plant growth. The experiment involved four fertilizer treatments and three replications:

- Formula 1: No fertilizer (Control)
- Formula 2: 0.2 kg NPK (20-20-15) per plant per year

Table 1: Experimental plot layout and planting density treatments.

Treatment	Treatment	Treatment
Formula 1	Formula 2	Formula 3
Formula 3	Formula 4	Formula 2
Formula 2	Formula 3	Formula 1
Formula 4	Formula 1	Formula 4

- Formula 3: 0.2 kg NPK (20-20-15) + 1 kg composted manure per plant per year
- Formula 4: 0.3 kg Song Gianh bio-organic fertilizer + 0.2 kg NPK (20-20-15) per plant per year.

Each experimental plot covered an area of 50 m^2, and the total experimental area was 600 m^2 (three replications × four treatments). The experimental design and layout were identical to those used in Experiment 1 [Table 1].

The same growth parameters (stem diameter and height) were measured 12, 24, and 30 months after planting to evaluate treatment effectiveness.

- Experiment 3: Monitoring pests and diseases

This experiment aimed to document pest and disease occurrences and assess their impact on *S. brachyandra* Diels under field conditions. Observations were conducted monthly over 3 consecutive years. The pest and disease investigation was performed in three stages: (1) Preliminary observation – Identification and marking of infected plants, recording damage levels and disease types, and collecting samples for laboratory analysis. (2) Symptom description – Documentation of affected plant parts (leaves, stems, roots), microscopic examination (×10 magnification), and comparison with reference materials for identification. (3) Cause determination – Analysis of disease characteristics, progression patterns, and environmental factors to identify underlying causes.

The percentage of infected plants (R%) was calculated using the formula:

$$R\% = \frac{n}{N} \times 100\%$$

Where:
n = number of infected plants in the experimental plot
N = total number of plants in the experimental plot

The severity of the infestation was classified as follows:
R% < 10%: Mild damage, healthy plants
10% ≤ R% < 15%: Moderate damage, normal plant growth
15% ≤ R% < 25%: Severe damage in clusters, visible plant stress
R% ≥ 25%: Critical damage, plant weakening, or mortality.

- Experiment 4: Harvesting and preservation assessment

The tubers of *S. brachyandra* Diels were harvested following standard procedures, with morphological characteristics, such as shape, color, taste, weight, and moisture content documented. These characteristics were compared against Vietnamese Pharmacopoeia (Volume V) standards.

For post-harvest processing, tubers were subjected to two drying methods:

Natural drying – Exposed to diffuse sunlight until the moisture content reaches ≤14%. Heat drying – Processed at 70°C until the sample weight remained constant. Evaluation criteria included post-processing weight, moisture content, color, taste, and condition, following the methods described by Vo Thi Que (2009).

2.3. Data Analysis

All collected experimental data were statistically analyzed using variance analysis (ANOVA) with PASW (SPSS) 2.0 software.

3. RESULTS

3.1. Effect of Planting Density on the Growth of *S. brachyandra* Diels

3.1.1. Effect of planting density on tuber diameter growth

The effect of four different planting densities on tuber diameter growth is presented in Table 2 below.

The analysis of variance results indicate that differences in the maximum tuber diameter of *S. brachyandra* Diels became increasingly pronounced among the treatments over time. Moreover, the trends observed in the comparisons between treatments varied considerably from year to year.

Statistically, in the 1st year, all four treatments produced tubers with similar diameters, ranging from 3.58 to 3.66 cm. By 2023, only treatments with formula 2 and formula 3 remained statistically comparable (5.18–5.22 cm), whereas formula 4 showed a significant increase in tuber diameter (5.72 cm), while formula 1 yielded the smallest tubers (5.05 cm).

In the final year, all treatments exhibited statistically significant differences, with the ranking completely reversed: formula 1 produced the largest tubers (8.89 cm), while formula 4, which had the highest planting density, resulted in the smallest tuber size among all treatments (8.47 cm).

3.1.2. Effect of planting density on stem height growth

The increasing planting densities had significant effects on stem height growth over the years, as shown in Table 3:

All recorded plant height values across treatments differed significantly at the 95% confidence level. A consistent trend was observed across all years: higher planting densities corresponded with taller plants. Accordingly, formula 4 (62,000 plants/ha) produced the tallest plants in each of the 3 years, followed by formula 3 (50,000 plants/ha), formula 2 (42,000 plants/ha), with formula 1 (28,000 plants/ha) yielding the shortest plants.

