4. Conclusion

Scale and rhizome characters with information on the previously described specimens suggested that the LVH-D19 sample collected in Phu Quoc National Park is of *Drynaria sparsisora*. This treatment was strongly supported by molecular evidence based on the plastid *rbcL* marker. According to our preliminary survey, *D. sparsisora* only naturally exists as isolated small populations scattered on Phu Quoc Island. Further investigations are thus required to gain a deeper insight into biological and cytological characteristics as well as the phytochemistry profile and medicinal potential usage of this newly recorded species for proper protection and sustainable promotion in traditional medicine.

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EFFECTS OF NITROGEN LEVELS ON GROWTH, YIELD AND QUALITY OF *CURCUMA AERUGINOSA* ROXB.

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Summary

Effects of Nitrogen Levels on Growth, Yield and Quality of *Curcuma aeruginosa* Roxb.

Nitrogen factor (N) is one of important major effect to yield and quality of *Curcuma aeruginosa* Roxb. The purpose of this research was to find the nitrogen fertilizer formula that is suitable for growth and development of *Curcuma aeruginosa* Roxb. in Vietnam. The result indicated that application of 150 kg N/ha could help *Curcuma aeruginosa* Roxb. to achieve high yield and quality with germination rate reached 80%, the time from planting to finishing sprouting was 20-25 days, time from planting to harvesting was from 252 to 260 days; The average plant height was 184.89 cm; number of leaves/main stem was 9-10 leaves; The average number of branches/cluster was 3-4 branches; the average yield was 8.87 - 10.37 tons/ha. The content of essential oil was 3.12 - 3.48 mL/100g; the yield of Germacrom content was 15.364 kg/ha; the yield of Curdion was 53.494 kg/ha; The yield of Furanodiene was 8.756 kg/ha. The results in the present study also could enable the production of *Curcuma aeruginosa* Roxb. obtain the high quality medicinal material in the future.

Keywords: *Curcuma aeruginosa* Roxb., *Nitrogen dosage on growth, Yield, Essential oil.*

1. Introduction

Curcuma aeruginosa Roxb. is a medicinal plant growning in mountainous areas in Indonesia, Malaysia, Cambodia and China. In Vietnam, *Curcuma aeruginosa* was found in some provinces, such as Ha Giang, Hung Yen, Hanoi and Thai Binh. *Curcuma aeruginosa* is an constituent in Vietnamease traditional medicine. The botanical characteristics of *Curcuma aeruginosa* Roxb. was described as Fig 1A, B. The extract from tuber of the plant is used to treat blood stasis, abdominal pain, enlarged liver and spleen, menstrual cramps and indigestion (Pham Hoang Ho, 2000; National Institute of Medicinal Materials, 2006).

More than 50 substances have been identified in the rhizome of *Curcuma aeruginosa* Roxb. and the main compounds have good effects in treatment disease such as 1,8-cineole, curzerenone, furanogermenone, camphor, (Z)-3-hexenol, furanodienone, curcumenol, isocurcumenol and β-elemene were listed by Leopold Jirovetz et al., 2000. Meanwhile, numerous study indicated that there is some essential oil of camphor (16.85%), curzerenone (16.81%) and epicurzerenone (3.5%) in the rhizomes of turmeric copper using High Performance Liquid Chromatography (HPLC) (Chantana Aromdee, Senee Polrat, Preeya Wangsomnuek, 2011).

In order to select good individual *Curcuma aeruginosa* Roxb. plant for cultivation, in 2019, samples of *Curcuma aeruginosa* Roxb. were collected in some locations (Ha Giang, Tuyen Quang, Thai Binh, Hung Yen and Hanoi) to planted and evaluated for more understanding about growth, development, yield and quality in Thanh Tri, Hanoi. The results showed that *Curcuma aeruginosa* Roxb. samples in Thai Binh and Hung Yen gave essential oil yields of 161.86 kg/ha and 166.05 kg/ha (Dao Van Nui et al., 2020).