At the final measurement, plants under formula 4 averaged 2.76 m in height. In contrast, those in formula 1 reached only about 2.05 m – 30 cm shorter than formula 2, 50 cm shorter than formula 3, and 71 cm shorter than formula 4.

3.2. Effect of fertilization on *S. brachyandra* diels growth

3.2.1. Effect of fertilization on tuber diameter growth

Tuber root diameter is a critical parameter for evaluating the growth and yield potential of *S. brachyandra* Diels. Among the factors influencing this trait, NPK fertilizer plays a significant role in nutrient uptake, biomass accumulation, and tuber quality [17,18]. In Experiment 2, four different fertilization treatments were applied to *S. brachyandra* Diels, and the tuber diameter growth data collected over 3 years are summarized in Table 4 below.

Table 2: Effect of planting density on tuber diameter growth.

Treatment	Density (plants/ha)	$\overline{D}_{00}$ (cm)		
		2022	2023	2024
Formula 1	28.000	3.66^{a}	5.05^{a}	8.89^{a}
Formula 2	42.000	3.65^{a}	5.18^{b}	8.76^{b}
Formula 3	50.000	3.60^{ab}	5.22^{b}	8.67^{c}
Formula 4	62.000	3.58^{b}	5.72^{c}	8.47^{d}

The diameter was measured at the widest part of the tuber (D00). The lowercase letters above the numerical values indicate statistically significant differences within the same column (α=0.05).

Table 3: Effect of planting density on stem height growth.

Treatment	Density (plants/ha)	Stem height (m)		
		2022	2023	2024
Formula 1	28.000	1.55^{a}	1.82^{a}	2.05^{a}
Formula 2	42.000	1.75^{b}	2.10^{b}	2.35^{b}
Formula 3	50.000	1.82^{c}	2.35^{c}	2.55^{c}
Formula 4	62.000	1.91^{d}	2.41^{d}	2.76^{d}

The lowercase letters above the numerical values indicate statistically significant differences within the same column (α=0.05).

Table 4: Effect of fertilization on tuber diameter growth.

Treatment	Fertilization regimen	$\overline{D}_{00}$ (cm)		
		2022	2023	2024
Formula 1	No fertilizer	3.32^{a}	4.87^{a}	7.82^{a}
Formula 2	0.2 kg NPK (20-20-15)/plant/year	3.67^{b}	5.24^{b}	8.78^{b}
Formula 3	0.2 kg NPK + 1 kg composted manure/plant/year	3.83^{c}	6.25^{c}	9.77^{c}
Formula 4	0.3 kg bio-organic fertilizer + 0.2 kg NPK (20-20-15)/plant/year	3.68^{b}	5.84^{d}	9.47^{d}

The diameter was measured at the widest part of the tuber (D00). The NPK fertilizer ratio was 20/20/15. The lowercase letters above numerical values indicate statistically significant differences within the same column (α=0.05).

The analysis of variance results indicate that all fertilization treatments produced statistically significant differences in tuber diameter each year, except for formula 2 and formula 4 in 2022. Across all years, formula 3 (a combination of NPK and organic manure) consistently resulted in the largest tuber size. In contrast, the absence of fertilization in formula 1 led to significantly smaller tubers compared to the other treatments.

Specifically, in 2022, the largest tuber diameter recorded was 3.83 cm, while the smallest was 3.32 cm. In the 2nd year, differences between treatments became more pronounced: formula 3 maintained the largest tuber diameter (6.25 cm), and formula 1 continued to have the smallest (4.87 cm). Formula 4 (bio-fertilizer + NPK) produced significantly larger tubers than formula 2 (5.84 cm vs. 5.24 cm).

By the 3rd year, the highest tuber diameter was 9.77 cm in formula 3, whereas formula 1 had the smallest at 7.82 cm. Among the other treatments, formula 4 produced considerably larger tubers than formula 2 but remained approximately 0.3 cm smaller than formula 3.

3.2.2. Effects of fertilizer types on plant height growth

Fertilization regimes also significantly impacted the height of *S. brachyandra* Diels, with each treatment generally producing statistically distinct results. The specific data are recorded in Table 5 below.

The analysis of variance results indicate significant differences in stem length among fertilization treatments for *S. brachyandra* Diels each year, except between formula 3 and formula 4 in 2024. In the 1st year, plants treated with formula 3 exhibited the greatest growth, averaging 1.92 m in height, while the unfertilized group (formula 1) showed the lowest growth at 1.45 m. Formula 4 (bio-fertilizer + NPK) also performed well, reaching 1.85 m – approximately 19 cm taller than formula 2 (NPK only).

Detailed soil assessments were conducted, measuring pH (6.0–6.5), organic matter content (2.1–2.5%), macronutrient levels (NPK), and texture (loamy). These analyses confirmed the soil's suitability for *S. brachyandra* Diels, which prefers well-drained, mildly acidic soils with moderate fertility. Such favorable soil conditions ensured that the observed fertilization effects were not confounded by underlying deficiencies.

By 2023, differences between treatments widened, with formula 3 maintaining its lead at 2.47 m, while formula 1 remained the slowest-growing group at 1.89 m. Formula 4 continued to outperform formula 2, reaching 2.35 m compared to 2.10 m, highlighting the positive impact of bio-fertilizers on plant growth.

In the final year, formula 3 plants achieved the greatest height at 2.88 m; however, the difference from formula 4 (2.77 m) was no longer statistically significant, suggesting that bio-fertilizers combined with NPK can be a relatively effective alternative. Formula 2 reached 2.55 m—33 cm shorter than formula 3—while formula 1 continued to have the lowest height at 2.15 m.

Table 5: Effects of fertilization on stem length growth.

Treatment	Fertilization regimen	Stem height (m)		
		2022	2023	2024
Formula 1	No fertilizer	1.45^a	1.89^a	2.15^a
Formula 2	0.2 kg NPK (20-20-15)/plant/year	1.66^b	2.10^b	2.55^b
Formula 3	0.2 kg NPK + 1 kg composted manure/plant/year	1.92^c	2.47^c	2.88^c
Formula 4	0.3 kg bio-organic fertilizer + 0.2 kg NPK (20-20-15)/plant/year	1.85^d	2.35^d	2.77^c

The NPK ratio is 20/20/15. Different letters indicate statistically significant differences within the same column (α = 0.05).

Table 6: Calculation of R% for pest damage level.

Month	R%			Damage assessment level
	Year 2022	Year 2023	Year 2024	
2	0	0	0	0
3	2.55	4.23	5.47	Healthy: R<10%
4	3.21	5.88	6.41	Healthy: R<10%
5	2.14	3.34	5.27	Healthy: R<10%
6	0	0	0	0

3.3. Results of survey on pest species and damage levels

After more than 2 years of observation, the study identified looper caterpillars as the primary pest affecting *S. brachyandra* Diels. These caterpillars predominantly feed on young and mature leaves from March to May, with peak densities observed in the early morning and late afternoon during cooler weather. Throughout the study period, no significant signs of disease were recorded on stems, roots, or tubers.

To evaluate the impact of looper caterpillars, the infestation rate (R%) was calculated over 3 years of experimentation. The detailed results are summarized in Table 6 below.

The pest infestation survey results indicate that the percentage of *S. brachyandra* Diels plants affected by looper caterpillars (R%) remained below 10% throughout the 3-year period, suggesting that the pest impact was minimal and did not significantly hinder plant growth. Overall, R% showed a slight upward trend over the years, with the highest infestation recorded in April 2024 at 6.41%. April 2023 also exhibited a relatively high infestation rate of 5.88%, second only to May 2024. Based on these data, the extent of damage appears to be insignificant, and cultivation areas of *S. brachyandra* Diels remain generally healthy. However, continuous monitoring of pest dynamics is essential to prevent potential outbreaks under unfavorable conditions.

3.4. Research Results on Harvesting, Processing, and Storing Products

3.4.1. Sensory characteristics of S. brachyandra Diels tubers after harvest

According to the Vietnamese Pharmacopoeia, *S. brachyandra* Diels tubers can be harvested throughout the year. Published studies suggest that the optimal harvesting age for medicinal cultivation is 5 years or older. To produce Rotundine, the tubers require only cleaning and storage in a well-ventilated area. However, for traditional herbal medicine preparation, the tubers should be thoroughly peeled, sliced, and dried under diffuse sunlight.