The basic raw material fertilizer and cultivartion played an important role in increasing yield and quality of *Curcuma aeruginosa* Roxb. medicinal content, however study on *Curcuma aeruginosa* Roxb. plant was very limited and there are still no scientific reports yet in Vietnam. Therefore, how to invest N fertilization for higher yields and quality of *Curcuma aeruginosa* Roxb. in Vietnam conditions. This study was evaluated the effect of nitrogen application doses on growth, yield and quality of *Curcuma aeruginosa* Roxb. in Hanoi.

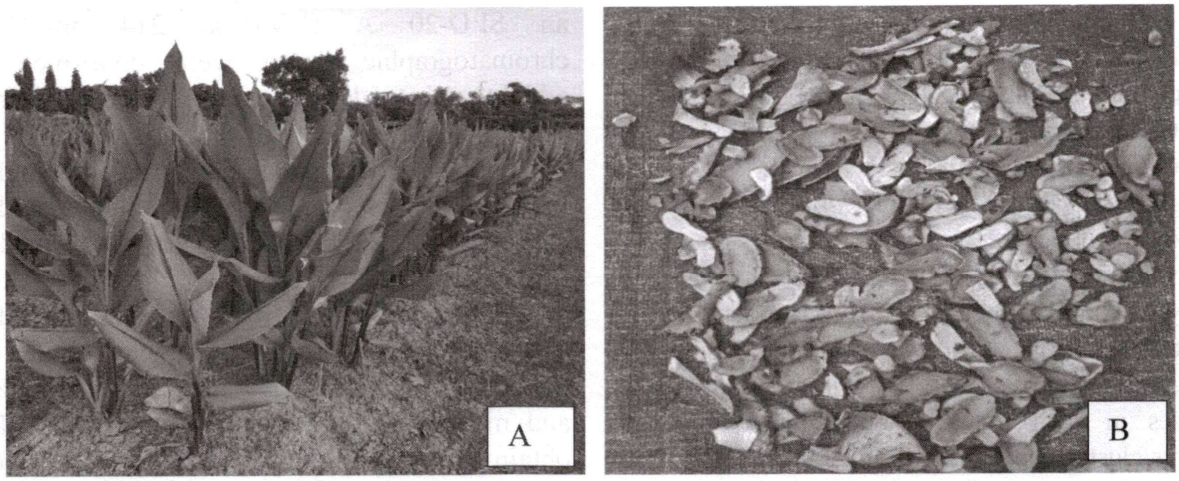

Fig.1. A. *Curcuma aeruginosa* Roxb. in the different nitrogen fertilizer treatment experiment
B. The *Curcuma aeruginosa* Roxb. dry rhizome was used as a traditional Vietnam medicine

2. Material and Methods

Plant material and experimental Site

Field experiments of this study was carried from 2020 to 2021 at Research Center for Medicinal Plants Hanoi, Vietnam (RCMP).

Curcuma aeruginosa Roxb. variety collected from Thai Binh was used in experiments, which was regarding productive performance in the Hanoi climatic conditions of Vietnam.

Experimental design and management

Two experimental were designed in this study.

Experiment 1: Determining the amount of nitrogen absorbed by Curcuma aeruginosa Roxb. *and the amount of nitrogen in the soil*

Sampling the crocus at the time of plant death (including 2 stems and 2 rhizomes), calculate the dry matter content and the nitrogen content (%) in the harvested product.

Take 2 soil samples before planting the nutrition experiment of *Curcuma aeruginosa* Roxb.

Total nitrogen (%) (TCVN 6498:1999) according to Kjeldahl Method. The samples were analyzed at the Institute of Soil and Agrochemistry.

Experiment 2: The effect of nitrogen doses on growth, yield and quality of Curcuma aeruginosa Roxb.

Total formula in this experiment was conducted from the result of the nitrogen based on the requirement of *Curcuma aeruginosa* Roxb. identified in experiment 1.

The experiment was consisted of 8 N treatments and was arranged in a randomized complete block design (RCBD) with 3 replications. The area of each experimental plot was $10m^2$.

Non-experimental factors: Planting distance 30 x 30cm. Planting season in March - April. After harvesting, the bulbs are stored in a shady place until the planting season.