Observation, Measurement, and Evaluation of the Morphological Characteristics and Taste of *S. brachyandra* Diels Medicinal Material: Results Presented in Table 7 Below.

The observed morphological characteristics and taste of *S. brachyandra* Diels medicinal material exhibit a high level of conformity with the standards outlined in the Vietnamese Pharmacopoeia V. The shape of the tubers varies depending on growing conditions, featuring a dark brown outer skin, grayish-white inner flesh, and a distinctive bitter taste. Tuber weights range from large to very large, with exceptional cases reaching up to 25 kg when harvested from natural forests in Thai Nguyen. The moisture content after drying ranged from 13.78% to 13.80%, remaining below the permissible limit of 14%, thereby ensuring compliance with storage standards. These findings are illustrated in Figure 1a (outer skin layer), Figure 1b (tuber morphology), and Figure 1c (sliced medicinal material), reaffirming the stability and quality of *S. brachyandra* Diels for practical applications.

3.4.2. Effects of natural air drying on the post-harvest quality of S. brachyandra Diels tubers

The following data present the research results on the characteristics of *S. brachyandra* Diels medicinal material during the drying and storage. The evaluated parameters include the fresh sample weight after harvest, the weight of the medicinal material after natural air drying, moisture content, color, taste, and pest infestation status.

The research results indicate that *S. brachyandra* Diels medicinal material exhibits high stability during drying and storage. From an initial fresh sample weight of 100 g, the average weight after natural air drying was 20.623 g, with a moisture content of 13.73%. The dried medicinal material had a grayish-white color, a characteristic bitter taste, and showed no signs of pest infestation or mold growth.

Table 7: Morphological characteristics and taste of *Stephania brachyandra* Diels medicinal material.

TT	Evaluation Indicator	Evaluation Indicator	Evaluation Indicator
1	Shape of the tuber	Shape varies depending on the location of tuber growth	Shape varies depending on the location of tuber growth
2	Tuber color	The outer skin is black-brown, and when peeled, the inner skin is gray-white	The outer skin is black-brown, and when peeled, the inner skin is gray-white
3	Taste of the tuber	Bitter	Bitter
4	Tuber weight	The root base develops into large tubers, with very large tubers	The root base develops into large tubers, with very large tubers
5	Moisture content (%)	≤14	13.78–13.80

Table 8: The effect of natural drying on the quality of medicinal material in *Stephania brachyandra* Diels roots.

Medicinal material	Evaluation Indicator	Sample 1	Sample 2	Sample 3	Average
Medicinal material after natural drying	Fresh sample weight after harvest (g)	100.0	100.0	100.0	100.0
	Sample weight after natural drying (g)	20.61	20.62	20.64	20.623
	Moisture content of the medicinal material (%)	13.7	13.8	13.7	13.73
	Color of the medicinal material	Grayish white			
	Taste of the medicinal material	Bitter			
	Damage by pests and molds	None			
Dry medicinal material after 6 months of storage	Sample weight after 6 months of storage (g)	20.63	20.65	20.66	20.65
	Weight difference between medicinal material after storage and after drying (g)	0.02	0.02	0.02	0.027
	Color of the medicinal material	Light yellow			
	Taste of the medicinal material	Bitter			
	Damage by pests and molds	None			

Table 9: The effect of heat treatment (drying) on the quality of medicinal material in *Stephania brachyandra* Diels roots.

Medicinal material	Evaluation Indicator	Sample 1	Sample 2	Sample 3	Average
Medicinal material after drying	Fresh sample weight after harvest (g)	100.0	100.0	100.0	100.0
	Dry sample weight after drying at 70°C (g)	20.61	20.60	20.61	20.607
	Moisture content of the medicinal material (%)	13.8	13.8	13.9	13.83
	Color of the medicinal material	Light yellow			
	Taste of the medicinal material	Bitter			
	Damage by pests and molds	None			
Dry medicinal material after 6 months of storage	Sample weight after 6 months of storage (gr)	20.63	20.61	20.63	20.63
	Weight difference between medicinal material after storage and after drying (g/sample)	0.02	0.01	0.02	0.017
	Color of the medicinal material	Light yellow			
	Taste of the medicinal material	Bitter			
	Damage by pests and molds	None			

After 6 months of storage, the average dry weight increased slightly by 0.027 g, with the color shifting to light yellow while retaining its characteristic bitterness, without any occurrence of pests or mold. These findings demonstrate that *S. brachyandra* Diels meets high storage standards, ensuring its quality and durability for medicinal use.

3.4.3. Effects of natural drying at 70°C on the post-harvest quality of S. brachyandra Diels tubers

Study the effects of drying methods (at 70°C) on the quality of *S. brachyandra* Diels medicinal material: results presented in Table 8.

The research results presented in Table 9 indicate that heat treatment (drying at 70°C) effectively preserves the quality of *S. brachyandra* Diels medicinal material. The average dry sample weight was 20.607 g, with a moisture content of 13.83%, meeting the storage standard (≤14%). After 6 months of storage, the sample weight increased by an average of 0.017 g/sample due to environmental moisture absorption; however, no pest infestations or mold growth were observed. The medicinal material retained its light yellow color and characteristic bitter taste.

Tubers were stored at 18–22°C, 60–70% relative humidity, and in darkness to reduce sprouting and maintain moisture content. These controlled conditions contributed to preserving the sensory and physicochemical stability of the samples throughout the storage period.

A comparison between the two processing methods (natural air drying and heat drying) reveals that both achieve the standard moisture

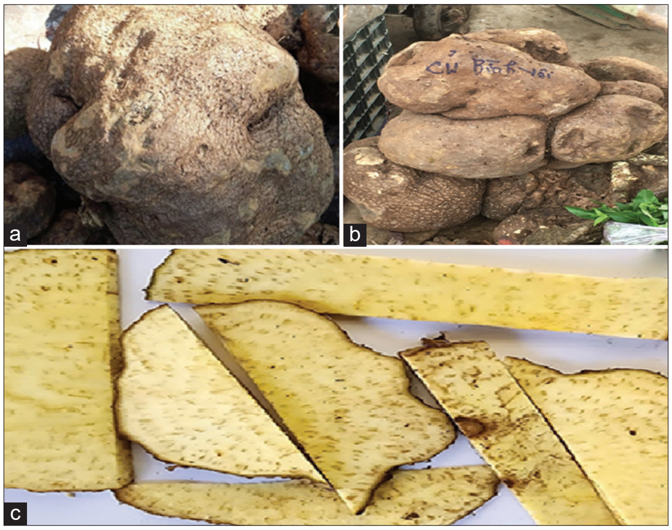

Figure 1: (a) The surface of the Pilewort tuber; (b) Pilewort tubers after harvest; (c) Cross-section of the tuber.

content and ensure medicinal quality over 6 months of storage. However, natural air drying offers economic advantages, especially under favorable weather conditions, and can be supplemented with final-stage heat drying to eliminate mold and pests. This combined approach minimizes fuel costs while maintaining long-term quality, making it a practical and widely applicable method for medicinal plant production.

4. DISCUSSION

Planting density is crucial in plant development, particularly for root crops, such as *S. brachyandra* Diels. Determining an optimal planting density not only maximizes yield but also contributes to the conservation strategy of valuable medicinal resources. Lower planting density gives plants more space for root system expansion, enhancing water and nutrient absorption, and increasing tuber size [19]. This finding aligns with the results in Table 2. Although medium density showed advantages in the first 2 years due to mutual reinforcement among plants, by the final year, low density resulted in the largest tuber size, confirming that lower density supports more effective root and tuber development [20]. Furthermore, high planting density produces intense competition for nutrients, restricting tuber growth [16,21]. According to Table 3, higher densities promote taller plant stems, but this does not necessarily translate into improved yield. Excessive stem height can cause an imbalance in plant growth, making them prone to lodging, which reduces nutrient allocation to the tuber and ultimately diminishes its medicinal value [22]. For long-term medicinal crops, such as *S. brachyandra* Diels, optimizing planting density enhances productivity and supports sustainable development strategies, ensuring a stable supply without depleting natural ecosystems.