Growth and yield analysis

Growth indexes included growth time, plant height and stem diameter of *Curcuma aeruginosa* Roxb. plants were recorded at harvest such as: Germination time (days); Planting-to-harvest time (days); The plant height (cm) was measured from root to tip of growth (once a month); Number of leaves/plant (leaves/plant): Count the number of leaves in each month of tracking; Number of plants/cluster (plants): Count the number of plants/cluster for each month of monitoring.

Biomass yield (rhizome yield) were determined in each plant. The fresh biomass and dry rhizome yield per plant were determined using one digital scale with precision of 0.01 g. Dry rhizome had an important role in determination of *Curcuma aeruginosa* Roxb. quality.

***Measurement of Curcuma aeruginosa* Roxb. Content**

Chemicals and materials: Acetonitrile (HPLC grade), deionized water. Ruranodiene was purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (CAS: 19912-61-9, HPLC $\geq$ 98%). Curdione and Germacrone were isolated in the laboratory from *Curcuma aeruginosa* Roxb. using column chromatography and their purity was determined by NMR.

Instrumentation and analytical conditions: Phytochemical analysis was conducted to identify major active compounds by using the high-performance liquid chromatography (HPLC) method, using an LC-20AD apparatus (Shimadzu Corp, Kyoto, Japan). The experiments were carried out using a reversed phase C18 Cosmosil column (250 mm × 4.6 mm; Nacalai Tesque Inc.). The samples were eluted with a stepwise elution of the mobile phase with solvent A (H_2O) and solvent B (acetonitrile), at a unique flow rate of 1 mL/min. The gradient elution program was as follows: 0 min 70% A:30% B; 15 min 53% A:47% B; 30 min 53% A:47% B; 40 min 40% A:60% B; 50 min 10% A:90% B, 60 min 0% A:100% B, 75 min 0% A:100% B, 90 min 70% A:30% B, 100 min 70% A:30% B. The sample injection volume was 20 µl and monitored with an SPD-20 A (UV) at 214 nm. The chromatographic peaks were confirmed by addition of standard compounds and comparing their retention times with those of the reference compounds. The amount of each compound in the extract was calculated from their standard curves. The HPLC analysis was performed in triplicate.

Preparation of standards: The standard solutions were prepared by dissolving in methanol and transferring in a volumetric flask and making up the volume using methanol to obtain the mixed standard solution of curdione (1.700 mg/mL), germacrone (0.720 mg/mL), and furanodiene (0.330 mg/mL). These stock solutions were further diluted to final concentrations of 0.085-1.700 mg/mL with curdione, 0.036-0.720 mg/mL with germacrone, and 0.0165-0.330 mg/mL with furanodiene in methanol and stored at 4°C until use.

Sample preparation: *Curcuma aeruginosa*

Roxb. were dried at 45°C by *freeze-drying*. Then, the samples were finely ground prior to extraction. Five hundred milligrams of the dried sample were extracted in 10 mL of methanol in an ultrasonic bath at room temperature for 30 min. After extraction, the sample solutions were filtered through a 0.45 µm membrane filter and injected into the chromatograph.

Quantification of essential oil content by steam distillation method (Attachment 12.7 DDVN V).

Test article solution preparation: *Curcuma aeruginosa* Roxb. were dried at 45°C by *freeze-drying* and powdered. 30 g powder of *Curcuma aeruginosa* Roxb., and added 500 mL in to flask of the medicinal essential oil dosing kit. And then Add 300 mL of water and continue to distill for 3 hours to measured.

Statistical Analysis

Data was analyzed using CropStat 7.2. and Excel 2010 software, and then strain and variety fertilizing amount were included as experimental factor to calculate the significant difference of oil content in *Curcuma aeruginosa* Roxb.

3. Results and discussion

3.1. Detemination the amount of N, P, K absorbed by Curcuma aeruginosa Roxb. *from the field and the amount of N, P, K in the soil*

3.1.1. Determination of the amount of nitrogen absorbed by Curcuma aeruginosa Roxb. from the field:

The percentage of nitrogen in crops generally varies from 1 to 6% of the dry matter weight. Nitrogen is the most important element for living organisms because it is the basic component of proteins which is essential for growth and development of plant. In order to determine the doses of nitrogen for the experiment on the response of turmeric to nitrogen, we sampled the leaves and roots of turmeric at the time of harvest to calculate the amount of nitrogen take up from the leaves and rhizomes.