NPK fertilizer is an essential component in the development of tuber crops. However, this study reveals that combining NPK with organic fertilizers is a key factor in promoting the sustainable growth of *S. brachyandra* Diels. Although baseline soil chemical parameters were not analyzed, we have added key site characteristics, such as soil type (Feralis on limestone, 40–50 cm depth), slope (27°, southeast-facing), and vegetation cover (30%) to support the interpretation of fertilizer effects [17,18]. In contrast, organic manure enhances soil structure, improves moisture retention, and supplies a sustainable organic source, creating favorable conditions for strong root development [23-25]. Bio-fertilizers containing beneficial bacteria aid in absorbing trace elements and promoting nutrient mineralization, enabling healthy plant growth without complete dependence on chemical fertilizers [26,27]. Overreliance on NPK without incorporating organic fertilizers may lead to soil degradation and a decline in medicinal quality over time [28-30]. Experimental results indicate that the combination of NPK with organic manure or bio-fertilizers produces larger tubers than NPK alone, reinforcing the importance of organic fertilizers in the sustainable development strategy for medicinal plants. This approach helps maintain soil fertility and protects the agricultural ecosystem.

Looper caterpillars (*Geometridae*) were the only recorded pests affecting *S. brachyandra* Diels in this study, with their damage concentrated between March and May. Compared to other root-bearing medicinal plants in the *Menispermaceae* family, such as *Fibraurea recisa* (Hoàng đằng) and *Tinospora cordifolia* (Dây ký ninh), *S. brachyandra* Diels has the advantage of relatively mild pest infestations, with a low damage rate (R% <10%) [31,32]. In contrast, some other medicinal plants, such as *F. recisa*, are highly susceptible to root rot caused by fungi or nematodes, leading to significant losses if not properly managed [14,33]. The absence of recorded diseases affecting the stems and roots of *S. brachyandra* Diels may be attributed to the plant's specific ecological adaptability, particularly its preference for limestone-rich soils. Such soils typically exhibit lower humidity and reduced fungal activity, creating less favorable conditions for common soil-borne pathogens [34]. Although looper caterpillar infestations have not caused significant damage, continuous monitoring during the spring-to-summer transition remains critical. Fluctuating environmental conditions could create temporary windows of vulnerability, making proactive pest management essential for ensuring the consistency and quality of *S. brachyandra* Diels crops [34,35].

In addition, findings from this study on tuber preservation methods align closely with previous research on medicinal plant storage. The use of sand or mesh bags has proven highly effective in extending shelf life, minimizing moisture loss, and lowering spoilage rates – practices that are consistent with established post-harvest handling strategies for medicinal roots [36]. These methods uphold the fundamental principles of herbal preservation, where maintaining moderate humidity and limiting air exposure are crucial in mitigating microbial growth and physical degradation [36]. When compared to other root-based medicinal crops, *S. brachyandra* Diels demonstrates superior post-harvest stability, exhibiting minimal quality loss over extended periods under appropriate conditions. This finding supports the broader goals of sustainable medicinal plant cultivation by enhancing supply chain efficiency, reducing storage and transport-related losses, and promoting the judicious use of raw herbal materials [36].

However, it is important to acknowledge a key limitation of this study. The experiment was conducted at a single location and over one cropping season. As such, findings may vary under different agro-climatic conditions or temporal contexts. We have now included a discussion of this limitation and emphasized the importance of conducting multi-location and multi-season trials to confirm the reproducibility and generalizability of the results.

5. CONCLUSION

This study evaluated the effects of planting density and fertilization methods on the growth of *S. brachyandra* Diels, contributing to the proposal of sustainable cultivation solutions. Low planting density facilitates optimal root and tuber development in the long term, whereas medium density offers advantages in the early stages due to balanced nutrient competition. Combining NPK with organic manure or bio-fertilizers enhances tuber size and maintains stable yield, emphasizing the crucial role of organic fertilizers in soil improvement and ecosystem protection. Pest monitoring experiments identified looper caterpillars as the primary pest species, but their impact was minimal, aligning with the goal of safe medicinal plant cultivation. In addition, storing tubers in sand or mesh bags effectively prolongs storage duration and reduces post-harvest losses.

Overall, these findings optimize *S. brachyandra* Diels production and preservation sustainably, ensuring a balance between yield, quality, and environmental conservation. However, further long-term studies are needed to assess the plant's adaptability to different climatic and soil conditions.

6. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to the conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the present journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This study on Stephania branchyandra Diels followed national regulations on biodiversity conservation and medicinal plant use. No endangered populations were disturbed; plant materials were responsibly sourced from permitted areas or research plots. Fieldwork was conducted with local consent, and all techniques aligned with sustainable practices. As no human or animal subjects were involved, IRB approval was not required.

10. DATA AVAILABILITY

Data are available from the corresponding author upon reasonable request.

11. PUBLISHER'S NOTE

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12. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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