Table 1. Content of nitrogen take up from the field

Number	Part	DM (%)	Dry yield (t/ha)	Fresh yield (t/ha)	Total nitrogen (%)	Total amount of nitrogen up take (kg/ha)
1	Rhizome	33.20	7.57	22.80	1.61	121.88
2	Leaf stem	29.94	10.09	33.70	0.86	86.77
Total		-	-	-	-	208.65

In this study, the results have shown in Table 1. Based on the results of the analysis for nitrogen content and yield of leaves and rhizomes, we calculated that the amount of nitrogen taken up from the field was 208.65 kg N/ha.

3.1.2. Contents of N, P, K of soil in nutrient experiment field:

To determine the content of N, P, K in the soil of *Curcuma aeruginosa* Roxb., the same sample of soil was collected to analyze the content of total N, P, K at the Institute of Soil and Agrochemistry. The results of this study were described in Table 2.

Table 2. Soil nutrient in the experiment field

Criterial	Sample		Average	N, P, K content in the soil (kg/ha)
	Soil 1	Soil 2		
NH_4^+ (mg/100g)	0.20	0.16	0.18	20.27
NO_3^- (mg/100g)	0.63	0.70	0.67	
P_2O_5 (mg/100g)	68.72	76.31	72.51	1,750.05
K_2O (mg/100g)	6.25	6.53	6.39	154.22

To determine nutrient content in soil to evaluate the ability of soil in this experiment. Soil samples were taken after harvesting *Leonurus japonicus* Houtt. Plant to absorb nutrients from soil and create evenly condition between experimental treatments as well as between replicates. The N, P and K values from two soil samples were consistent.

The results of Table 1 and Table 2, it shows the nitrogen demand of the plant (208.65 kg N/ha) and the nitrogen supply capacity of the soil (20.27 kg N/ha). Hence, to evaluate the response of *Curcuma aeruginosa* Roxb. to nitrogen doses and suitable amounts of nitrogen for plants to achieve the highest yield and quality, an experiments was conducted with 9 nitrogen application rates from 0, 60, 90, 120, 150, 180, 210 and 240 kg N per ha.

3.2. Identify the effect of nitrogen dosage on growth, development and yield quality of Curcuma aeruginosa Roxb. *medicinal herbs*

3.2.1. Effect of nitrogen dose on growing time and growth rate of *Curcuma aeruginosa* Roxb.:

Table 3. Effect of different nitrogen dosage on growing time and growth rate of *Curcuma aeruginosa* Roxb.

Treatment	Year 2020				Year 2021			
	Plant to sprout (day)	Plant to harvest (day)	Growth rate		Plant to sprout (day)	Plant to harvest (day)	Growth rate	
			%	√x+0,5			%	√x+0,5
N1 (0N)	25	260	93.33	10.16	20	252	78.95	9.39
N2 (60N)	25	260	83.33	9.63	20	252	86.11	9.78
N3 (90N)	25	260	87.50	9.85	20	252	81.25	9.51
N4 (120N)	25	260	81.67	9.54	20	252	82.35	9.57
N5 (150N)	25	260	85.83	9.76	20	252	83.33	9.63
N6 (180N)	25	260	84.17	9.67	20	252	92.11	10.10
N7 (210N)	25	260	90.00	9.99	20	252	97.22	10.36
N8 (240N)	25	260	89.17	9.94	20	252	89.47	9.96
$LSD_{0,05}$	-	-	-	*1.49*	-	-	-	*0.94*
CV%	-	-	-	*8.60*	-	-	-	*7.60*

The results in Table 3 showed that there was no interaction between different fertilizer treatments to growth rate and growing time of *Curcuma aeruginosa* Roxb. All data are means ± LSD calculated from three replicates. Three biological experiments were performed, which produced similar results. Evaluating the factors isolated, different nitrogen fertilizer treatment did not show significance on growth time of *Curcuma aeruginosa* Roxb.

3.2.2. Effect of nitrogen doses on growth parameters of *Curcuma aeruginosa* Roxb.:

The difference in fertilizer uptake affected in growth may be because of the higher absorption of water and mineral nutrients due to extensive colonization of roots or rhizome (Benhmimou A et al., 2017).

To identify the effect of nitrogen level on growth parameters, this study evaluated plant height, number of leaves/main stem, number of clusters/plant when harvested over 2 consecutive years 2020 and 2021.

Table 4. Effect of nitrogen doses on growth parameters *of Curcuma aeruginosa* Roxb.

Treatment	Year 2020			Year 2021		
	Plant height (cm)	Number of leaves/main stem	Number of plants/cluster	Plant height (cm)	Number of leaves/main stem	Number of plants/cluster
N1 (0N)	97.30	9.47	3.33	146.66	9.07	3.07
N2 (60N)	94.74	8.63	3.00	162.33	9.33	3.83
N3 (90N)	104.16	9.40	3.20	165.22	9.40	2.53
N4 (120N)	102.56	9.13	3.17	169.77	9.52	3.56
N5 (150N)	111.08	9.43	3.07	184.89	10.78	4.22
N6 (180N)	102.98	9.37	3.03	185.56	10.11	4.33
N7 (210N)	105.69	9.27	3.17	183.00	9.91	4.30
N8 (240N)	110.23	9.40	3.37	184.89	9.78	4.57
CV (%)	*8.70*	*9.10*	*12.2*	*7.8*	*12.00*	*13.70*
$LSD_{0.05}$	*15.80*	*0.87*	*0.31*	*23.62*	*2.86*	*1.23*

During growing period of *Curcuma aeruginosa* Roxb., the data for growth and development was collected and presented in Table 4. All data were means ± LSD calculated from three replicates. Symbols LSD indicate significant difference between of growth parameters at 0.05.

The result in Table 4 indicated that plant height in the second year showed superiority to the first year of planting. It can be seen that in the second year of planting turmeric has gradually stabilized along with the growing time and good for plant growth. The plant heighest reached 184.89 cm in second year while in the first year the highest reached 111.08 cm. However, plant height between the treatments for each year was not statistically significant with 95% confidence.

3.2.3. Effect of different nitrogen dosage on yield in *Curcuma aeruginosa* Roxb.:

Yield is the goal of the *Curcuma aeruginosa* Roxb. production, and also affects the economic efficiency and quality of plant in cultivation. In this study, the results in Table 5 showed that *Curcuma aeruginosa* Roxb. responded differently with nitrogen dosage. All data are means ± LSD calculated from three replicates. Three biological experiments were performed, which produced similar results.

The biomass yield of *Curcuma aeruginosa* Roxb. was highest at application of 150 kg N/ha (Table 5) and was not significant different between N5, N6, N7 and N8 treatment. So N5 was maybe suitable for *Curcuma aeruginosa* Roxb. cultivated in Hanoi.

Table 5. Effect of different nitrogen dosage on yield of *Curcuma aeruginosa* Roxb.

Formula	Year 2020			Year 2021		
	Individual weight (g/plant)	Fresh/ Dry Ratio	Dry yield (tonnes /ha)	Individual weight (g/plant)	Fresh/ Dry Ratio	Dry yield (tonnes /ha)
N1 (0N)	597.77	2.93 ± 0.40	5.31	645.70	3.31 ± 0.43	6.50
N2 (60N)	720.58	3.25 ± 0.22	5.62	778.30	3.02 ± 0.19	6.63
N3 (90N)	769.95	3.42 ± 0.19	5.41	836.33	3.28 ± 0.29	7.17
N4 (120N)	780.33	3.44 ± 0.06	6.44	861.30	2.92 ± 0.31	7.19
N5 (150N)	1013.07	3.06 ± 0.06	8.87	1199.00	3.38 ± 0.14	10.37
N6 (180N)	1017.57	3.09 ± 0.12	8.77	1200.00	3.08 ± 0.45	9.23
N7 (210N)	1021.57	3.52 ± 0.54	8.82	1221.33	3.30 ± 0.41	9.37
N8 (240N)	1081.08	3.19 ± 0.32	8.26	1224.60	3.04 ± 0.61	9.21
CV (%)	*19.23*		*10.04*	*21.20*		*10.70*
$LSD_{0.05}$	*232.56*		*1.96*	*337.62*		*1.54*

3.2.4. Effect of different nitrogen dosage on quality in *Curcuma aeruginosa* Roxb.:

In this study, as shown in Table 6 *Curcuma aeruginosa* Roxb. plants under eight nitrogen fertilization treatment. After treatment, *Curcuma aeruginosa* Roxb. Tube were collected for measurements of yield content. The means and LSD were calculated from three replicates.

For germacrom and curdion content, at the treatment N8 yield was highest. However, the application of N in treatments N5, N6, N7 and N8 was not significant different (with $LSD_{0.05}$= 2.26, 12.48 respectively). Similarly, for furanodiene content, N7 obtained the highest yield of active ingredient furanodiene, but there was no significant difference in the application of N in treatments N5, N6, N7 and N8 with $LSD_{0.05}$ = 1.18.

The result in table 5 and 6 showed that the applying fertilizer could increase yield and quality in *Curcuma aeruginosa* Roxb. Hence, application of N5 was more suitable for *Curcuma aeruginosa* Roxb. cultivation.

Table 6. Effect of different nitrogen dosage on quality in *Curcuma aeruginosa* Roxb.

Treatment	Curdion Content (g/100g)	Curdion yield (kg/ha)	Germacrom Content (g/100g)	Germacrom yield (kg/ha)	Furanodiene Content (g/100g)	Furanodiene yield (kg/ha)
N1 (0N)	-	-	0.096 ± 0.006	6.23	0.029 ± 0.003	1.90
N2 (60N)	1.060 ± 0.011	70.30	0.187 ± 0.004	12.43	0.102 ± 0.002	6.77
N3 (90N)	1.057 ± 0.009	75.77	0.182 ± 0.011	13.03	0.093 ± 0.0005	6.67
N4 (120N)	0.902 ± 0.020	64.80	0.162 ± 0.001	11.60	0.088 ± 0.002	6.33
N5 (150N)	1.017 ± 0.005	93.67	0.186 ± 0.004	17.13	0.106 ± 0.001	9.77
N6 (180N)	0.919 ± 0.005	84.83	0.161 ± 0.0002	14.87	0.083 ± 0.002	7.70
N7 (210N)	0.940 ± 0.010	87.47	0.176 ± 0.003	16.37	0.105 ± 0.0002	9.80
N8 (240N)	0.985 ± 0.008	92.00	0.180 ± 0.004	16.80	0.099 ± 0.0002	9.23
CV (%)	-	*10.00*	-	*9.50*	-	*9.30*
$LSD_{0.05}$	-	*12.48*	-	*2.26*	-	*1.18*

Table 7 showed that essential oil content of *Curcuma aeruginosa* Roxb. was significant difference between N2 and N7. The essential oil content in treatment 1 was only from 1.16 (2020) to 1.52 mL/100 g dry matter (2021) and was lower than that of other treatments. The highest oil content in the 2nd year was at the treatment N8 (240N), however, over 2 consecutive years, essential oil content in treatment 5 was more stable. Therefore, the treatment N5 (150N) was chosen as the most optimal application for *Curcuma aeruginosa* Roxb.

Table 7. Evaluation of essential oil content in turmeric of treatments

Treatments	Essential oil content (mL/100 g)	
	Year 2020	Year 2021
N1 (0N)	1.16	1.52
N2 (60N)	2.20	3.04
N3 (90N)	1.28	3.14
N4 (120N)	1.40	2.74
N5 (150N)	3.48	3.12
N6 (180N)	1.84	2.77
N7 (210N)	1.91	3.04
N8 (240N)	2.88	3.33

4. Conclusion

This research indicated that different N treatments had significant effect on the growth parameters and crop yield, however it does not affect the time from planting to sprouting and harvesting. Application of 150 kg N/ha gave the highest the germination rate (80%), plant height (184.89 cm), the average number of leaves/main stem (9-10 leaves) and average number of plants/cluster (3-4 plant). In addition, applied 150 kg N/ha gave the highest yield (8.87 - 10.37 tonnes/ha), highest essential oil (3.48-3.12 mL/100g). Therefore, by using suitable fertilizer for cultivation of *Curcuma aeruginosa* Roxb. could be targeted for obtaining high qualititative medicinal materials.

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